Single plate separation of lung phospholipids including disaturated phosphatidylcholine

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Summary We have developed an improved thin-layer chromatographic method for separation of lung phospholipids. Individual phospholipids are completely separated in the first dimension. All phospholipids, except phosphatidylcholine, are then removed. The phosphatidylcholine-containing area is reacted with osmium tetroxide and saturated phosphatidylcholine species are separated from the unsaturated oxidation products by subsequent chromatography in the second dimension. This method should prove useful in studies on lung surfactant metabolism and secretion, in prediction of fetal lung maturity by analysis of amniotic fluid phospholipids, and in studies on surfactant obtained by bronchoscopy in adult lung diseases.—**Gilfillan, A. M., A. J. Chu, D. A. Smart, and S. A. Rooney.** Single plate separation of lung phospholipids including disaturated phosphatidylcholine. *J. Lipid Res.* 1983. **24:** 1651–1656.

Supplementary key words disaturated phosphatidylcholine • phosphatidylglycerol • lung surfactant • amniotic fluid • bronchoalveolar lavage

Lung surfactant has a characteristic phospholipid composition which is distinctly different from that of whole lung or other tissues (1–3). Phosphatidylcholine accounts for about 80% of the total phospholipid in surfactant and over half of this is disaturated. Phosphatidylglycerol is the second most abundant phospholipid and accounts for up to 11% of the total (2, 3). Other common membrane phospholipids, such as phosphatidylethanolamine and sphingomyelin, are characteristically present only in very low amounts in surfactant. Small amounts of phosphatidylserine, phosphatidylinositol, cardiolipin, lysophosphatidylcholine, and lysobisphosphatidic acid have also been reported in surfactant preparations (4–6).

Since there is no exclusive chemical marker for it, the phospholipid profile has been used to identify surfactant, for instance in secretions from newborn lung slices (7) and isolated type II pneumocytes (6). Measurement of surfactant phospholipids in amniotic fluid is used clinically to predict the degree of fetal lung maturity (8) while alterations in surfactant phospholipids have also been studied in adult lung disease (9–12).

Most conventional procedures for complete separation of surfactant phospholipids involve two-dimensional thin-layer chromatography (6, 8, 13, 14). Separation of disaturated phosphatidylcholine usually requires elution and an additional chromatographic step (6, 15). In this study we describe a procedure that permits complete resolution of surfactant phospholipids by one-dimensional thin-layer chromatography and separation of saturated and unsaturated phosphatidylcholine species on the same plate by chromatography in the second dimension; the entire separation can be carried out in 2–3 hr. An abstract of this work has been published (16).

MATERIALS AND METHODS

Adult female New Zealand White rabbits (Pineacres Rabbitry, West Brattleboro, VT) were killed with an overdose of sodium pentobarbital and the lungs were lavaged in situ with cold saline via an endotracheal tube (4). The lavage liquid was centrifuged at 800 *g* for 15 min, to remove cellular material, and lyophilized.

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Human lavage was obtained from healthy adults as described previously (17). Human amniotic fluid was obtained by amniocentesis. Human lavage and amniotic fluids were centrifuged at 500 *g* for 10 min.

Type II pneumocytes were isolated from adult male Sprague-Dawley rats (Charles River, Cambridge, MA), weighing 150–175 g, essentially by the procedure of Brown and Longmore (18) except that the trypsin concentration was 0.06% and the trypsin inhibitor consisted of 30% fetal bovine serum and 0.05% soybean trypsin inhibitor in Hepes buffered salt solution (19). The cells were cultured for 18–20 hr in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) containing 10% fetal bovine serum, streptomycin (100 U/ml), and penicillin (100 U/ml). The cells consisted of over 95% type II pneumocytes as determined by electron microscopy. Over 99.8% of the cells excluded trypan blue. Rates of choline and palmitate incorporation into phosphatidylcholine, leucine into protein, and thymidine into DNA were linear with time for at least 6 hr after the 18–20 hr culture period. Type II cell phospholipids were labeled by inclusion of sodium [1-^{14}C]acetate (5 μCi/ml) in the culture medium. The cells were washed with fresh medium, and 2 hr later the medium was aspirated and the cells were lysed with ice-cold water for phospholipid extraction.

