Cell Tissue Res (1981) 220:17–25

Cell and Tissue Research

Differentiation of type II cells of human fetal lung in vitro*

Jeanne M. Snyder, John M. Johnston, and Carole R. Mendelson

Cecil H. and Ida Green Center for Reproductive Biology Sciences; and Departments of Biochemistry, Cell Biology, and Obstetrics-Gynecology. The University of Texas Health Science Center at Dallas, Dallas, Texas, USA

Summary. Lung tissue explants from mid-trimester human abortuses were maintained for 8 days in organ culture in medium with or without serum. Before the start of culture the cells lining the pre-alveolar ducts were undifferentiated and contained no lamellar bodies, the intracellular organelle that contains surfactant. After 4 days in organ culture, the epithelium lining the pre-alveolar ducts was composed of differentiated type II cells containing numerous lamellar bodies. During the 8-day culture period there was increased incorporation of [^{3}H]choline into phosphatidylcholine and disaturated phosphatidylcholine. In addition, the specific activity of phosphatidate phosphohydrolase, a regulatory enzyme in lung phospholipid synthesis, increased 4-fold during the culture period. Lamellar bodies isolated by differential centrifugation from explants maintained in culture for 7 days had the characteristic ultrastructure described for this organelle. Lamellar bodies were isolated from explants which had been incubated with [^{14}C]glycerol. When the glycerophospholipid composition of lamellar bodies was analyzed it was found that the majority of the radiolabeled glycerol (74%) was incorporated into phosphatidylcholine and into the anionic phospholipids, phosphatidylglycerol (5%) and phosphatidylinositol (6%). Thus, human fetal lung explants maintained in organ culture contain differentiated type II cells which synthesize surfactant characteristic of human fetal lung at 36 to 38 weeks of gestation.

Key words: Lung – Human – Lamellar bodies – Phospholipids – Differentiation

Respiratory distress syndrome, the leading cause of death in premature human newborns, is caused by an immaturity in the mechanism of synthesis and secretion

Send offprint requests to: Jeanne M. Snyder, Ph.D., Department of Biochemistry, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235, USA

* This work was supported by USPH Grant 1 PO1 HD13912 and the Robert A. Welch Foundation, Houston, Texas

Acknowledgements. The authors express thanks to Sue E. Gallaspy, Sybille Galosy, and Virginia Herndon for expert technical assistance and acknowledge the excellent editorial assistance of Dolly Tutton. We also thank Sue MacDonald and Susan Cutrer for assistance in obtaining the tissues used for this study

0302-766X/81/0220/0017/$01.80

of surfactant by the type II cell of the lung (Farrell and Avery 1975). Surfactant, a lipoprotein that acts to reduce the surface tension at the air-alveolar interface, is stored within the type II cell in a specific organelle, the lamellar body.

Surfactant is particularly rich in a specific phosphatidylcholine, dipalmitoyl-phosphatidylcholine. The second most abundant glycerophospholipid in adult lung surfactant is phosphatidylglycerol. During the latter stages of gestation, the phospholipid composition of surfactant changes from one enriched in phosphatidylinositol to one enriched in phosphatidylglycerol (Hallman et al. 1976). It has been suggested that phosphatidylglycerol is necessary for the proper functioning of surfactant (Ikegami et al. 1979) and that its absence in newborn lung surfactant may predispose the infant to develop respiratory distress syndrome (Hallman et al. 1977).

Phosphatidate phosphohydrolase (PAPase; EC 3.1.3.4) activity has been shown to be associated with the lamellar body and surfactant, and both PAPase and surfactant are secreted into the fluid bathing the fetal lung alveoli and eventually into the amniotic fluid (Bleasdale and Johnston 1981). PAPase specific activity in amniotic fluid increases after the 30th gestational week in the human, and this increase in enzyme activity precedes or is concomitant with an increase in the amniotic fluid lecithin-to-sphingomyelin ratio, an index of fetal lung maturation.

There is considerable evidence that hormones may regulate type II cell differentiation in the fetal lung (c.f. Farrell and Hamosh 1978). In order to study the direct effect of hormones on fetal lung maturation it is necessary to use an in vitro model, preferably one that does not require serum. Rousseau-Merck et al. (1972) and Ekelund et al. (1975) have studied human fetal lung explants maintained in serum-containing medium. In the present study, we report that when lung tissue from mid-trimester human abortuses is maintained in organ culture in serum-free medium the ductular epithelium differentiates into type II cells which contain numerous lamellar bodies. The phospholipid composition, as determined by [^{14}C]glycerol incorporation, of lamellar bodies isolated from these explants is similar to that of lamellar bodies isolated from amniotic fluid at 36 to 38 weeks gestation.

