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# Studies on the formation of dipalmitoyl species of phosphatidylcholine and phosphatidylethanolamine in pulmonary type II cells

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Endogenous content of and incorporation of labelled glycerol into alkenylacyl-, alkylacyl- and diacyl-glycerol, -glycero-3-phosphocholine and -glycero-3-phosphoethanolamine of pulmonary type II cells were measured. On prolonged incubation of type II cells with labelled glycerol, the proportion of label incorporated into the diacyl subclass of these glycerolipids increased and the proportion of label incorporated into the ether lipids declined. Endogenous phosphatidylcholine (PtdCho) of type II cells contained 38.4% of the dipalmitoyl species, but endogenous phosphatidylethanolamine (PtdEtn) only 2.5%. In contrast, similar proportions of labelled glycerol were incorporated into dipalmitoyl-PtdCho and -PtdEtn after short-time incubation but, with prolonged incubation time the proportion of labelled dipalmitoyl-PtdCho increased from 11.3 to 18.8%, whereas that of dipalmitoyl-PtdEtn did not change significantly. Type II cell membranes were found to exhibit cofactor-independent and CoA-mediated transacylations of [1-$^{14}$C]palmitoyl-lyso-PtdCho and -lyso-PtdEtn. The distribution of label among the palmitic acid-containing species of PtdCho and PtdEtn formed by both transacylation activities was determined. Cofactor-independent and CoA-mediated transacylation showed a strong selectivity for palmitate and arachidonate and a strong discrimination against oleate. The amount (nmol) of dipalmitoyl-PtdEtn formed by both transcylation activities after short-time incubation (2 min) decreased with prolonged incubation time (60 min). In contrast, the nmol of dipalmitoyl-PtdCho formed by cofactor-independent transacylation remains nearly the same after short-time and longer incubation. The nmol of dipalmitoyl-PtdCho formed by CoA-mediated transacylation increased strongly in the same time interval. Beside synthesis *de novo* via the CDP-choline pathway and reacylation of lyso-PtdCho with palmitoyl-CoA, the CoA-mediated transacylation of lyso-PtdCho may be an effective pathway for the formation of dipalmitoyl-PtdCho in pulmonary type II cells.

## INTRODUCTION

Lung surfactant, a phospholipid-rich surface-active material lining the alveolar surface, is formed and secreted by pulmonary type II cells. PtdCho and PtdGro (in some animals PtdIns) are the major lipid constituents of pulmonary surfactant, whose physiological properties are determined by the relative proportions of these phospholipids (for reviews see Wright & Clements, 1987; Van Golde *et al.*, 1988; Post & Van Golde, 1988). Both surfactant phospholipids are characterized by a relatively high proportion of the dipalmitoyl species (Goerke, 1974; Schlame *et al.*, 1988).

In order to explain the high content of dipalmitoyl-PtdCho, three different mechanisms have been suggested:

(i) synthesis *de novo* (Crecelius & Longmore, 1984*b*);

(ii) reacylation of lyso-PtdCho with palmitoyl-CoA by the forward reaction of the acyl-CoA:lysoPtdCho acyltransferase (Mason & Dobbs, 1980; Post *et al.*, 1983); and

(iii) acyl exchange between unsaturated fatty acid in the *sn*-2 position of PtdCho and palmitoyl-CoA, involving the reverse reaction of the acyltransferase, as shown in lung microsomes (Stymne & Stobert, 1985), and possibly also indirectly by transacylation of unsaturated fatty acids from PtdCho to lyso-PtdEtn (Nijssen & Van den Bosch, 1986*b*). In contrast with the surfactant lipids PtdCho, PtdGro and PtdIns (Goerke, 1974; Hallman *et al.*, 1985; Schlame *et al.*, 1986; Rüstow *et al.*, 1988), surfactant PtdEtn contained only trace amounts of dipalmitoyl species. Thus it appears that endogenous dipalmitoylglycerol of lung microsomes (Rüstow & Kunze, 1984) and of type II cells (Post *et al.*, 1983) is hardly used to synthesize dipalmitoyl-PtdEtn. It has to be taken into account, however, that both endogenous diacylglycerols and those formed *de novo* represent different substrate pools for the biosynthesis of PtdCho and PtdEtn (Binaglia *et al.*, 1982; Rüstow & Kunze, 1985, 1987). Therefore it may be assumed that dipalmitoyl-PtdEtn is formed *de novo* but is preferentially hydrolysed to lyso-PtdEtn, which in turn could serve as acyl acceptor of unsaturated acyl groups from PtdCho.

