The influence of *myo*-inositol on phosphatidylglycerol synthesis by rat type II pneumonocytes

John E. BLEASDALE,*†‡ Nancy E. TYLER,* Fredric N. BUSCH* and J. Gerald QUIRK†‡

Departments of *Biochemistry and †Obstetrics-Gynecology and ‡The Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, TX 75235, U.S.A.

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Type II pneumonocytes isolated from adult rat lung were incubated in a serum-free medium containing [^{14}C]glycerol and the incorporation of ^{14}C into glycerophospholipids was measured. After 24 h, more than 80% of the ^{14}C incorporated into total lipids or into phosphatidylcholine and approx. 90% of the ^{14}C incorporated into phosphatidylglycerol after 24 h was recovered in the glycerophosphoester moieties of these molecules. Supplementation of the incubation medium with foetal-bovine serum (10%, v/v) did not alter the incorporation of [^{14}C]glycerol by type II pneumonocytes after 24 h into either a total lipid extract or phosphatidylcholine. In the presence of foetal-bovine serum, however, the incorporation of ^{14}C into phosphatidylglycerol was decreased and the incorporation of ^{14}C into phosphatidylinositol was increased. In the absence of foetal-bovine serum, the incorporation of ^{14}C into phosphatidylglycerol was decreased progressively as the concentration of *myo*-inositol in the incubation medium was increased. The range of concentration (0.04–0.50 mM) over which *myo*-inositol had the greatest influence on [^{14}C]glycerol incorporation into phosphatidylglycerol by type II pneumonocytes *in vitro* encompassed the concentration range measured in foetal-rat serum late in gestation. At 4 days before birth, the concentration of *myo*-inositol in foetal-rat serum was 0.36 mM and decreased to 0.23 mM 1 day before birth. The concentration of *myo*-inositol in adult rat serum increased from 0.03 mM to 0.06 mM during pregnancy. Isolated rat type II pneumonocytes were found to take up *myo*-inositol by a saturable process. A half-maximal rate of *myo*-inositol uptake occurred at a concentration of *myo*-inositol of 0.29 mM. The results of this investigation are consistent with the hypothesis that late in gestation there is a decreasing availability of *myo*-inositol to the foetal lungs and that this favours the biosynthesis of phosphatidylglycerol for surfactant at the expense of phosphatidylinositol biosynthesis.

The glycerophospholipid composition of pulmonary surfactant is unusual in at least two respects. First, the most abundant glycerophospholipid and principal surface-active component of surfactant is 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (King & Clements, 1972). Such a disaturated phosphatidylcholine is uncommon in most mammalian tissues. Secondly, phosphatidylglycerol is a major glycerophospholipid of surfactant (Body, 1971; Pfeiffer & Thomas, 1971), even though this lipid is found in only trace amounts in most mammalian tissues. The biophysical properties of phosphatidylglycerol in surfactant and its physiological role in surfactant function are not well understood. In several species, however, it has been

observed that during foetal-lung development the earliest surfactant produced by immature lungs contains the full complement of disaturated phosphatidylcholine, but is deficient in phosphatidylglycerol (Hallman & Gluck, 1975; Hallman *et al.*, 1977). In place of phosphatidylglycerol, the surfactant from immature lungs is enriched with phosphatidylinositol. As the lung develops, there is a change from the production of a surfactant rich in phosphatidylinositol to one rich in phosphatidylglycerol. A number of investigators have found that pre-term infants, delivered before the appearance of surfactant that is rich in phosphatidylglycerol, are at an increased risk of succumbing to respiratory distress syndrome (Hallman *et al.*, 1976).