Materials and methods

Culture techniques

Lung tissues from 12 human abortuses (16 to 22 weeks gestational age) were obtained aseptically in accordance with the Donors Anatomical Gift Act of the State of Texas after obtaining consent in writing from the woman undergoing abortion. The consent form and protocol were approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas, Texas. The lung tissue was rinsed several times with sterile tissue culture medium, Waymouth's MB 752/1, which contained 100 units/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml fungizone (Gibco, Grand Island, NY). Major airways and blood vessels were dissected from the lungs and the tissue was minced into small fragments (approximately 1 mm^3) with a razor blade. The tissue pieces were placed on lens paper supported by a stainless steel grid in a 35 mm plastic organ culture dish (Falcon, Oxnard, CA) containing tissue culture medium. In some experiments heat-inactivated fetal calf serum (FCS) (Gibco, Grand Island, NY) was added at a concentration of 10% (v/v). Cultures were maintained at 37° C in an atmosphere of 95% air and 5% CO_2. Media were changed daily.

Biochemical analyses

The synthesis of phosphatidylcholine by lung explants was assayed by incubating the tissue with [methyl-^{3}H]choline (2 μCi/ml, 84 Ci/mmol, New England Nuclear, Boston, MA) for 24 h after 1, 3, 5 or 7 days of culture. The explants were then homogenized in 1 ml H_2O (4° C), an aliquot removed for protein determination according to the method of Lowry et al. (1951), and 0.7 ml extracted with chloroform-methanol according to the method of Bligh and Dyer (1959). We found that greater than 90% of the label was incorporated into phosphatidylcholine after analysis of the glycerophospholipids by thin layer chromatography (Skipski and Barclay 1969). In addition, we determined the percent of [^{3}H]choline incorporated into disaturated phosphatidylcholine (DSPC) (Mason et al. 1976).

The relative amount of [^{14}C]glycerol incorporated into the various glycerolipids of isolated lamellar bodies was also determined. Lung tissue explants that had been cultured for 6 days were incubated with [U-^{14}C]glycerol (1 μCi/ml, 100 mCi/mmol, New England Nuclear, Boston, MA) for 24 h. The explants were then homogenized, lamellar bodies isolated as described below, and the phospholipids extracted, Lipid extracts were analyzed by the two-dimensional thin layer chromatography system described by Yavin and Zutra (1977). Phospholipid spots were visualized with iodine vapor, scraped into scintillation vials, and counted by liquid scintillation techniques. The data are expressed as the percentage of the cpm in each lipid class compared to the total cpm recovered from the plate. The percent incorporation reflects the amount of each glycerolipid class synthesized during the 24 h pulse with [^{14}C]glycerol for all of the glycerolipids with the exception of phosphatidylglycerol. Since phosphatidylglycerol contains two glycerol molecules within its structure, the relative amount of phosphatidylglycerol is computed by the percent glycerol incorporation divided by two. Recoveries of the original sample spotted on the plate averaged 85%.

PAPase specific activity was assayed in human fetal lung explants harvested every 24 h up to 6 days of culture. The tissue was homogenized in Tris (0.01 M), sucrose (0.25 M) buffer, pH 7.4, and the enzyme activity was measured in the 600 × *g* supernatant fraction according to a method described previously (Johnston et al. 1978).