In this paper we present data which provide indirect support for the hypothesis that transacylation reactions are involved in the formation of the characteristic species patterns found in endogenous type II cell phospholipids. We compared the incorporation of labelled glycerol into the palmitic acid-containing species of PtdCho and PtdEtn, using pulmonary type II cells of adult rats, and the patterns of molecular species synthesized by transacylation of 1-palmitoyl-lyso-PtdCho and -lyso-PtdEtn, with endogenous lipids of type II cell membranes serving as acyl donor. The results indicate that in type II cells the dipalmitoyl species of PtdCho and PtdEtn are synthesized *de novo* to the same extent. But dipalmitoyl-PtdCho increased with prolonged incubation time, whereas dipalmitoyl-PtdEtn did not increase. With endogenous lipids of type II cell membranes as acyl donor, both 1-[1-$^{14}$C]palmitoyl-lyso-PtdCho and 1-[1-$^{14}$C]-palmitoyl-lyso-PtdEtn are transacylated, yielding preferently dipalmitoyl and polyunsaturated species. After prolonged incubation, the dipalmitoyl species formed by the transacylation activities are

Abbreviations used: PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; Gro-3-*P*, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate.

**Table 1. Endogenous and newly formed subclasses of diradyl-glycerol, -PtdCho and -PtdEtn and their dipalmitoyl species isolated from pulmonary type II cells**

Values are given as percentages from a total of three independent experiments, with S.D. in parentheses.

| | Diradylglycerol | | | Diradyl-PtdCho | | | Diradyl-PtdEtn | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Newly formed* | | | Newly formed* | | | Newly formed* | |
| | Endogenous | 10 min | 90 min | Endogenous | 10 min | 90 min | Endogenous | 10 min | 90 min |
| Alkenyl-acyl | 2.6 | 7.0 | 3.1 | 2.4 | 11.9 | 1.3 | 28.8 | 17.4 | 22.0 |
| | (1.0) | (2.0) | (0.8) | (0.6) | (2.3) | (0.4) | (5.5) | (5.4) | (5.8) |
| Alkyl-acyl | 18.0 | 44.2 | 38.5 | 11.4 | 12.7 | 5.4 | 13.8 | 28.1 | 19.5 |
| | (3.9) | (2.8) | (7.6) | (1.9) | (1.6) | (3.9) | (0.6) | (2.1) | (5.1) |
| Diacyl | 78.3 | 48.4 | 56.7 | 89.1 | 75.4 | 95.3 | 58.9 | 54.6 | 60.2 |
| | (6.0) | (5.3) | (2.8) | (2.2) | (3.3) | (4.5) | (4.4) | (1.7) | (11.9) |
| Dipalmitoyl† | 15.4 | 15.7 | 16.6 | 38.4 | 11.3 | 18.8 | 2.5 | 10.5 | 8.9 |
| | (2.7) | (0.3) | (2.1) | (1.8) | (1.2) | (3.0) | (0.2) | (0.5) | (2.6) |

* 'Newly formed' means labelled lipids formed by incubation of type II cells with [1(3)-$^3$H]glycerol for 10 or 90 min.
† Values are given as percentages of the total diacyl fractions.

degraded. Only dipalmitoyl-PtdCho formed by CoA-mediated transacylation increased, indicating that this pathway may be effective for the formation of dipalmitoyl-PtdCho in type II cells.