The regulation of the amount of phosphatidylinositol and phosphatidylglycerol in pulmonary surfactant is not well understood. Phosphatidylinositol and phosphatidylglycerol are each synthesized from a common precursor, namely CDP-diacylglycerol. The reciprocal developmental changes in the amounts of phosphatidylinositol and phosphatidylglycerol in surfactant are suggestive of regulation at the level of CDP-diacylglycerol utilization. However, the developmental increase in phosphatidylglycerol biosynthesis that occurs at the expense of phosphatidylinositol biosynthesis does not appear to be a consequence of a decreased total activity of CDP-diacylglycerol:inositol phosphatidyltransferase (Bleasdale *et al.*, 1979b) or of an increased total activity of CDP-diacylglycerol:glycerol 3-phosphate phosphatidyltransferase in the lung (on the basis of optimal activity *in vitro*). For this reason alternative explanations have been sought for the developmental increase in the phosphatidylglycerol content of surfactant.

It has been proposed that the relative rates of synthesis of phosphatidylinositol and phosphatidylglycerol for surfactant may be influenced by the availability of *myo*-inositol in the lung (Bleasdale *et al.*, 1982; Hallman *et al.*, 1982). In many tissues, the limited amount of CDP-diacylglycerol that is present likely restricts the biosynthesis of both phosphatidylinositol and phosphatidylglycerol and it has been observed *in vitro* that the metabolic fate of CDP-diacylglycerol is influenced by the extracellular concentration of *myo*-inositol (Freinkel *et al.*, 1975; Eichberg *et al.*, 1979; Esko & Raetz, 1980). We have examined previously *myo*-inositol homeostasis in the human foetus (Quirk & Bleasdale, 1982) and in the foetal rabbit (Bleasdale *et al.*, 1982). Based on the results of those studies we concluded that lung development in the human and in the rabbit is accompanied by decreased availability of *myo*-inositol for surfactant phosphatidylinositol biosynthesis. In addition, we have observed that surfactant phosphatidylinositol biosynthesis by explants of foetal-rabbit lung *in vitro* is stimulated at the expense of surfactant phosphatidylglycerol synthesis when the concentration of *myo*-inositol in the culture medium is increased (Longmuir *et al.*, 1982). Since the foetal-rabbit lung explants contained many lung cell types, it was not possible to determine whether the primary site of action of *myo*-inositol was on type II pneumonocytes or even whether *myo*-inositol was taken up by type II pneumonocytes. The objectives of the present investigation were (1) to examine the effects of *myo*-inositol on the incorporation of [^{14}C]glycerol into glycerophospholipids by isolated type II pneumonocytes, (2) to compare the concentration of *myo*-inositol at which any effect on [^{14}C]glycerol incorporation into

glycerophospholipids was observed with the concentrations found in foetal-rat serum and (3) to examine the uptake of *myo*-inositol by type II pneumonocytes.

Experimental

Materials

The animals used in this investigation were male Sprague-Dawley rats (200–225 g) free from specific pathogens and were obtained from Blue Spruce Farms, Inc., Altamont, NY, U.S.A. Rats were housed inside a laminar-flow hood and were fed *ad libitum* until they were killed. The materials used in the procedure for isolating type II pneumonocytes were obtained from the sources described by Mason *et al.* (1977b). [$\text{U-}^{14}\text{C}$]Glycerol (sp. radioactivity 100–150 Ci/mol) in ethanol/water (1:1, v/v), *myo*-[2- ^3H]inositol (10–20 Ci/mmol) and D-[1- ^{14}C]mannitol (45 Ci/mol) were supplied by New England Nuclear, Boston, MA, U.S.A. Foetal-bovine serum (heat-inactivated) and antibiotic/antimycotic solution [containing penicillin (10000 units/ml), fungizone (25 $\mu\text{g}/\text{ml}$) and streptomycin (10 mg/ml)] were obtained from GIBCO, Grand Island, NY, U.S.A. Dulbecco's modified Eagle's medium was obtained from KC Biological Inc., Lenexa, KS, U.S.A. Gentamycin sulphate was from Schering Corp., Kenilworth, NJ, U.S.A. Lipid standards were supplied by Serdary Research Laboratories, London, Ontario, Canada. Silica-gel G t.l.c. plates (Uniplate G; 200 μm) were obtained from Analtech Inc., Newark, DE, U.S.A. All other chemicals were commercially available reagent grade.

