A comparison of the specificity of phosphatidylcholine synthesis by human fetal lung maintained in either organ or organotypic culture

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Human fetal lung (14–18 weeks gestation) was maintained in either organ or organotypic culture. By 4 days in organ culture or 14 days in organotypic culture, epithelial cells within both culture systems exhibited well-developed apical microvilli and possessed numerous intracellular lamellar bodies characteristic of surfactant phospholipid stores. However, analysis of the pattern of synthesis of individual molecular species of phosphatidylcholine by [¹⁴C]choline incorporation and reversed-phase h.p.l.c. showed that this apparent maturation was not paralleled by an increased synthesis of the dipalmitoyl species in either culture system. By contrast, the fractional synthesis of dipalmitoyl phosphatidylcholine, expressed as a percentage of total [¹⁴C]choline incorporation, decreased with time in both organ and organotypic culture. Moreover, these fractions were not significantly different from those measured in parallel monolayer cultures of mixed human fetal lung cells that displayed mainly fibroblast morphology. These results suggest that the synthesis pattern of phosphatidylcholine species by lung cells in culture is determined principally by their incubation conditions and not by their state of apparent maturation.

INTRODUCTION

Fetal lung maturation and development are characterized by a number of well-defined morphological and biochemical changes. These include both the architectural development of the lung and the maturation of the pulmonary surfactant system. The accumulation of cytoplasmic lamellar bodies within type II alveolar epithelial cells [1] and the increased fractional contribution of the dipalmitoyl species to lung phosphatidylcholine [2] are both closely related *in vivo*. As lamellar bodies are the sites of surfactant assembly and storage before secretion [3,4], and PC16:0/16:0 is responsible for the surface-tension-lowering properties of surfactant [5], both these parameters have been extensively used for the assessment of tissue culture models of fetal lung development. Two such models are organ [6–8] and organotypic [9–11] cultures of immature fetal lung, for both of which changes have been described that resemble apparent developmental maturations *in vitro*. Results in the present paper compare for the first time analyses of morphology and phosphatidylcholine metabolism in parallel organ and organotypic cultures derived from human fetal lung.

A variety of techniques have been described for the organ culture of lung derived from animal [7,12] and human [6,8,13,14] fetuses. The cells within such cultures in general remain viable for about 1 week, over which time they display a spontaneous apparent maturation. The epithelial cells within the pseudo-glandular structures of the immature lung acquire in culture many of the characteristics of differentiated type II alveolar epithelial

cells. They form enlarged structures surrounding a central lumen, assume a more cuboidal shape, develop extensive apical microvilli and accumulate numerous intracellular lamellar bodies. Also, a number of studies have described a stimulation of phosphatidylcholine synthesis in organ cultures of human fetal lung by hormones such as glucocorticoids [6,13,14], tri-iodothyronine [14], prolactin [13,14] and insulin [13] that are thought to be involved in surfactant maturation *in utero*. Further evidence for the physiological relevance of this apparent maturation was provided by the demonstration of the expression of type II epithelial cell-specific surface lectins and surfactant apoproteins in cells derived from such organ cultures [15].

Comparable morphological changes have been described for rat [9,10] and human [11] lung maintained in organotypic culture. This technique exploits the tendency of isolated cells from proteolytically dissociated fetal tissues to re-aggregate with other similar cell types *in vitro* [16,17]. When dissociated fetal lung cells are allowed to re-aggregate in culture within a collagen-sponge matrix, epithelial cells form structures, surrounding a central lumen, that have been compared with the alveolus *in vivo*. The cells within these alveolar-like structures display changes that are essentially identical with those described above for the equivalent cells in organ culture, including the accumulation of intracellular lamellar bodies. One potential advantage of the organotypic cultures is the greater duration of cell viability in an apparently differentiated form [18]. Metabolic integrity and morphological structure were maintained for up to 4 weeks for human fetal lung in organotypic culture [11].

Abbreviations used: the molecular species of phosphatidylcholine are designated by PC sn -1-acyl/ sn -2-acyl: PC14:0/16:0, PC16:0/18:3, PC16:0/16:1, PC16:0/18:2, PC16:0/16:0, PC16:0/18:1, PC18:1/18:1 are respectively myristoyl/palmitoyl, palmitoyl/linolenoyl, palmitoyl/palmitoleoyl, palmitoyl/linoleoyl, dipalmitoyl, palmitoyl/oleoyl and dioleoyl molecular species.