Isolation of lamellar bodies

Lung tissue explants that had been maintained in culture for 7 days in a 100 mm petri dish containing 10 ml of tissue culture medium (approximately 0.5 g of tissue) were harvested and homogenized in 2.0 ml buffer [NaCl (0.15 M), EGTA (0.10 mM), Tris HCl (0.05 M), pH 7.4] with 10 strokes in a 5 ml Potter-Elvehjem teflon-glass homogenizer. All procedures were performed at 4° C. Lamellar bodies were isolated as described previously (Spitzer et al. 1975) with some modifications. The homogenate was diluted to 5 ml with buffer, then layered over 5 ml of sucrose (0.75 M) in buffer in a 15 ml test tube. The test tube was centrifuged at 18,000 × *g* for 10 min and the white material at the interface between the buffer and sucrose was removed, diluted with 10 ml of buffer and then centrifuged at 18,000 × *g* for 20 min. The resulting pellet was resuspended in 1.5 ml of buffer and layered over a discontinuous gradient made up of 2.0 ml of sucrose (0.65 M) and 1.5 ml of sucrose (0.41 M) in buffer. The gradient was centrifuged at 75,000 × *g* for 1 h at 4° C in a Beckman SW 60 Ti rotor. Two lamellar bodies fractions, designated lamellar body fraction I and lamellar body fraction II, were isolated. Lamellar body fraction I banded at the buffer-0.41 M sucrose interface while fraction II banded at the 0.41 M sucrose-0.65 M sucrose interface. The lamellar body fractions were either pelleted by centrifugation for electron microscopy or extracted for phospholipid analysis (Bligh and Dyer 1959).

Morphological analyses

Samples were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M), pH 7.4. Fixation for light and electron microscopy was carried out before and after culture. Subcellular fractions were pelleted at 100,000 × *g* in cellulose nitrate tubes, the supernatant fluid removed and replaced with fixative. After fixation, the tissues were post-fixed in 2% OsO_4 in sodium cacodylate buffer (0.1 M) for 1 h and then dehydrated in ethanol. Subcellular fractions were treated with OsO_4 as described above and then incubated with 1% tannic acid in sodium acetate buffer (0.1 M), pH 7.4 for 1 h before dehydration with an acetone series. Tissues and pellets were embedded in Epon 812. Thick sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate, and viewed with a Phillips 301 electron microscope.

Results

Morphology

The epithelial cells lining the pre-alveolar ducts of the fetal lung tissue before culture were filled with glycogen, contained relatively few cytoplasmic organelles, had a few small microvilli on their luminal surface, and contained no lamellar bodies (Fig. 1A). After 4 days in organ culture the cells contained numerous lamellar bodies, decreased amounts of glycogen, and increased amounts of rough endoplasmic reticulum and Golgi apparatus (Fig. 1B). Many microvilli projected from the apical surface of the cells. The type II cells were joined by tight junctions at their lateral surfaces and rested on a basal lamina. There were no morphological differences between explants cultured in medium with or without FCS. The lamellar bodies observed within the type II cells of the human fetal lung explants frequently contained the unique triangular core region described by Stratton (1978). Lamellar bodies were rarely observed within the lumina of the pre-alveolar ducts, neither was tubular myelin.

Two lamellar body fractions were isolated from explants that had been maintained in control medium for 7 days. The intact lamellar bodies of fraction I had no discernable contamination from other subcellular material. The lamellar bodies were large, with many concentric lamellae having a typical lipid bilayer structure (Fig. 2A). Lamellar body fraction II also contained many membranous profiles, perhaps due to unfolding of the lamellar bodies (Fig. 2B).

Biochemical studies

Explants of human fetal lung incorporated increased amounts of choline into phosphatidylcholine with increasing time in culture (Fig. 3A). In explants cultured in control medium the rate of choline incorporation increased significantly ($p < 0.05$) from 19.8 ± 5.4 nmol choline $\times 24\,h^{-1} \times mg^{-1}$ protein on day 2 to 35.7 ± 6.0 nmol choline $\times 24\,h^{-1} \times mg^{-1}$ protein on day 8, an 80% increase. The addition of 10% FCS to the medium did not influence the rate of choline incorporation by the fetal lung explants (Fig. 3A). The data presented in Fig. 3A are from 7 experiments utilizing lung tissue from 7 different abortuses. The percent of DSPC synthesized by fetal lung explants maintained in control medium (Fig. 3B) increased from 25% of the phosphatidylcholine on day 2 to 31% on day 8 of the culture period. The addition of 10% FCS to the medium did not affect the DSPC content of the phosphatidylcholine synthesized by fetal lung explants. The data presented in Fig. 3B are the mean of 2 experiments with tissue from 2 different abortuses.

The specific activity of PAPase, an important regulatory enzyme in the synthesis of glycerophospholipids by the lung (Bleasdale and Johnston 1981), increased three-fold during the 6-day culture period (Fig. 4). There was no difference in the specific activity of PAPase in explants maintained in either control or FCS-containing medium. The data presented in Fig. 4 are the mean of 2 experiments with tissue from 2 different abortuses.