Methods

Isolation of rat type II pneumonocytes. Type II pneumonocytes were isolated from adult rat lungs by a procedure utilizing cell dispersion with trypsin and deoxyribonuclease, discontinuous-density-gradient centrifugation on gradients prepared from serum albumin solutions, and differential adherence as described by Mason *et al.* (1977a,b) and Mason & Dobbs (1980). The first differential adherence step (3 h) (during which macrophages became attached to the plastic culture flask) was performed as described by Mason & Dobbs (1980). The second differential adherence step (20 h) (during which type II pneumonocytes become attached to the plastic culture flask) was routinely omitted from the procedure of Mason & Dobbs (1980). This omission did not significantly affect the purity of the type II pneumonocyte preparation but improved markedly the yield of cells. Purity of the final cell suspension was assessed by light microscopy using a modified Papanicolaou stain (Kikkawa & Yoneda, 1974) and by electron microscopy (Snyder *et al.*, 1981). Approx. 4×10^7 cells, of which 75–85% were type

II pneumonocytes, were isolated routinely from five rats. The major contaminating cells were lymphocytes.

Measurement of [^{14}C]glycerol incorporation into glycerophospholipids by type II pneumonocytes. The cell suspension resulting after the 3 h differential adherence step was centrifuged under sterile conditions at 150 g for 6 min. The pellet of cells was resuspended in Dulbecco's modified Eagle's medium containing 1 ml of antibiotic/antimycotic solution per 100 ml and was then recentrifuged at 150 g for 6 min. The washed cell pellet was resuspended in Dulbecco's modified Eagle's medium containing antibiotics and antimycotics, with or without foetal-bovine serum (10%, v/v). Cells (approx. 2×10^6 in 1 ml of medium) were incubated in glass screw-cap culture tubes (150 mm $\times$ 20 mm) in an incubator at 37°C. [^{14}C]Glycerol was added to cell suspensions to give a final concentration of 0.1 mM (approx. 10 Ci/mol) and in standard experiments the cells were incubated for 24 h. After the incubation period, 4.5 ml of chloroform/methanol (1:2, v/v) and 0.1 ml of HCl (6 M) were added to each tube containing cells. After mixing, the contents of each tube were sonicated (1 min in a Bransonic 220 bath-type sonicator). Lipids were then extracted as described previously (Bleasdale *et al.*, 1979a) and separated by two-dimensional t.l.c. (Yavin & Zutra, 1977). In some experiments the intramolecular distribution of incorporated ^{14}C was determined. For this purpose, areas of thin-layer chromatograms containing separated lipids were scraped into glass centrifuge tubes and lipids were eluted employing the extraction procedure of Bligh & Dyer (1959). Each eluted lipid was evaporated to dryness under N_2 and then subjected to alkaline hydrolysis (Dawson, 1967). Only non-esterified fatty acids and water-soluble glycerophosphoesters were produced in this procedure. After hydrolysis, the pH of each hydrolysis mixture was adjusted to pH 2 with HCl and then non-esterified fatty acids were extracted from the mixture using hexane (2 $\times$ 2 ml) and glycerophosphoesters were recovered in the aqueous phase.