Lipid extraction and fractionation

Lipids were extracted with mixtures of chloroform and methanol. For lung tissue we used essentially the Folch procedure (4) and for lung lavage, amniotic fluid and type II cell materials a modification (20) of the procedure of Bligh and Dyer (21). Phospholipids were separated from neutral lipids and glycolipids by chromatography on silicic acid (Unisil; Clarkson Chemical Co., Williamsport, PA) columns (4).

Thin-layer chromatography

We used 0.25-mm-thick, 20 × 20 cm K6 silica gel plates (Whatman, Clifton, NJ) without any pretreatment other than activation at 100°C for 90 min to avoid any problems with humidity (22). Phospholipids were separated in the first dimension in chloroform–methanol–petroleum ether (bp 35–60°C)–acetic acid–boric acid 40:20:30:10:1.8 (v/v/v/v/w). The boric acid was dissolved in the methanol–acetic acid before addition of the other components. The solvent was freshly prepared and the chromatogram was developed to within 1 cm of the top of the plate in tanks lined with solvent-saturated filter paper. After drying under a stream of N_2, the lipids were visualized by brief exposure to iodine vapor. The gel in the entire first dimension strip, with the exception of the area containing phosphatidylcholine, was removed and the plate was developed with acetone to a height of 10 cm in the second dimension. This procedure removed traces of iodine and acetic acid which interfere with the subsequent oxidation and separation of phosphatidylcholine species. The plate was thoroughly dried with N_2. If any trace of iodine remained, this step was repeated. Phosphatidylcholine was oxidized directly on the gel with 20–60 μl (sufficient to cover the entire spot) of 5% osmium tetroxide in carbon tetrachloride. After 3 min, excess osmium tetroxide was removed in a fume hood under a stream of N_2 and the plate was developed in chloroform–methanol–conc. NH_4OH 65:35:5 (v/v/v) to a height of 10 cm in a solvent-saturated atmosphere. After drying, the phosphatidylcholine spots were visualized with iodine vapor.

The complete separation can be achieved in 2–3 hr. Chromatography in the first dimension takes approximately 1.5 hr, each acetone wash approximately 15 min, and the final separation of the phosphatidylcholine species takes approximately 30 min.

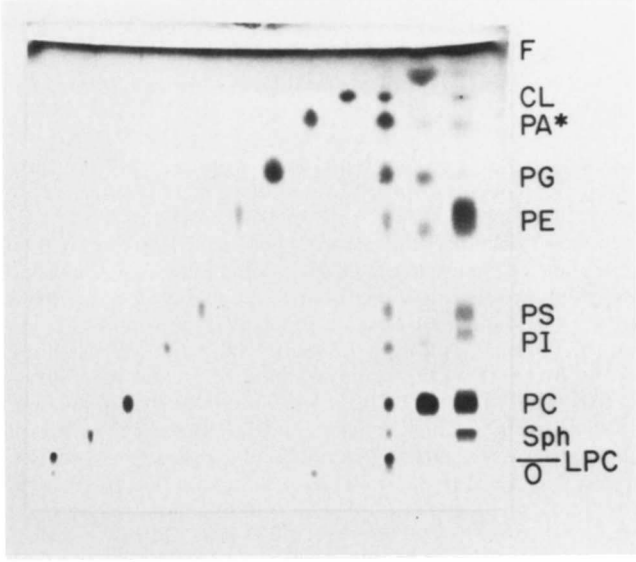

Fig. 1. Phospholipid separation in the first dimension in chloroform–methanol–petroleum ether (bp 35–60°C)–acetic acid–boric acid 40:20:30:10:1.8 (v/v/v/v/w). Abbreviations: O, origin; LPC, lysophosphatidylcholine; Sph, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; CL, cardiolipin; F, solvent front. From left, lanes 1–9 contain individual phospholipid standards (5–25 μg of phospholipid, assuming a phosphorus content of 4%), lane 10 contains a mixture of standards (90 μg of phospholipid), and lanes 11 and 12 contain total lipid extracts from adult rabbit lung lavage (4 μg of phosphorus) and lung tissue (5 μg of phosphorus), respectively. Lipids were applied in 2–10 μl and visualized by exposure to iodine vapor. The chromatogram was photographed with a Polaroid MP3 camera and type 51 high contrast film (25). *The lipid in lung tissue and lung lavage samples that migrated to the same position as standard PA may be lysobisphosphatidic acid (4, 25). It was separated from standard PA on chromatography in the second dimension in chloroform–methanol–water 65:35:5 (v/v/v) (25).