Lamellar bodies were isolated from explants previously labeled with 1 μCi/ml [^{14}C]glycerol for 24 h. Lamellar body fraction I contained approximately twice the

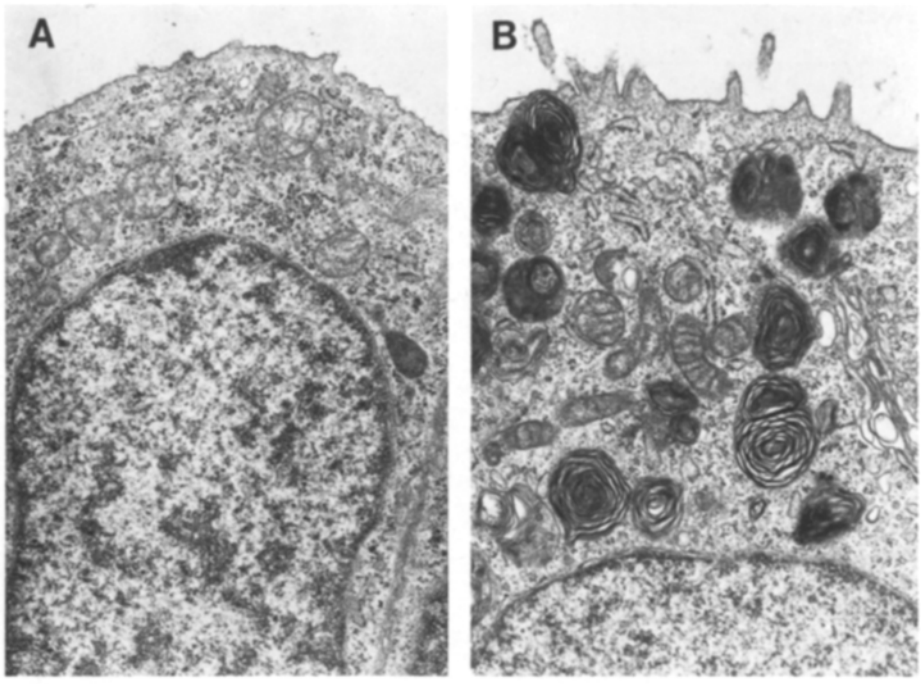

Fig. 1 A, B. Electron micrographs of ductular epithelial cells of human fetal lung in glandular stage (**A**) and after organ culture for 4 days (**B**). × 17,500

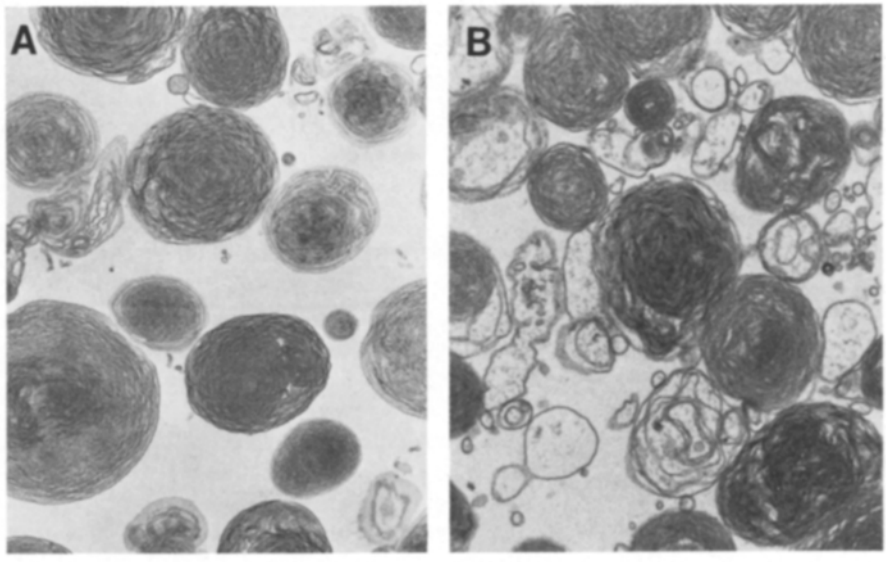

Fig. 2 A, B. Electron micrographs of lamellar bodies isolated from cultured human fetal lung tissue. **A** Lamellar body fraction I, **B** Lamellar body fraction II, containing smaller proportion of intact lamellar bodies. × 30,000