Measurement of *myo*-inositol uptake by type II pneumonocytes. Type II pneumonocytes (approx. 2.5×10^6 cells) were suspended in 0.5 ml of Dulbecco's modified Eagle's medium supplemented with [$1\text{-}^{14}\text{C}$]mannitol (carrier-free; approx. 8 μM ; 0.05 Ci/mmol) and *myo*-[2- ^3H]inositol (approx. 5 μCi , at various concentrations) and were incubated for 20 min at 37°C in a Dubnoff metabolic shaker. After incubation, type II pneumonocytes were collected by vacuum filtration on Millipore GS filters (0.22 μm) that had been prewashed with *myo*-inositol (100 mM). Filters holding type II pneumonocytes were washed twice with 2 ml of NaCl (0.154 M) and then placed in a scintillation vial containing 0.85 ml of Triton X-100 (5%, w/v) and

stored at room temperature overnight. Radioactivity was assayed as described previously (Bleasdale *et al.*, 1979a) and the amount of *myo*-inositol taken up by type II pneumonocytes was calculated by using [$1\text{-}^{14}\text{C}$]mannitol to estimate the amount of extracellular *myo*-inositol associated with the filters (Bleasdale *et al.*, 1982). Under the experimental conditions described, less than 10% of total *myo*-[2- ^3H]inositol associated with filters was extracellular. Uptake of *myo*-inositol by type II pneumonocytes occurred at a constant rate for at least 30 min at 37°C.

Other procedures. *myo*-Inositol in serum was measured employing g.l.c. of its trimethylsilyl derivative as described previously (Bleasdale *et al.*, 1982). Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as the reference standard. Lipid phosphorus was determined as described by Ames & Dubin (1960). Radioactivity was assayed as described previously (Bleasdale *et al.*, 1979a). Differences between mean values were determined by the Newman-Keuls multiple-range comparison test (Zar, 1974).

Results

Type II pneumonocytes that were incubated in Dulbecco's modified Eagle's medium supplemented with foetal-bovine serum (10%, v/v) incorporated [^{14}C]glycerol into glycerophospholipids for at least 36 h, but the rate of incorporation decreased as the incubation period increased (Fig. 1). The decreasing rate of incorporation of [^{14}C]glycerol was most pronounced for phosphatidylglycerol. The percentage of ^{14}C incorporated into total lipid that was associated with phosphatidylglycerol was 12.8 ± 1.4 after 1 h of incubation but only 6.6 ± 0.4 after 24 h of incubation (means $\pm$ S.E.M. from three determinations). Expressed as a percentage of ^{14}C incorporated into total lipid, the incorporation into individual lipids after 15 h was as follows: phosphatidylcholine, 53.9%; neutral lipid plus non-esterified fatty acids, 13.5%; lysophosphatidylethanolamine, 8.7%; phosphatidylglycerol, 6.9%; sphingomyelin plus lysophosphatidylcholine, 5.9%; phosphatidylethanolamine, 3.9%; phosphatidylinositol, 3.4%; phosphatidylserine, 1.8%; bis(monoacylglycerol)-phosphate, 0.8%; phosphatidic acid, 0.6%; unidentified, 2.2%; average total recovery, 101.6% (average values from two determinations; the two values for each individual lipid differed by not more than 10%). The unexpectedly high recovery of ^{14}C in lysophosphatidylethanolamine may be an artefact resulting from hydrolysis of ethanolamine plasmalogen that would be facilitated by the acidic extraction conditions employed. To determine the intramolecular distribution of ^{14}C within individual glycerophospholipids, lipids were separated by using

Fig. 1. Incorporation of [^{14}C]glycerol into lipids by rat type II pneumonocytes

Rat type II pneumonocytes were incubated in the presence of foetal-bovine serum (10%, v/v) and [^{14}C]glycerol (0.1 mM; approx. 10 Ci/mol) for various time periods and then lipids were extracted as described in the Experimental section. Lipids were separated by using two-dimensional t.l.c. and ^{14}C incorporated into individual lipids was measured. Symbols: ▲, total lipids; △, phosphatidylcholine; ●, phosphatidylglycerol; ○, phosphatidylinositol. Data are average values derived from duplicate measurements in two separate experiments.

two-dimensional t.l.c. and were then hydrolysed as described above. The hydrolysis products (fatty acids and glycerophosphoesters) were separated by phase distribution and radioactivity in the products was measured. After 15 h of incubation with [^{14}C]glycerol, the ratios of radioactivity in the glycerophosphoester backbone of lipids to that in the fatty acyl substituents were as follows: total lipid, 4.8; phosphatidylcholine, 4.2; phosphatidylglycerol, 9.5 (means from three determinations; in each case the range did not exceed 20% of the mean).