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Other procedures

Phospholipids were quantitated by phosphorus assay. Phosphorus was measured directly on the gel by the procedure of Bartlett (23) after digestion with H_2SO_4 (13). Fatty acids were analyzed as methyl esters by gas–liquid chromatography as described previously (24). Radioactivity was measured directly on the gel in Aquasol (New England Nuclear, Boston, MA) containing 3% water.

Chemicals

Phosphatidylinositol and phosphatidylserine standards were obtained from Avanti (Birmingham, AL); other phospholipid standards and osmium tetroxide were from Sigma (St. Louis, MO). Other chemicals were reagent grade.

RESULTS AND DISCUSSION

Separation of phospholipids in the first dimension is shown in **Fig. 1.** The separation was highly reproducible as indicated by the low variation in R_f values (**Table 1**). Neutral lipid standards including free fatty acids, cholesterol, cholesteryl oleate, triolein, and monoolein migrated close to the solvent front ahead of cardiolipin. We routinely applied 100–150 μg of phospholipid from lung tissue or lavage, but up to 250 μg did not result in appreciable loss of resolution.

Fig. 2 illustrates separation of disaturated and unsaturated phosphatidylcholine species after removal of the other phospholipids separated in the first dimension. Presumably the three spots derived from unsaturated phosphatidylcholines are oxidation products derived from molecules with different degrees of saturation since oxidation products of 1-palmitoyl-2-oleoylphosphatidylcholine and dioleoylphosphatidylcholine were separated in this system. More than 98% of the fatty acids in the disaturated spot were saturated. Duplicate analyses of four different samples revealed the following fatty acid composition (% of total expressed as mean ± SE): myristic acid, 3.3 ± 0.3; palmitic acid, 93.1 ± 0.2; stearic acid, 2.4 ± 0.3; unidentified, 1.2 ± 0.1. Recoveries of mixtures of standard saturated and unsaturated phosphatidylcholines were close to expected (**Table 2**).

TABLE 1. Phospholipid composition of lung and surfactant-rich materials[a]

Phospholipid	R_f[b]	Adult Rabbit Lung Tissue	Adult Rabbit Lung Lavage	Adult Human Lung Lavage[c]	Human Amniotic Fluid[d]	Adult Rat Type II Pneumocytes[e]		
						Cells	Cells	Medium
		% total phospholipid phosphorus					*% total radioactivity*	
n[f]		3	4	3	4	4	3	3
Origin		0	0.2 ± 0.1	0.5 ± 0.2	0.7 ± 0.4	0.1 ± 0.1	0.6 ± 0.3	0.6 ± 0.5
LPC[g]	0.04 ± 0.004	0.6 ± 0.2	1.0 ± 0.5	1.7 ± 0.5	1.0 ± 0.4	0.3 ± 0.3	1.7 ± 0.4	1.0 ± 0.1
Sph	0.10 ± 0.008	10.9 ± 0.8	1.4 ± 0.4	4.0 ± 2.0	4.2 ± 0.7	3.2 ± 0.2	3.9 ± 1.1	3.5 ± 1.1
PC[h]	0.18 ± 0.014	50.5 ± 1.1	80.9 ± 0.9	67.5 ± 3.3	74.6 ± 2.2	71.1 ± 2.9	76.7 ± 3.6	80.1 ± 2.3
DSPC		18.6 ± 1.7	50.0 ± 2.4	49.7 ± 6.4	47.8 ± 3.1	31.2 ± 2.3	43.2 ± 3.4	52.6 ± 4.4
USPC		31.9 ± 0.7	30.9 ± 1.8	17.7 ± 3.3	26.8 ± 4.0	39.9 ± 1.2	33.4 ± 2.2	27.5 ± 3.5
PI	0.28 ± 0.013	4.6 ± 0.3	3.2 ± 0.2	3.6 ± 0.4	6.9 ± 1.4	2.8 ± 0.3	2.9 ± 0.6	1.7 ± 0.2
PS	0.35 ± 0.013	7.3 ± 0.4	0.5 ± 0.1	1.6 ± 0.7	2.1 ± 0.5	1.5 ± 0.3	1.3 ± 0.4	0.6 ± 0.3
PE	0.55 ± 0.019	21.2 ± 0.6	3.2 ± 0.8	5.3 ± 1.3	2.8 ± 1.1	11.8 ± 1.2	4.4 ± 1.1	2.8 ± 0.8
PG	0.64 ± 0.014	1.7 ± 0.5	7.1 ± 0.9	10.0 ± 1.0	3.7 ± 0.7	6.0 ± 0.7	4.6 ± 0.1	6.5 ± 0.4
Unknown[i]	0.76 ± 0.012	1.0 ± 0.03	1.1 ± 0.2	3.6 ± 1.0	2.1 ± 0.4	0.5 ± 0.4	1.0 ± 0.1	1.5 ± 0.3
CL	0.83 ± 0.013	0.8 ± 0.04	0.2 ± 0.04	0.9 ± 0.5	0.3 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	0.9 ± 0.5
Front[j]		1.8 ± 0.5	1.3 ± 0.1	1.3 ± 0.3	1.8 ± 0.1	1.6 ± 0.9	1.8 ± 0.7	0.8 ± 0.7
DSPC as % of PC		36.6 ± 2.5	61.6 ± 2.4	73.1 ± 5.9	64.4 ± 5.0	43.8 ± 1.9	56.3 ± 2.9	65.6 ± 4.7