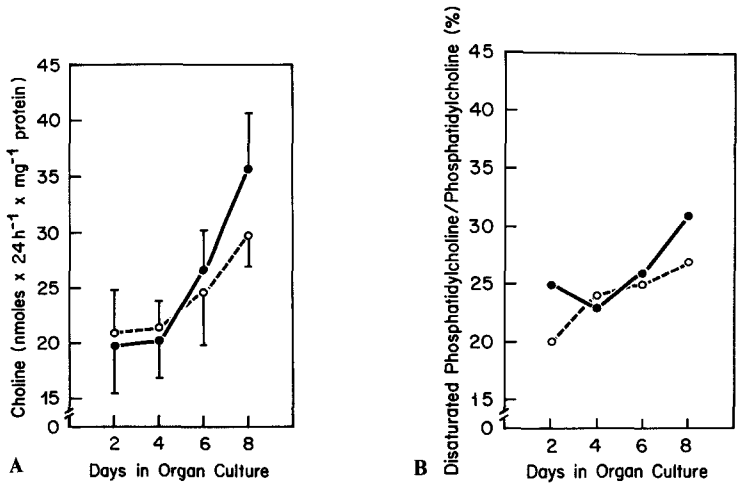

Fig. 3A, B. Choline incorporation into phosphatidylcholine (**A**) and disaturated phosphatidylcholine content (**B**) of lung explants from abortuses of 16- to 22-weeks gestational age as a function of days in culture. Explants incubated for up to 8 days in medium with (●—●) or without serum (o---o). **A** Choline incorporation into phosphatidylcholine. Each data point represents mean of 21 determinations (3 replicate cultures from 7 different experiments) plus or minus standard error of the mean. **B** Disaturated phosphatidylcholine content of human fetal lung explants. Each data point represents mean of 2 determinations. Lipid extracts of 3 replicate cultures pooled for each determination

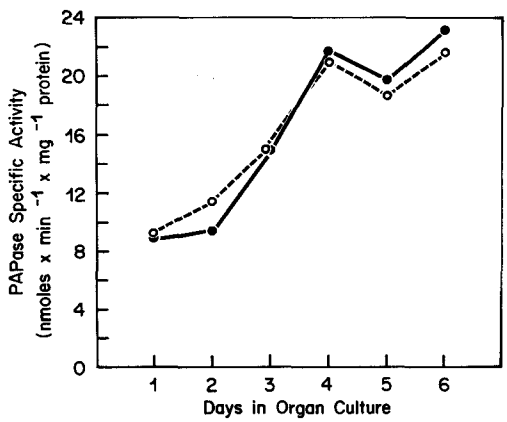

Fig. 4. PAPase specific activity in human fetal lung explants as a function of days in culture. Explants cultured in medium with (●—●) or without (o---o) serum. Each data point represents mean of 2 determinations. Explants from 10 replicate cultures pooled for each determination

amount of lipid phosphorus and [^{14}C]glycerol incorporated into phospholipids as that in lamellar body fraction II (Table 1). The specific activity of [^{14}C]glycerol incorporated into the total phospholipid fraction averaged approximately 150 cpm/nmol lipid phosphorus and was similar in fractions I and II.

The distribution of [^{14}C]glycerol in the different phospholipids of lamellar body fractions I and II did not differ significantly from each other (Table 2). The proportion of label incorporated into neutral lipids was higher in fraction II. Most

Table 1. [^{14}C]glycerol incorporated and lipid phosphorus content of lamellar bodies isolated from human fetal lung explants

	Fraction I	Fraction II
cpm/mg protein[a]	10,080 ± 4,030	5,590 ± 2,940
nmol PO_4/mg protein[a]	92.2 ± 25.3	53.3 ± 9.3

The data presented are the mean plus or minus standard error of the mean of 4 experiments

[a] Based on the protein content of the original homogenate (mean = 8.5 ± 1.2 mg)

Table 2. Incorporation of [^{14}C]glycerol into various lipids of lamellar bodies isolated from human fetal lung explants

	Percent Incorporation[a]	
	Fraction I	Fraction II
Phosphatidylcholine	76.52 ± 2.12	72.51 ± 1.20
Phosphatidylinositol	6.19 ± 1.42	6.76 ± 1.25
Phosphatidylglycerol[b]	5.04 ± 2.27	5.74 ± 3.28
Phosphatidylserine	0.58 ± 0.13	0.95 ± 0.08
Phosphatidylethanolamine	3.15 ± 0.95	3.02 ± 0.69
Neutral lipids	2.36 ± 1.02	6.04 ± 0.14
Phosphatidic acid	0.09 ± 0.03	0.04 ± 0.04
Lysophosphatidylethanolamine	1.81 ± 0.24	1.89 ± 0.26
Lysophosphatidylcholine	2.54 ± 0.72	2.56 ± 0.26
Lysobisphosphatidic acid	0.48 ± 0.17	0.42 ± 0.23