The effect of foetal-bovine serum on the incorporation of [^{14}C]glycerol into glycerophospholipids by type II pneumonocytes after 1 h, 12 h or 24 h in culture was next examined. Omission of foetal-bovine serum from the culture medium did not affect significantly the incorporation of [^{14}C]glycerol into total glycerophospholipids or phosphatidylcholine (Fig. 2). After 12 h or 24 h in the presence of serum, however, incorporation of [^{14}C]glycerol into phosphatidylinositol was increased, whereas incorporation into phosphatidylglycerol was decreased. We examined the possibility that *myo*-inositol in foetal-bovine serum was responsible for the decreased incorporation of [^{14}C]glycerol into phosphatidylglycerol and increased [^{14}C]glycerol incorporation into phosphatidylinositol observed in the presence of foetal-bovine serum. The concentration of *myo*-inositol in the foetal-bovine serum employed

Fig. 2. The influence of foetal-bovine serum on the incorporation of [^{14}C]glycerol into lipids by rat type II pneumonocytes

Rat type II pneumonocytes were incubated for 1, 12 or 24 h with [^{14}C]glycerol in the absence (open columns) or in the presence (hatched columns) of foetal-bovine serum (10%, v/v) and lipids were extracted, then separated and incorporated radioisotope was measured as described in the Experimental section. (a) Total lipids; (b), phosphatidylcholine; (c), phosphatidylglycerol; (d), phosphatidylinositol. Data are expressed either as 10^{-3} c.p.m./ 10^6 cells (a) or as percentages of ^{14}C incorporated into total lipids (b–d) and are means $\pm$ S.E.M. (indicated by the bars) derived from duplicate measurements in four experiments. A statistically significant effect ($P < 0.05$) of foetal-bovine serum on ^{14}C incorporation was observed only for phosphatidylglycerol (12 h and 24 h) and for phosphatidylinositol (12 h and 24 h).

in this investigation was measured and found to be 0.33 mM. Therefore, when the incubation medium was supplemented with foetal-bovine serum (10%, v/v) the concentration of *myo*-inositol in which the type II pneumonocytes were exposed was increased from 0.04 mM (the concentration of *myo*-inositol in Dulbecco's modified Eagle's medium) to 0.069 mM. The effect of *myo*-inositol on [^{14}C]glycerol incorporation into glycerophospholipids by type II pneumonocytes was investigated by incubating cells in a serum-free medium in the presence of *myo*-inositol at various concentrations. It was observed that in the absence of serum, but in the presence of *myo*-inositol at concentrations up to 2 mM, incorporation of [^{14}C]glycerol into total lipids remained essentially unchanged. However, as illustrated in Fig. 3, the incorporation of [^{14}C]glycerol into phosphatidylglycerol and phosphatidylinositol was affected markedly by *myo*-inositol. Expressed as a percentage of incorporation into total lipids, the incorporation of [^{14}C]glycerol into phosphatidylglycerol decreased from 13% to 2% as the concentration of *myo*-inositol in the medium was

Fig. 3. The effect of *myo*-inositol on the incorporation of [^{14}C]glycerol into phosphatidylglycerol and phosphatidylinositol by rat type II pneumonocytes

Rat type II pneumonocytes were incubated with [^{14}C]glycerol for 24 h in Dulbecco's modified Eagle's medium containing *myo*-inositol at various concentrations. Lipids were extracted, then separated and ^{14}C incorporation was measured as described in the Experimental section. ●, Phosphatidylglycerol; ○, phosphatidylinositol. Data are expressed as percentages of ^{14}C incorporation into total lipids and are means $\pm$ S.E.M. derived from duplicate measurements in three experiments. The addition of *myo*-inositol (at all concentrations up to 2.04 mM) did not affect significantly the incorporation of [^{14}C]glycerol into total lipids (results not shown).