[a] The phospholipid fraction of the radiolabeled and human lavage lipids and total lipids from the other samples were analyzed. The data are means ± SE. Recoveries, as determined by phosphorus assay, were 99.3% ± 2.0 in 14 analyses.

[b] R_f values were measured in the first dimension. These data are means ± SE from 10 analyses chosen randomly over a period of 4 months.

[c] Human lavage samples were obtained from 20–22-year-old healthy females. A spot which co-chromatographed with lidocaine, used as a local anesthetic in the bronchoscopy procedure, was removed on silicic acid column chromatography.

[d] Amniotic fluid was collected at 36–38-weeks (mean = 37.25) gestation.

[e] Acutely isolated type II cells after 3 hr in culture were used for phospholipid analysis by phosphorus assay. Cells cultured for 18–20 hr were used in the radioactive experiments.

[f] n = number of individual rabbits, humans, or type II cell preparations from five to ten rats. Each sample was analyzed at least in duplicate.

[g] Abbreviations are as in Figs. 1 and 2.

[h] The PC value was calculated by addition of the DSPC and USPC values.

[i] The unknown phospholipid may be lysobisphosphatidic acid (see Fig. 1 footnote).

[j] The front includes all material ahead of CL.

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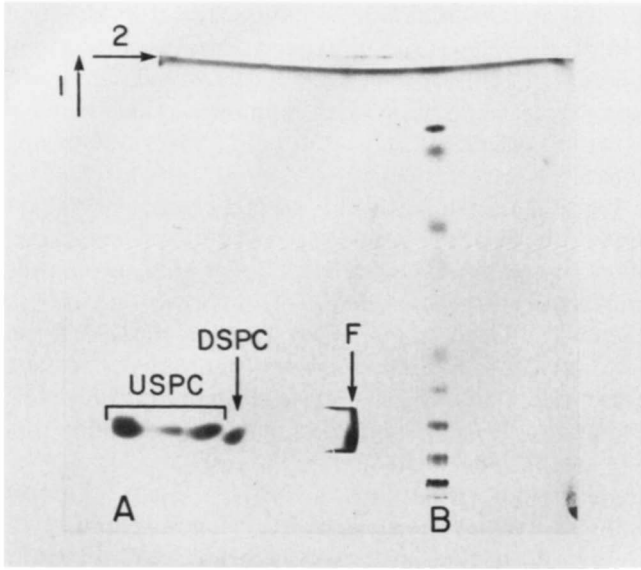

Fig. 2. Separation of saturated and unsaturated phosphatidylcholines in the second dimension. Rabbit lung tissue lipids (8 μg of phosphorus) were applied to lane A and a mixture of standard phospholipids (Fig. 1) was applied to lane B and the chromatogram was developed as in Fig. 1. After all silica gel in lane A (with the exception of the area containing phosphatidylcholine) was scraped off, the phosphatidylcholine spot was oxidized with osmium tetroxide as described in Materials and Methods, and the chromatogram was developed in the second dimension with chloroform–methanol–conc. NH_4OH 65:35:5 (v/v/v). The lane was scored to prevent lateral migration of the solvent. First and second dimensions are indicated by arrows. Abbreviations: USPC, oxidation products of unsaturated phosphatidylcholines; DSPC, disaturated phosphatidylcholine; F, second dimension solvent front.