In contrast with the apparent morphological differentiation of fetal lung in culture, results from biochemical measurements have been more equivocal. Phosphatidylcholine synthesis and concentration in organotypic cultures of rat fetal lung were both shown to increase over 10 days in culture, but the proportion present as the disaturated species remained constant [10,19]. Moreover, the observation that the activity of CTP:choline-phosphate cytidyltransferase increased as a function of time in culture and was not related to the original gestational age of the fetus questioned the suitability of organotypic culture as a model for biochemical studies concerning development. There have been no reports about the detailed analysis of phosphatidylcholine metabolism in organotypic cultures of human fetal lung. Organ culture of human fetal lung has been more extensively used to study the developmental regulation of phosphatidylcholine metabolism. There have been consistent reports of a spontaneous increase in phosphatidylcholine synthesis after 4–8 days in culture, which was stimulated some 2–4-fold by glucocorticoid and thyroid hormones [6,13,14]. However, reports of the proportion of phosphatidylcholine synthesized as disaturated species in these cultures range from 25 to 51 %, and these proportions showed only slight variations at times when total phosphatidylcholine synthesis was increasing.

One limitation placed on many of these studies has been the availability of suitably detailed analytical methods. Studies of surfactant metabolism have almost universally relied on the measurement of total disaturated phosphatidylcholine after oxidation of unsaturated species with OsO_4 [20]. Such analyses have proved invaluable, but provide no information about either unsaturated species or specific molecular species such as PC16:0/16:0. Such information is essential to test mechanisms purported to regulate the selective enrichment of PC16:0/16:0 in differentiated type II epithelial cells. Techniques for the analysis of individual molecular species of phosphatidylcholine by h.p.l.c. have recently been devised [21,22] to replace the more laborious argentation t.l.c. methods [23]. We have analysed by h.p.l.c. the patterns of synthesis of molecular species of phosphatidylcholine by human fetal lung maintained in both organ and organotypic culture. The results obtained provide good evidence for the respective roles of phenotypic expression and culture conditions in phosphatidylcholine metabolism by these cells.

MATERIALS AND METHODS

Human fetal lung cell culture

Human fetal material was obtained from therapeutic abortion after prostaglandin induction at 12–20 weeks of gestation. Lungs were removed and the lung parenchyma was aseptically dissected free from bronchiolar material. This tissue was diced into 1 mm³ pieces and washed free from adherent blood cells with Hanks buffered saline. Organ cultures were established by placing about 20 of these lung pieces on a collagen sponge (1 cm × 1 cm × 0.7 cm) in a 60 mm-diam. tissue-culture dish [6]. These cultures were maintained at 37 °C under 5 % CO_2 in air in RPMI 1640 medium without serum but containing antibiotics (100 µg of kanamycin/ml, 100 µg of fungizone/ml, 50 units of penicillin/ml, 50 ng of streptomycin/ml, 5 units of nystatin/ml and 8 µg of

gentamycin/ml). Cultures were placed on a rocking platform at 6 oscillations/min, and medium was replaced every 2 days.

For the establishment of organotypic and monolayer cultures, lung pieces were proteolytically digested by three consecutive incubations at 37 °C for 15 min with 0.125 % (w/v) trypsin and 0.1 % (w/v) collagenase in Hanks buffered saline [11]. The combined digests were filtered through 0.45 µm nylon mesh into 20 ml of RPMI 1640 medium at 4 °C containing 10 % (v/v) fetal-calf serum. The cell suspension was centrifuged at 200 *g* for 10 min and washed three times with RPMI medium containing 10 % fetal-calf serum. For monolayer cultures, 1×10^6 of these cells were plated directly on to 60 mm-diam. tissue-culture dishes and incubated in RPMI 1640 medium containing 10 % fetal-calf serum and antibiotics. For the establishment of organotypic cultures, the washed cell pellet was incubated at 37 °C for 1 h, and then 50 µl portions, containing a minimum of 1×10^6 cells (mean 4.8×10^6 ; range 1.2×10^6 – 1.3×10^7 cells, $n = 11$) were inoculated on to sterile collagen sponges (2 cm × 1 cm × 0.7 cm) in 60 mm-diam. tissue-culture dishes. After aggregation at 37 °C for 1 h, culture medium (5 ml) was added, comprising RPMI 1640 medium, 10 % fetal-calf serum and antibiotics. Cultures were incubated at 37 °C in a 5 % CO_2 atmosphere, initially statically but after 2 days on a rocking platform at 6 oscillations/min. Medium was replaced every 2 days.