increased. Conversely, with increasing *myo*-inositol concentration in the medium the incorporation of [^{14}C]glycerol into phosphatidylinositol increased from 3% to 7%. As the concentration of *myo*-inositol in the medium was increased, the decrease in incorporation into phosphatidylglycerol was greater than the increase in incorporation into phosphatidylinositol. This was to be expected since phosphatidylglycerol contains two glycerol moieties, whereas phosphatidylinositol contains only one.

Since *myo*-inositol in increased amounts appeared to alter CDP-diacylglycerol utilization by type II pneumonocytes in favour of phosphatidylinositol biosynthesis at the expense of phosphatidylglycerol biosynthesis, we examined the ability of glycerol in increased amounts to restore phosphatidylglycerol biosynthesis in the presence of large amounts of *myo*-inositol. Type II pneumonocytes were incubated for 24 h in serum-free incubation medium in the presence of *myo*-inositol (0.04 mM or 1.04 mM) and [^{14}C]glycerol (0.1 mM or 1.1 mM). When the concentration of glycerol in the medium was 1.1 mM, the ability of *myo*-inositol to diminish phosphatidylglycerol synthesis and to stimulate phosphatidylinositol synthesis by type II pneumonocytes was not affected (Table 1). In a further attempt to influence CDP-diacylglycerol availability, the concentration of either choline or cytidine in the incubation medium was increased, but the effect of *myo*-inositol

Table 1. Effects of various treatments on the relative incorporation of [^{14}C]glycerol into phosphatidylglycerol and phosphatidylinositol by type II pneumonocytes

Type II pneumonocytes were incubated in Dulbecco's modified Eagle's medium containing [^{14}C]glycerol (approx. 10 Ci/mol) with or without additional *myo*-inositol. Additional glycerol was added to some incubations, raising the concentration from 0.1 mM to 1.1 mM. In other incubations additional choline or cytidine was added. After incubation the incorporation into individual glycerophospholipids was measured as described in the text. The data are expressed as the ratio of ^{14}C incorporated into phosphatidylglycerol to ^{14}C incorporated into phosphatidylinositol and are means $\pm$ S.E.M. for the number of determinations shown in parentheses.

Addition to standard medium	^{14}C incorporated into phosphatidylglycerol / ^{14}C incorporated into phosphatidylinositol	
	No added <i>myo</i> -inositol (0.04 mM)	Added <i>myo</i> -inositol (1.04 mM)
None	5.17 $\pm$ 0.64 (5)	0.63 $\pm$ 0.06 (6)
Glycerol (1 mM)	5.44 (2)	0.56 (2)
Choline (1 mM)	4.72 (2)	0.68 $\pm$ 0.03 (4)
Cytidine (1 mM)	4.79 $\pm$ 0.77 (3)	0.69 $\pm$ 0.06 (3)

on the relative rates of incorporation of [^{14}C]glycerol into phosphatidylinositol and phosphatidylglycerol remained unaltered (Table 1).