The phospholipid composition of various pulmonary materials as determined by this procedure is shown in Table 1. These data are similar to those previously published for adult rabbit lung tissue (1, 4, 5, 26) and lavage (2, 4, 26), adult human lung lavage (12), human amniotic fluid at or close to term (27, 28), adult rat type II cells as determined by phosphorus assay (29–31), or [^{14}C]acetate incorporation (6, 31) and material secreted from type II cells (6). The disaturated phosphatidylcholine values are also in good agreement with previous values, except that Hallman et al. (12) reported less disaturated phosphatidylcholine (63% of total phosphatidylcholine) in human lung lavage than in our study (73%). Disaturated phosphatidylcholine in human amniotic fluid is usually quantitated by densitometry rather than phosphorus assay and, in addition, is measured in an acetone-precipitated phospholipid fraction (8), which makes comparison with our data difficult. Nevertheless, the percentage of amniotic fluid phosphatidylcholine that is disaturated in our study (64%) is similar to the values (65% and 60%, respectively) reported for term newborn human lung effluents (32) and rhesus monkey amniotic fluid (33).

This new system has the advantage over existing systems that the entire separation can be carried out on one thin-layer plate. The complete separation can be achieved in 2–3 hr with complete recovery of the applied phospholipid. Since neutral lipids migrate close to the solvent front in the first dimension and glycolipids are only minor components in lung (4) and lung lavage (34) lipid extracts, preliminary separation of phospholipids from neutral lipids and glycolipids should only be necessary when radiolabeled lipids are analyzed. Most previous systems for complete separation of lung phospholipids require two-dimensional thin-layer chromatography of the individual phospholipids (6, 8, 13, 14) followed by separation of phosphatidylcholine species in additional steps (6, 15, 26). Recently, one-dimensional systems have been improved by use of boric acid-impregnated plates (35) or inclusion of triethylamine (36, 37) or petroleum ether (38) in conventional solvent systems. Our system incorporates some of these features but it avoids the necessity of pretreating the plates by inclusion of boric acid in the developing solvent. Separation of all commonly detectable lung phospholipids in amounts sufficient for conventional phosphorus assay is considerably improved over that in other recently developed one-dimensional systems (35–41).

Since it allows simultaneous measurement of the phosphatidylcholine/sphingomyelin ratio and percentages of disaturated phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol, this system may be particularly useful in determining the 'lung profile' (8) used in the prediction of fetal lung maturity (8) and assessments of surfactant in adult lung disease (12).

TABLE 2. Separation of mixtures of saturated and unsaturated phosphatidylcholines[a]

Disaturated Phosphatidylcholine as % of the Mixture	
Expected	Found
0	5.8 ± 2.6
25	24.5 ± 0.7
50	52.2 ± 0.5
75	76.2 ± 0.8
100	97.9 ± 1.0

[a] Mixtures (a total of 2.0 μg phosphorus) of dipalmitoylphosphatidylcholine and 1-palmitoyl-2-oleoylphosphatidylcholine-dioleoylphosphatidylcholine 1:1 were applied to thin-layer plates that had been previously developed in the first solvent and dried thoroughly. After oxidation with osmium tetroxide, the chromatograph was developed in the second dimension and the disaturated and unsaturated phosphatidylcholine spots (Fig. 2) were quantitated by phosphorus assay. The phosphatidylcholine standards (99% pure) were purchased from Sigma (St. Louis, MO) and were used without further purification. The data are means ± SE of 4 analyses. Recovery of phospholipid phosphorus was 97.7% ± 2.8 in 15 analyses.