Microscopy

Cultures were fixed in 5 % (w/v) glutaraldehyde in cacodylate/sucrose buffer, pH 7.4, for 8 h, then treated with 2 % (w/v) OsO_4 for 2 h and with 1.5 % uranyl acetate for 30 min. After dehydration in a graded series of ethanol, they were cleared in HistoSol Clearing Agent (Shandon Southern Products, Runcorn, Cheshire, U.K.) and embedded in Spurr resin. Sections (90 nm thick) for transmission electron microscopy were stained with lead citrate and examined in an Hitachi H7000 microscope. Relative numbers of epithelial cells in these sections displaying morphological characteristics of type II cells, expressed as a percentage of total cell nuclei, were estimated at a magnification of 1000×. Cells were quantified in five randomly chosen grid squares (150 nm × 150 nm) for each section, and in duplicate for each series of cultures.

Incorporation of [¹⁴C]choline

Phosphatidylcholine synthesis was monitored by measurement of the rate of [¹⁴C]choline chloride incorporation. Fresh medium (5 ml) was added to the cultures, followed by 100 µl of [¹⁴C]choline chloride (58 µCi/µmol) to give final concentrations and specific radioactivities in the medium of respectively 400 nCi/ml and 16.3 µCi/µmol. Cells were incubated with label at 37 °C in 5 % CO_2 for 18 h on a rocking platform. The cultures were then washed extensively and suspended in Hanks saline. [³H]PC16:0/16:0 (77 mCi/µmol; 50 nCi) was added as internal standard, and the cultures were disrupted by sonication with a MSE Soniprep 150 instrument. Total cellular lipids were extracted with chloroform/methanol, dried under N_2 and stored in 100 µl of chloroform at –20 °C until analysis [24].

Fig. 1. Phosphatidylcholine synthesis by human fetal lung after 4 days in organ culture

After incubation with 2 μ Ci of [14 C]choline for 18 h, the isolated phosphatidylcholine was analysed by reversed-phase h.p.l.c. and radiochemical detection. The upper trace represents incorporated [14 C]choline, and the lower trace the internal standard [3 H]PC16:0/16:0. The identity of the peaks was determined by g.l.c. analysis of the fatty acid composition of eluted peaks.

Analysis of phosphatidylcholine molecular species

Phosphatidylcholine was purified from the lipid extract by using disposable aminopropyl-Bondelut columns (Jones Chromatography, Hengoed, Mid Glamorgan, Wales, U.K.). The chloroform extract (1 ml) was applied to a 100 mg column, which was then washed with 2 ml of chloroform. Bound phosphatidylcholine was selectively eluted by 1 ml of chloroform/methanol (3:2, v/v), dried under N_2 and dissolved in trifluoroethanol. Recovery of internal standard was $81.7 \pm 3.0\%$ ($n = 17$). The rate of total phosphatidylcholine synthesis was calculated from the measurement of radioactivity in a portion of this sample, and was found in preliminary experiments to be linear between 3 and 18 h in culture. Individual molecular species of phosphatidylcholine were separated by reversed-phase h.p.l.c. on a 25 cm $\times$ 4.6 mm Apex ODS 2 column (Jones Chromatography) thermostatically maintained at 50 $^{\circ}$ C [22]. The mobile phase contained 40 mM-choline chloride in methanol/water (37:3, v/v) delivered at 1 ml/min by a Waters 6000A pump. Eluted peaks were detected by one of two alternative techniques, either a flow-through radioactivity detector or a post-column fluorescent-derivative-formation procedure. Peaks were identified by g.l.c. analysis of their methyl fatty acid derivatives.