The ability of type II pneumonocytes to take up *myo*-inositol from the incubation medium was investigated. Type II pneumonocytes were found to take up *myo*-inositol by a saturable process and the rate of uptake was half-maximal at a *myo*-inositol concentration of 0.29 mM (Fig. 4). In many species the concentration of *myo*-inositol is higher in foetal serum than in adult serum and a decline in the concentration of *myo*-inositol in foetal serum occurs during late gestation (Campling & Nixon, 1954; Burton & Wells, 1974; Bleasdale *et al.*, 1982). We observed that 4 days before birth, the concentration of *myo*-inositol in foetal-rat serum was 0.36 $\pm$ 0.04 mM ($n = 4$) and at 1 day before birth was 0.23 $\pm$ 0.01 mM ($n = 8$). In contrast, the concentration of *myo*-inositol in adult-rat serum was 0.034 $\pm$ 0.002 mM ($n = 54$) and increased to 0.064 $\pm$ 0.001 mM ($n = 65$) during pregnancy (mean values $\pm$ S.E.M.). The concentration of *myo*-inositol in foetal-rat serum at the end of gestation is much lower than that required to support maximal uptake of *myo*-inositol by isolated rat type II pneumonocytes. Therefore, the decline in the concentration of *myo*-inositol in foetal-rat serum during development may result in restricted availability of *myo*-inositol for phosphatidylinositol biosynthesis in the foetal

Fig. 4. The uptake of myo-inositol by rat type II pneumonocytes

Rat type II pneumonocytes (approx. 2.5×10^6 cells) were incubated in 0.5 ml of serum-free Dulbecco's modified Eagle's medium containing [1- 14 C]mannitol (approx. $8 \mu\text{M}$; 0.05 Ci/mmol) and myo-[2- ^3H]inositol (approx. $5 \mu\text{Ci}$, at various concentrations) for 20 min at 37°C . Cells were then collected by vacuum filtration and intracellular uptake of myo-inositol was calculated as described in the Experimental section. The data were derived from duplicate measurements in a typical experiment. The rate of myo-inositol uptake was half-maximal at a myo-inositol concentration of 0.29 mM (average of two experiments).

lung. As a consequence the change from a phosphatidylinositol-rich surfactant characteristic of immature lungs to a phosphatidylglycerol-rich surfactant characteristic of mature lungs would be facilitated.

Discussion

Rat type II pneumonocytes maintained *in vitro* incorporated [^{14}C]glycerol into glycerophospholipids and initially the fastest rates of incorporation were for the two most abundant glycerophospholipids of pulmonary surfactant, namely phosphatidylcholine and phosphatidylglycerol. The rate of incorporation of [^{14}C]glycerol into glycerophospholipids by type II pneumonocytes decreased over time *in vitro* and this effect was most pronounced for phosphatidylglycerol. Mason & Dobbs (1980) observed that the rate of incorporation of [^{14}C]palmitate into phosphatidylglycerol by type II pneumonocytes decreased strikingly after 24 h *in vitro*. The cause of this decreased capacity to incorporate [^{14}C]glycerol into glycerophospholipids is unknown, but it has been reported that a protein

secreted from fibroblasts (Tanswell & Smith, 1979) and several hormones (Snyder *et al.*, 1981) are necessary for optimal lipid synthesis by type II pneumonocytes *in vitro*.