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REFERENCES

1. Sanders R. L. 1982. The chemical composition of the lung. *In* Lung Development: Biological and Clinical Perspectives. Vol. 1, Biochemistry and Physiology. P. M. Farrell, editor. Academic Press, New York. 179–192.
2. Sanders, R. L. 1982. The composition of pulmonary surfactant. *In* Lung Development: Biological and Clinical Perspectives. Vol. 1, Biochemistry and Physiology. P. M. Farrell, editor. Academic Press, New York. 193–210.
3. Rooney, S. A. 1983. Biochemical development of the lung. *In* The Biological Basis of Reproductive and Developmental Medicine. J. B. Warshaw, editor. Elsevier Biomedical, New York. 239–287.
4. Rooney, S. A., P. M. Canavan, and E. K. Motoyama. 1974. The identification of phosphatidylglycerol in the rat, rabbit, monkey and human lung. *Biochim. Biophys. Acta.* **360:** 56–67.
5. Hallman, M., and L. Gluck. 1980. Formation of acidic phospholipids in rabbit lung during perinatal development. *Pediatr. Res.* **14:** 1250–1259.
6. Dobbs, L. G., R. J. Mason, M. C. Williams, B. J. Benson, and K. Sueishi. 1982. Secretion of surfactant by primary cultures of alveolar type II cells isolated from rats. *Biochim. Biophys. Acta.* **713:** 118–127.
7. Marino, P. A., and S. A. Rooney. 1980. Surfactant secretion in a newborn rabbit lung slice model. *Biochim. Biophys. Acta.* **620:** 509–519.
8. Kulovich, M. V., M. Hallman, and L. Gluck. 1979. The Lung Profile. I. Normal pregnancy. *Am. J. Obstet. Gynecol.* **135:** 57–63.
9. von Wichert, P., and F. V. Kohl. 1977. Decreased dipalmitoyllecithin content found in lung specimens from patients with so-called shock-lung. *Intens. Care Med.* **3:** 27–30.
10. Low, R. B., G. S. Davis, and M. S. Giancola. 1978. Biochemical analyses of bronchoalveolar lavage fluids of healthy human volunteer smokers and nonsmokers. *Am. Rev. Respir. Dis.* **118:** 863–875.
11. Ryan, S. F., D. F. Liau, A. L. L. Bell, S. A. Hashim, and C. R. Barrett. 1981. Correlation of lung compliance and quantities of surfactant phospholipids after acute alveolar injury from N-nitroso-N-methylurethane in the dog. *Am. Rev. Respir. Dis.* **123:** 200–204.
12. Hallman, M., R. Spragg, J. H. Harrell, K. M. Moser, and L. Gluck. 1982. Evidence of lung surfactant abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. *J. Clin. Invest.* **70:** 673–683.
13. Khosla, S. S., A. Brehier, A. J. Eisenfeld, L. D. Ingleson, P. A. Parks, and S. A. Rooney. 1983. Influence of sex hormones on lung maturation in the fetal rabbit. *Biochim. Biophys. Acta.* **750:** 112–126.
14. Aloia, R. C. 1981. Phospholipid composition of the lung and liver of the hibernating ground squirrel, *Citellus lateralis. Comp. Biochem. Physiol.* **68B:** 203–208.
15. Mason, R. J., J. Nellenbogen, and J. A. Clements. 1976. Isolation of disaturated phosphatidylcholine with osmium tetroxide. *J. Lipid Res.* **17:** 281–284.
16. Gilfillan, A. M., A. J. Chu, and S. A. Rooney. 1983. Separation of total and disaturated lung phospholipids on a single thin-layer plate. *Federation Proc.* **42:** 1911 (Abstract).
17. Merrill, W., E. O'Hearn, J. Rankin, G. Naegel, R. A. Matthay, and H. Y. Reynolds. 1982. Kinetic analysis of respiratory tract proteins recovered during a sequential lavage protocol. *Am. Rev. Respir. Dis.* **126:** 617–620.
18. Brown, L. A. S., and W. J. Longmore. 1981. Adrenergic and cholinergic regulation of lung surfactant secretion in the isolated perfused rat lung and in the alveolar type II cell in culture. *J. Biol. Chem.* **256:** 66–72.
19. Dobbs, L. G., and R. J. Mason. 1979. Pulmonary alveolar type II cells isolated from rats. Release of phosphatidylcholine in response to β-adrenergic stimulation. *J. Clin. Invest.* **63:** 378–387.
20. Rooney, S. A., L. I. Gobran, P. A. Marino, W. M. Maniscalco, and I. Gross. 1979. Effects of betamethasone on phospholipid content, composition and biosynthesis in the fetal rabbit lung. *Biochim. Biophys. Acta.* **572:** 64–76.
21. Bligh, E. G., and W. G. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
22. Tsao, F. H. C. 1982. The effect of humidity on the determination of lecithin/sphingomyelin ratio and phosphatidylglycerol by thin-layer chromatography. *Clin. Chim. Acta.* **122:** 75–78.
23. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
24. Rooney, S. A., B. A. Page-Roberts, and E. K. Motoyama. 1975. Role of lamellar inclusions in surfactant production: studies on phospholipid composition and biosynthesis in rat and rabbit lung subcellular fractions. *J. Lipid Res.* **16:** 418–425.
25. Poorthuis, B. J. H. M., P. J. Yazaki, and K. Y. Hostetler. 1976. An improved two-dimensional thin-layer chromatography system for the separation of phosphatidylglycerol and its derivatives. *J. Lipid Res.* **17:** 433–437.
26. Rooney, S. A., L. L. Nardone, D. L. Shapiro, E. K. Motoyama, L. Gobran, and N. Zaehringer. 1977. The phospholipids of rabbit type II alveolar epithelial cells: comparison with lung lavage, lung tissue, alveolar macrophages, and a human alveolar tumor cell line. *Lipids.* **12:** 438–442.
27. Hallman, M., M. Kulovich, E. Kirkpatrick, R. G. Sugarman, and L. Gluck. 1976. Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: indices of lung maturity. *Am. J. Obstet. Gynecol.* **125:** 613–617.
28. Oulton, M., A. E. Bent, J. H. Gray, E. R. Luther, and L. J. Peddle. 1982. Assessment of fetal pulmonary maturity by phospholipid analysis of amniotic fluid lamellar bodies. *Am. J. Obstet. Gynecol.* **142:** 684–691.
29. Mason, R. J., and M. C. Williams. 1980. Phospholipid composition and ultrastructure of A549 cells and other cultured pulmonary epithelial cells of presumed type II cell origin. *Biochim. Biophys. Acta.* **617:** 36–50.
30. Dobbs, L. G., E. F. Geppert, M. C. Williams, R. D. Greenleaf, and R. J. Mason. 1980. Metabolic properties and ultrastructure of alveolar type II cells isolated with elastase. *Biochim. Biophys. Acta.* **618:** 510–523.