[a] The data are the percent of the total cpm recovered from the thin-layer chromatograph which were found in each lipid class. Data are from 3 independent experiments and are expressed as the mean ± SEM

[b] Since each molecule of phosphatidylglycerol contains 2 molecules of glycerol the actual relative synthesis of phosphatidylglycerol is approximately one half the percent incorporation, i.e. 2.52% for fraction I and 2.87% for fraction II

of the [^{14}C]glycerol was incorporated into phosphatidylcholine (74%). Approximately 5% of the label was incorporated into phosphatidylglycerol and 6% into phosphatidylinositol. Because each phosphatidylglycerol molecule contains 2 molecules of glycerol, the relative abundance of phosphatidylglycerol is approximately half of the percent [^{14}C]glycerol incorporated, i.e. 2.5%. Smaller amounts of [^{14}C]glycerol were incorporated into the other phospholipids detected in the lamellar body phospholipid extract.

The percent of lamellar body phosphatidylcholine that was disaturated was determined in lung explants incubated with [^{3}H]choline. The DSPC content of lamellar bodies was 44 percent of the total phosphatidylcholine fraction of lamellar bodies. There was no difference in the percent of disaturated phophatidylcholine in lamellar body fractions I and II.

Discussion

Type II cells normally appear in fetal lung only after the 24th week of gestation (Stahlman and Gray 1978). We found that after 4 to 6 days of culture, the epithelial cells lining the pre-alveolar ducts of explants of human fetal lung of 16 to 22 weeks gestational age had the typical ultrastructural morphology of lung type II cells. Biochemical studies confirmed that the cells in the explants synthesized surfactant rich in phosphatidylcholine. PAPase specific activity in the human fetal lung explants increased 3-fold during the culture period. It should be noted that the accelerated differentiation of human fetal lung tissue explants in organ culture occurred even in the absence of serum in the culture medium. Thus, the effect of individual hormones or combinations of hormones on fetal lung development can be directly assessed in this organ culture system.

Several possible explanations for the accelerated differentiation of human fetal lung in vitro can be offered. One explanation is that the change from an in vivo to an in vitro environment initiates the differentiation phenomenon. Adamson and Bowden (1974) reported that adult lung epithelium responds to injury with a hypertrophy of lung type II cells, resulting in an epithelium composed primarily of type II cells. Another possible explanation is that the lung of the human fetus is normally under an inhibitory influence during mid-gestation and that its removal from this postulated inhibitory influence results in accelerated differentiation. Differentiation of fetal lung tissue of other species in vitro has been reported previously. When undifferentiated fetal lung tissue from rats (Funkhouser et al. 1976) or rabbits (Snyder et al. 1981) was maintained in organ culture, the cells lining the pre-alveolar ducts differentiated into an epithelium composed primarily of type II cells.

Lamellar bodies, the organelles containing surfactant, have been isolated from adult and fetal lung tissues of a number of species (Gil and Reiss 1973; Hallman and Gluck 1980). The phospholipid composition of lamellar bodies is similar to that of isolated surfactant, i.e., high in phosphatidylcholine content, with the anionic phospholipids, phosphatidylinositol or phosphatidylglycerol, being the next most abundant phospholipid class. The lamellar body lipids of adult rabbit lung are enriched in phosphatidylglycerol while lamellar bodies isolated from fetal rabbit lung are enriched in phosphatidylinositol (Hallman and Gluck 1980). Structures resembling lamellar bodies have been isolated from human amniotic fluid (Hook et al. 1978). The major source of the phospholipids of amniotic fluid is the surfactant secreted by the fetal lung. The phospholipid composition of amniotic fluid phospholipids is similar to that of fetal lung surfactant and its content of disaturated phosphatidylcholine is high (Oulton et al. 1980). Hallman et al. (1976) and Oulton et al. (1980) have analyzed human amniotic-fluid phospholipids throughout gestation and found that, as gestation proceeds, the content of phosphatidylinositol decreases and the content of phosphatidylglycerol increases. The phospholipid composition of lamellar bodies isolated from human fetal lung maintained in organ culture in our studies most closely resembles that of surfactant produced late in gestation, i.e., from weeks 36 to 38 (Hallman et al. 1976; Oulton et al. 1980). Since we can isolate large quantities of lamellar bodies from these explants we can address questions concerning the specific effects of hormones on the synthesis of individual surfactant phospholipids.