The h.p.l.c. radioactivity detector (Berthold LB505;

Berthold Instruments U.K., Hemel Hempstead, Herts., U.K.) consisted of a post-column pump followed by a flow-through counter fitted with a 2 ml homogeneous flow cell. The column eluate was mixed with scintillation fluid (Scintran; BDH) in the ratio 1:2. The detector was calibrated for dual-labelled counting of 3 H/ 14 C, and the results were analysed by a Berthold LB510 data station. A representative display of one such analysis is shown in Fig. 1, the upper trace being incorporated [14 C]choline and the lower trace being the [3 H]PC16:0/16:0 internal standard. Internal-standard recovery facilitated both the calculation of absolute rates of radioactivity incorporation and the relative calibration of h.p.l.c. retention times to confirm the identities of eluted peaks.

Phosphatidylcholine concentrations in the h.p.l.c. eluate were measured by using 1,6-diphenylhexa-1,3,5-triene as a post-column fluorescent-derivative-formation agent [22]. The methanol-based h.p.l.c. eluate at 1 ml/min was admixed with an aqueous stream at 3 ml/min containing 150 μ l of 3 mM-diphenylhexatriene solution in tetrahydrofuran/l. The post-column stream was passed through a Teflon string mixer (3 m $\times$ 0.5 mm internal diam.) at 50 $^{\circ}$ C and then through a Perkin-Elmer LS1 fluorescence spectrophotometer. The excitation and emission wavelengths were 340 nm and 460 nm respectively. Areas under eluted peaks were calculated with an Hitachi 2000 integrator (Baird and Tatlock, Chadwell

Heath, Essex, U.K.) and were proportional to phosphatidylcholine concentration over the range 0.5–100 nmol, irrespective of the degree of acyl unsaturation.

DNA concentration

The DNA concentration of the homogenized cells were measured by an automated fluorescence-binding method using the fluorochrome Hoechst 33258 [25].

Chemicals

Tissue-culture materials were generally supplied by Flow Laboratories, Rickmansworth, Herts., U.K., except for fetal-calf serum (Sera Lab, Crawley Down, Sussex, U.K.), nystatin (Gibco, Uxbridge, Middx., U.K.) and gentamycin (David Bull Laboratories, Warwick, U.K.). General chemicals were supplied by Sigma

Chemical Co., Poole, Dorset, U.K., and h.p.l.c. solvents by Rathburn Chemicals, Walkerburn, Scotland, U.K. Radiochemicals were obtained from Amersham International, Amersham, Bucks., U.K. Collagen sponge was supplied by Allan and Hanburys, Greenford, Middx., U.K.

RESULTS

Establishment of human fetal lung in tissue culture

Variables inherent in the culture of human fetal tissue include maternal clinical history and the time of fetal death in relation to the duration of the induction of labour. Such variables can potentially exert a considerable influence on the viability and characteristics of subsequent cell cultures. Consequently, the success of

Fig. 2. Transmission-electron-microscopic appearance of human fetal lung and lung cultures

(a) Fetal lung at 15 weeks gestation (magnification $\times 3000$); (b) organ culture after 4 days (magnification $\times 1500$); (c) organotypic culture after 14 days (magnification $\times 1500$); (d) lamellar bodies in an epithelial cell after 14 days in organotypic culture (magnification $\times 1500$). Key: LB, lamellar bodies; MV, microvilli; CL, central lumen; V, lipid vesicles; C, collagen; GA, Golgi apparatus.

establishing organ and organotypic cell cultures from the lungs of 83 consecutive therapeutically aborted fetuses was assessed. The range of gestational age was estimated by crown-rump length measurements to be 12–20 weeks, with most (87%) being between 14 and 18 weeks. For 27 fetal lungs (32.5%), cell viability after proteolytic digestion, estimated by Trypan Blue exclusion, was less than 25%, and no cultures were initiated. A combination of organ, organotypic and monolayer cultures were established from the remaining 56 lungs. Criteria for the assessment of culture viability included measurement of DNA concentration, Trypan Blue exclusion of cells liberated by collagenase digestion of the cultures and phase-contrast microscopy of the monolayer cultures. There was little evidence for cell attachment or growth in 18 cultures (22%), whereas 38 cultures (46%) were regarded as acceptable for further analysis.