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31. Mason, R. J., L. G. Dobbs, R. G. Greenleaf, and M. C. Williams. 1977. Alveolar type II cells. *Federation Proc.* **36:** 2697–2702.
32. Hallman, M., B. H. Feldman, E. Kirkpatrick, and L. Gluck. 1977. Absence of phosphatidylglycerol (PG) in respiratory distress syndrome in the newborn. Study of the minor surfactant phospholipids in newborns. *Pediatr. Res.* **11:** 714–720.
33. Curbelo, V., D. B. Gail, and P. M. Farrell. 1978. Determination of disaturated lecithin in rhesus monkey amniotic fluid as an index of fetal lung maturity. *Am. J. Obstet. Gynecol.* **131:** 764–769.
34. Slomiany, A., F. B. Smith, and B. L. Slomiany. 1979. Isolation and characterization of a sulfated glyceroglucolipid from alveolar lavage of rabbit. *Eur. J. Biochem.* **98:** 47–51.
35. Fine, J. B., and H. Sprecher. 1982. Unidimensional thin-layer chromatography of phospholipids on boric acid-impregnated plates. *J. Lipid Res.* **23:** 660–663.
36. Touchstone, J. C., J. C. Chen, and K. M. Beaver. 1980. Improved separation of phospholipids in thin-layer chromatography. *Lipids.* **15:** 61–62.
37. Korte, K., and M. L. Casey. 1982. Phospholipid and neutral lipid separation by one-dimensional thin-layer chromatography. *J. Chromatogr.* **232:** 47–53.
38. Pappas, A. A., R. E. Mullins, and R. H. Gadsden. 1982. Improved one-dimensional thin-layer chromatography of phospholipids in amniotic fluid. *Clin. Chem.* **28:** 209–211.
39. Vitiello, F., and J. P. Zanetta. 1978. Thin-layer chromatography of phospholipids. *J. Chromatogr.* **166:** 637–640.
40. Painter, P. C. 1980. Simultaneous measurement of lecithin, sphingomyelin, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, and phosphatidylserine in amniotic fluid. *Clin. Chem.* **26:** 1147–1151.
41. Gentner, P. R., M. Bauer, and I. Dieterich. 1981. Thin-layer chromatography of phospholipids. Separation of major phospholipid classes of milk without previous isolation from total lipid extracts. *J. Chromatogr.* **206:** 200–204.

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