The inclusion of foetal-bovine serum (10%) in the culture medium did not result in a significantly altered incorporation of [^{14}C]glycerol into either total lipid or phosphatidylcholine but did result in increased incorporation into phosphatidylinositol and decreased incorporation into phosphatidylglycerol. Such a reciprocal relationship between the synthesis of phosphatidylglycerol and that of phosphatidylinositol has been observed in several tissues (Freinkel *et al.*, 1975; Eichberg *et al.*, 1979; Esko & Raetz, 1980; Longmuir *et al.*, 1982). Although direct interconversion of phosphatidylglycerol and phosphatidylinositol in a transphosphatidylation reaction has been detected in some tissues (for review, see Kanfer, 1980), no evidence for this reaction in lung tissue was obtained (Bleasdale & Wallis, 1981). It seems more likely that the observed relationship between biosynthesis of phosphatidylglycerol and that of phosphatidylinositol is a reflection of competition for the limited amount of CDP-diacylglycerol that is the common precursor of both glycerophospholipids. Competition for CDP-diacylglycerol appears to be influenced by the availability of myo-inositol, such that an increase in the extracellular concentration of myo-inositol results in a stimulation of phosphatidylinositol biosynthesis at the expense of phosphatidylglycerol biosynthesis. In the present investigation, when foetal-bovine serum (10%, v/v) was added to the incubation medium the concentration of myo-inositol to which the type II pneumonocytes were exposed was approximately doubled and this can account, in part, for the increased incorporation of [^{14}C]glycerol into phosphatidylinositol that was observed. In the absence of foetal-bovine serum, the incorporation of [^{14}C]glycerol into phosphatidylinositol by type II pneumonocytes was increased and that into phosphatidylglycerol was decreased when the concentration of myo-inositol in the incubation medium was raised. The dependence of phosphatidylinositol synthesis by type II pneumonocytes on the concentration of myo-inositol in the medium was similar to the concentration-dependence of myo-inositol uptake by these cells. The finding that the relative rates of phosphatidylglycerol and phosphatidylinositol biosynthesis by type II pneumonocytes was influenced by the extracellular concentration of myo-inositol may be physiologically significant since it was also observed that the concentration of myo-inositol in foetal-rat serum decreased during late gestation. A similar developmental decline in the concentration of myo-inositol in the serum of other species has been observed (Campling & Nixon, 1954; Bleasdale *et al.*, 1982) and the present

observation complements the finding by Burton & Wells (1974) of a post-natal decline in the concentration of *myo*-inositol in rat serum.

Although the experiments reported here were performed using type II pneumonocytes from adult rats it is likely that foetal type II pneumonocytes respond in a similar manner. We have observed previously that the characteristics of *myo*-inositol uptake by slices of adult- and foetal-rabbit lung were very similar (Bleasdale *et al.*, 1982). The amount of *myo*-inositol found in either foetal- or adult-rabbit lung (assuming an even distribution) was approximately 10-times greater than that required for optimal synthesis of phosphatidylinositol by lung microsomes *in vitro* (Bleasdale *et al.*, 1982). In spite of an apparent excess of intracellular *myo*-inositol, phosphatidylinositol synthesis in lung tissue is responsive to changes in the extracellular concentration of *myo*-inositol (Longmuir *et al.*, 1982; the present paper). There are at least two possible explanations for this finding. First, intracellular *myo*-inositol may be unevenly distributed and only a small intracellular pool (responsive to changes in extracellular *myo*-inositol concentration) may be available for phosphatidylinositol biosynthesis. Secondly, the increased synthesis of phosphatidylinositol observed in the presence of increased extracellular concentrations of *myo*-inositol may be a reflection not of the affinity of CDP-diacylglycerol:inositol phosphatidyltransferase for *myo*-inositol in the catalysis of phosphatidylinositol formation, but rather an indication of the larger amount of *myo*-inositol necessary to inhibit the reverse reaction catalysed by this enzyme (Bleasdale *et al.*, 1979a). We have proposed previously that the reverse reaction catalysed by CDP-diacylglycerol:inositol phosphatidyltransferase proceeds under physiological conditions and that during lung development the CMP accumulating as a co-product of enhanced surfactant phosphatidylcholine biosynthesis facilitates the reverse reaction. As a result, net synthesis of phosphatidylinositol from newly synthesized CDP-diacylglycerol is restricted and more CDP-diacylglycerol is now available for phosphatidylglycerol synthesis (Bleasdale *et al.*, 1979a; Quirk *et al.*, 1980; Bleasdale & Johnston, 1982). Therefore, the influence of a developmental decline in *myo*-inositol availability on surfactant phosphatidylglycerol biosynthesis is complementary to the role envisioned for CMP in the regulation of surfactant lipid composition.

On the basis of these and previous observations (Longmuir *et al.*, 1982; Bleasdale *et al.*, 1982) it is concluded that factors that influence *myo*-inositol availability to the type II pneumonocyte during foetal-lung development could potentially alter the relative rates of phosphatidylglycerol and phosphatidylinositol biosynthesis for surfactant.

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