References

Adamson IYR, Bowden DH (1974) the type 2 cell as progenitor of alveolar epithelial regeneration: A cytodynamic study in mice after exposure to oxygen. Lab Invest 30:35–42

Bleasdale JE, Johnston JM (1981) Phosphatidic acid production and utilization. In: Farrell P (ed) The Developmental Biology of the Lung and the Pathobiology of Hyaline Membrane Disease. Academic Press, New York (In press)

Bligh EC, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Ekelund L, Arvidson G, Emanuelsson H, Myhrberg H, Astedt B (1975) Effect of cortisol on human fetal lung in organ culture. A biochemical, electron microscopic, and autoradiographic study. Cell Tissue Res 163:262–272

Farrell PM, Avery ME (1975) Hyaline membrane disease. Am Rev Resp Dis 111:657–688

Farrell PM, Hamosh M (1978) The biochemistry of fetal lung development. Clin. Perinatol. 5:197–229

Funkhouser JD, Hughes ER, Peterson RDA (1976) An organ culture system for study of fetal lung development. Biochem Biophys Res Commun 70:630–637

Gil J, Reiss OK (1973) Isolation and characterization of lamellar bodies and tubular myelin from rat lung homogenates. J Cell Biol 58:152–171

Hallman M, Kulovich M, Kirkpatrick E, Sugarman RG, Gluck L (1976) Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: Indices of lung maturity. Am J Obstet Gynecol 125:613–617

Hallman M, Feldman BH, Kirkpatrick E, Gluck L (1977) Absence of phosphatidylglycerol (PG) in respiratory distress syndrome in the newborn. Study of the minor surfactant phospholipids in newborns. Pediat Res 11:714–720

Hallman M, Gluck L (1980) Formation of acidic phospholipids in rabbit lung during perinatal development. Pediatr Res 14:1250–1259

Hook GER, Gilmore LB, Tombropoulos EG, Fabro SE (1978) Fetal lung lamellar bodies in human amniotic fluid. Am Rev Respir Dis 117:541–550

Ikegami M, Silverman J, Adams FH (1979) Restoration of lung pressure-volume characteristics with various phospholipids. Pediatr Res 13:777–780

Johnston JM, Reynolds G, Wylie MB, MacDonald PC (1978) The phosphohydrolase activity in lamellar bodies and its relationship to phosphatidylglycerol and lung surfactant formation. Biochim Biophys Acta 531:65–71

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Mason RJ, Nellenbogen J, Clements JA (1976) Isolation of disaturated phosphatidylcholine with osmium tetroxide. J Lipid Res 17:281–284

Oulton M, Martin TR, Faulkner GT, Stinson D, Johnson JP (1980) Developmental study of a lamellar body fraction isolated from human amniotic fluid. Pediatr Res 14:722–728

Rousseau-Merck MF, Wyllie L, Basset F, Nezelof C (1976) In vitro behavior of human fetal lung maintained in organ culture. Virchows Arch (Pathol Anat) 371:305–321

Skipski VP, Barclay M (1969) Thin layer chromatography of lipids. In: Lowenstein JM (ed) Methods in Enzymology. Academic Press, N.Y., Vol. 14, pp. 530–598

Snyder JM, Mendelson CR, Johnston JM (1981) The effect of cortisol on rabbit fetal lung maturation in vitro. Devel Biol 85:129–140

Spitzer HL, Rice JM, MacDonald PC, Johnston JM (1975) Phospholipid biosynthesis in lung lamellar bodies. Biochem Biophys Res Comm 66:17–23

Stahlman MT, Gray ME (1978) Anatomical development and maturation of the lungs. Clin Perinatol 5:181–196

Stratton CJ (1978) The ultrastructure of multilamellar bodies and surfactant in the human lung. Cell Tissue Res 193:219–229

Yavin E, Zutra A (1977) Separation and analysis of ^{32}P-labeled phospholipids by a simple and rapid thin-layer chromatographic procedure and its application to cultured neuroblastoma cells. Anal Biochem 80:430–437

Accepted May 6, 1981