Morphology of cultured human fetal lung

A typical section through the parenchyma of human fetal lung at 15 weeks of gestation (Fig. 2a) shows epithelial cells surrounding a central lumen. These cells are characteristically immature and contain no intracellular lamellar bodies. The changes in the morphology of these cells with time in organ and organotypic culture were comparable with previous reports and closely resembled parameters of developmental maturation observed *in vivo* [1–3]. After 4 days in organ culture (Fig. 2b) and 14 days in organotypic culture (Fig. 2c) these epithelial cells became more cuboidal, possessed numerous apical microvilli and consistently contained intracellular cytoplasmic lamellar bodies. Such lamellar bodies were absent from non-epithelial cells of mesenchymal origin, which, by contrast, contained numerous amorphous lipid inclusions. The variety in appearance of epithelial lamellar bodies is clearly shown in Fig. 2(d). At these times in culture, morphometric analysis provided estimates (mean $\pm$ S.E.M.) of $40.3 \pm 1.9\%$ ($n = 5$) and $31.7 \pm 3.8\%$ ($n = 6$) for percentages of type II cells in organ and organotypic cultures respectively. Inspection of monolayer cultures of mixed human fetal lung cells by phase-contrast microscopy displayed principally cells of fibroblast morphology.

Phosphatidylcholine composition of human fetal lung

The molecular species composition of human fetal lung phosphatidylcholine at 15 weeks of gestation is shown in Table 1. The predominant species was PC16:0/18:1, although the PC16:0/16:0 content at 32% was already significant. Instead of increasing with time in organ culture, the content of PC16:0/16:0 decreased to 26.7% after 4 days. Therefore there was no correlation between the accumulation of numerous lamellar bodies (Fig. 2a) and any increased content of PC16:0/16:0.

Phosphatidylcholine synthesis by organ cultures of human fetal lung

The rate of [14 C]choline incorporation into phosphatidylcholine increased significantly with time in organ culture, from 490 ± 47 d.p.m./18 h per μ g of DNA on day 1 to 996 ± 105 d.p.m./18 h per μ g of DNA on day 4 ($n = 7$, $P < 0.01$). However, the pattern of [14 C]choline incorporation into phosphatidylcholine molecular species (Table 2) showed no increased fractional synthesis of PC16:0/16:0, and supported the concentration measure-

Table 1. Phosphatidylcholine molecular-species distribution of human fetal lung at 15 weeks of gestation and after 4 days in organ culture

The results reported comprise > 90% of detected species; unidentified h.p.l.c. peaks each contributed < 2% to the total phosphatidylcholine. Values are means $\pm$ S.E.M. ($n = 4$).

Molecular species	Relative retention time	Phosphatidylcholine concn. (% of total)	
		Fetal lung, 15 weeks	Fetal lung in organ culture for 4 days
PC14:0/16:0 } PC16:0/18:3 }	0.69	7.9 ± 0.4	9.9 ± 1.7
PC16:0/16:1	0.74	7.4 ± 3.3	14.2 ± 3.4
PC16:0/16:0	1.00	32.0 ± 1.2	26.7 ± 2.8
PC16:0/18:1	1.08	43.2 ± 2.3	42.9 ± 2.4
PC18:1/18:1	1.18	9.0 ± 2.2	8.2 ± 1.9

Table 2. Phosphatidylcholine synthesis by organ cultures of human fetal lung

The pattern of [14 C]choline incorporation into phosphatidylcholine molecular species over 18 h was analysed by h.p.l.c. Results are means $\pm$ S.E.M.

Molecular species	Relative retention time	Time in culture...	[14 C]Choline incorporation (% of total)	
			1 day ($n = 4$)	4 days ($n = 5$)
PC14:0/16:0 } PC16:0/18:3 }	0.69		2.4 ± 0.2	6.5 ± 0.3
PC16:0/16:1	0.74		3.8 ± 0.8	11.6 ± 1.1
PC16:0/18:2	0.84		23.0 ± 0.8	14.5 ± 1.2
PC16:0/16:0	1.00		17.0 ± 0.9	14.2 ± 0.8
PC16:0/18:1	1.08		38.4 ± 1.1	40.7 ± 1.6
PC18:1/18:1	1.18		15.5 ± 2.0	12.1 ± 0.8

ments in Table 1. At both 1 and 4 days in culture, PC16:0/18:1 was the major species synthesized, whereas the synthesis of PC16:0/16:0 decreased with time in culture. The lower percentage [14 C]choline incorporation compared with the concentration distribution at 4 days in culture demonstrated that newly synthesized phosphatidylcholine was progressively less disaturated.

Phosphatidylcholine synthesis by organotypic and monolayer cultures of human fetal lung

Measurements of total phosphatidylcholine synthesis by organotypic cultures showed no significant changes with time in culture. The rate at 4 days (637 ± 41 d.p.m./18 h per μ g of DNA) was unchanged at 14 days (589 ± 59 d.p.m./18 h per μ g of DNA) and was still maintained at 28 days in culture (532 ± 68 d.p.m./18 h per μ g of DNA). The patterns of [14 C]choline incorporation into phosphatidylcholine molecular species by

Table 3. Phosphatidylcholine synthesis by organotypic cultures of human fetal lung

Conditions for [14 C]choline incorporation were the same as described in Table 2. Results are means $\pm$ S.E.M.

Molecular species	Relative retention time	Time in culture...	[14 C]Choline incorporation (% of total)	
			4-6 days (n = 11)	14-28 days (n = 10)
PC14:0/16:0}	0.69		6.1 $\pm$ 0.4	8.7 $\pm$ 0.6
PC16:0/18:3}				
PC16:0/16:1	0.74		9.9 $\pm$ 0.7	11.9 $\pm$ 1.0
PC16:0/18:2	0.84		12.3 $\pm$ 0.7	11.5 $\pm$ 0.5
PC16:0/16:0	1.00		17.6 $\pm$ 0.9	11.7 $\pm$ 0.8
PC16:0/18:1	1.08		40.4 $\pm$ 0.8	37.9 $\pm$ 1.4
PC18:1/18:1	1.18		13.4 $\pm$ 1.1	18.3 $\pm$ 1.0

Table 4. Phosphatidylcholine synthesis by monolayer cultures of human fetal lung

Conditions for [14 C]choline incorporation were the same as described in Table 2. Results are means $\pm$ S.E.M.

Molecular species	Relative retention time	Time in culture...	[14 C]Choline incorporation (% of total)	
			4-6 days (n = 11)	14-28 days (n = 10)
PC14:0/16:0}	0.69		9.1 $\pm$ 0.4	11.9 $\pm$ 0.5
PC16:0/18:3}				
PC16:0/16:1	0.74		11.1 $\pm$ 1.0	12.2 $\pm$ 0.3
PC16:0/18:2	0.84		8.2 $\pm$ 1.0	7.6 $\pm$ 2.7
PC16:0/16:0	1.00		13.7 $\pm$ 2.5	9.9 $\pm$ 2.0
PC16:0/18:1	1.08		44.7 $\pm$ 2.5	40.0 $\pm$ 2.7
PC18:1/18:1	1.18		13.0 $\pm$ 0.8	18.3 $\pm$ 1.0

organotypic (Table 3) and monolayer (Table 4) cultures followed trends with time in culture that were similar to those in organ cultures. In both culture systems there was a progressive decrease in the incorporation into PC16:0/16:0 between 4-6 days and 14 days in culture, with no further change up to 28 days. Again, at all times PC16:0/18:1 was the major species synthesized, and there were no significant differences in the incorporation of [14 C]choline into any individual molecular species between 14 and 28 days in culture. The observation that the synthesis patterns of molecular species of phosphatidylcholine by organotypic and parallel monolayer cultures were identical clearly showed that the state of the apparent differentiation of type II cells within these culture systems had little effect on their patterns of synthesis of phosphatidylcholine.

DISCUSSION

The electron-microscope analyses (Fig. 2) clearly show the apparent morphological maturations *in vitro* previously described for human fetal lung maintained in organ [6,8,13] and organotypic [11] cell-culture systems.

The physiological relevance of these changes as a model of lung differentiation is supported by the reported demonstration of surface lectin-binding proteins and surfactant apoproteins characteristic of differentiated type II epithelial cells in cells derived from organ cultures of human fetal lung [15]. This conclusion was supported by our observations that the accumulation of lamellar bodies in both organ (Fig. 2a) and organotypic (Fig. 2b) cultures was restricted to epithelial cell types.

In contrast with the morphological changes, the analysis of phosphatidylcholine metabolism by these cultures provided no evidence for a comparable biochemical maturation. At 4 days in organ culture (Table 2) and 14 days in organotypic culture (Table 3), periods of significant accumulation of lamellar bodies in epithelial cells, the fractional incorporations of [14 C]choline into PC16:0/16:0 were decreasing rather than increasing. These results question the use of either absolute or fractional measurements of disaturated phosphatidylcholine as markers of biochemical differentiation in cultured lung cells [26-28]. The identical patterns of molecular species of phosphatidylcholine synthesized by cells in organotypic culture (Table 3) and monolayer culture (Table 4), despite their different morphologies, suggest that the culture conditions and not developmental determinants were the primary factors regulating phosphatidylcholine synthesis in these cultures. Comparison with previous results from organ cultures of human fetal lung support this concept. For instance, Mendelson & Snyder [13] described an accumulation of lamellar bodies and increased total phosphatidylcholine synthesis with time in culture comparable with our results, whereas Gonzales *et al.* [14] only demonstrated changes in either parameter under the influence of hormone exposure. As the gestational ages of the fetuses from which cultures were established were identical, such discrepancies probably reflect differences in the culture techniques or conditions, for instance the use of either collagen or plastic substrates for the cultures.

These results are consistent with the regulation of phosphatidylcholine synthesis in a wide range of other types of cultured cells [29]. Tissues other than lung also maintain specific profiles of molecular phospholipid species *in vivo*, but the phospholipid fatty acid compositions of cells derived from those tissues can readily be modified in culture [29,30]. For instance, human endothelial cells cultured in serum-containing medium had PC16:0/18:1 as 20.8% and PC16:0/16:0 as 11.7% of their total phosphatidylcholine [31], values directly comparable with the results in Tables 3 and 4. Incubation of many cultured cell types with low concentrations of exogenous fatty acids can radically alter their membrane phospholipid fatty acid compositions [30,32]. Similar results have been presented detailing the changes in disaturated phosphatidylcholine that can be achieved by incubation of fetal rabbit [26] and adult rat lung type II cells [33] with either palmitate or oleate.

Our results do not discriminate between phosphatidylcholine synthesis *de novo* by the CDP-choline pathway or by acyl re-modelling [34]. However, the acyl groups incorporated into phosphatidylcholine by all three culture systems were primarily saturated and monoenoic, characteristic of fatty acid synthesis and desaturation *de novo* [27]. Moreover, although acyl-CoA compositions are unknown, considerable evidence suggests that the pattern of synthesis *de novo* of lung phosphatidylcholine

species is not acyl-specific. Microsomal glycerol 3-phosphate acylation in isolated type II alveolar epithelial cells showed no preferential incorporation into phosphatide of palmitoyl-CoA over oleoyl-CoA [35]. Similarly, the synthesis of phosphatidylcholine by lung microsomes prelabelled with [^{14}C]glycerol 3-phosphate showed no selectivity in terms of molecular species over that of the endogenous diacylglycerol substrate [34]. These results support the concept that the pattern of phosphatidylcholine species synthesized by lung cells in culture is determined largely by their acyl-CoA composition. Consideration of the distribution of PC16:0/16:0 within the lung reaches similar conclusions. It has long been recognized that only some 30–50% of lung disaturated phosphatidylcholine is directly related to the surfactant pool [36,37], whereas the results presented in Table 1 and previous reports [38] demonstrate that fetal lung already has a significant PC16:0/16:0 content considerably in advance of initiation of surfactant biosynthesis. Evidently, the factors regulating the enhanced synthesis of PC16:0/16:0 are common to lung cells other than type II epithelial cells. The study of the mechanisms regulating the acyl-CoA compositions both of whole lung and of type II cells should be important in the investigation of surfactant PC16:0/16:0 metabolism. The combination of cell culture and chromatography techniques described in the present paper will prove useful in studying the responses of both 'de novo' and re-modelling pathways of phosphatidylcholine synthesis to a variety of hormones and to a range of alterations in culture conditions.

We are grateful for support from the Children Nationwide Medical Research Fund and to the Wessex Medical School Trust, and thank the Electron Microscopy Unit, Southampton General Hospital, for the electron photomicroscopy.

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