## MATERIALS AND METHODS

### Materials

Pulmonary type II cells of adult male Wistar rats (body weight 180–250 g) were isolated as described by Mason *et al.* (1977), with some modifications (Schlame *et al.*, 1988). [1(3)-$^3$H]Glycerol (sp. radioactivity 3.0 Ci/mmol), 1-palmitoyl-2-[1-$^{14}$C]linoleoyl-PtdEtn (sp. radioactivity 55.6 mCi/mmol) and 1,2-di[1-$^{14}$C]-palmitoyl-PtdCho (sp. radioactivity 114.0 mCi/mmol) were purchased from The Radiochemical Centre (Amersham, Bucks, U.K.). Labelled dipalmitoyl-PtdEtn was synthesized by incubation of 1,2-di[1-$^{14}$C]palmitoyl-PtdCho with phospholipase D in the presence of ethanolamine (Comfurius & Zwaal, 1977). [1-$^{14}$C]Palmitoyl-2-lyso-PtdCho and -lyso-PtdEtn were formed by treatment of the corresponding dipalmitoyl compounds with phospholipase $A_2$ (Waku & Nakazawa, 1972). The reaction products were extracted as described by Bligh & Dyer (1959) and purified by t.l.c. Phospholipase C (*Bacillus cereus*), phospholipase D (cabbage), phospholipase $A_2$ (*Naja naja*), trypsin and soya-bean trypsin inhibitor were obtained from Boehringer (Mannheim, Germany). Precoated silica-gel H60 plates were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade, and the organic solvents were purified by distillation.

### Methods

**Incubation of type II cells with labelled glycerol.** Type II cells [$(10–18 \times 10^6)$] were incubated in 1 ml of Dulbecco's modified minimal essential medium containing 10% (v/v) fetal-calf serum and 50–100 $\mu$Ci of [$^3$H]glycerol at 37 °C for 10 or 90 min. The type II cell suspension was then centrifuged at 1500 *g* for 10 min to remove excess radioactivity. Finally, the reaction was terminated by addition of 3 ml of chloroform/methanol (1:2, v/v).

**Incubation of membranes of type II cells with labelled lysophospholipids.** Type II cells [$(10–18) \times 10^6$] were homogenized in 5 ml of 0.25 M-sucrose/1 mM-EDTA/10 mM-Tris (pH 7.4) in a glass/Teflon Potter homogenizer. The homogenates were centrifuged at 100000 *g* at 4 °C for 60 min. The supernatant was concentrated to 1–2 ml containing 2–4 mg of protein/ml and used as type II-cell cytosol. The sedimented membranes of type II cells were resuspended in 0.25 M-sucrose/1 mM-EDTA/10 mM-Tris (pH 7.4) and then incubated with labelled lyso-PtdCho or lyso-PtdEtn at 37 °C for 60 min. The incubation mixture contained 0.1 M-Tris, 5 mM-EDTA, 5 mM-dithiothreitol, 15 $\mu$M-[1-$^{14}$C]palmitic acid, -1-[1-$^{14}$C]palmitoyl-lyso-PtdCho or -1-[1-$^{14}$C]-palmitoyl-lyso-PtdEtn (sp. radioactivity 100–500 d.p.m./nmol), 1.2–2.0 mg of membrane protein/ml and, if indicated, 50 $\mu$M-CoA, 3.5 mM-ATP, 15 $\mu$M-[1-$^{14}$C]palmitic acid (sp. radioactivity 300–1000 d.p.m./nmol) or 0.1–0.6 mg of cytosolic protein/ml. The final volume was 0.25 ml. To deplete type II cell membranes of endogenous acyl-CoA and CoA, preincubations with Gro-3-*P* and succinate/succinyl-CoA synthetase were carried out as described in the legend of Table 2.

**Methylation and base exchange.** Type II cell membranes were incubated with labelled lyso-PtdEtn to produce membrane-bound labelled PtdEtn as described above. To measure the rate of PtdEtn methylation, 0.1 ml of 0.5 M-Tris (pH 9.5) and *S*-adenosylmethionine were added (final concn. 20 $\mu$M) and the incubation was continued for 20 min. To measure the base-exchange reaction, 10 mM-$Ca^{2+}$ and 8 mM-choline were added and the incubation was continued for 20 min.

In each case the incubations were terminated by addition of 3 ml of chloroform/methanol (1:2, v/v).

**Lipid analysis.** Lipids were extracted as described by Bligh & Dyer (1959) and separated by two-dimensional t.l.c. on precoated silica-gel H60 plates with the solvents described previously (Rüstow *et al.*, 1987). The areas containing PtdCho, PtdEtn and neutral lipids were made visible with 0.001% primulin in acetone, scraped off and extracted from silica gel. The PtdEtn fraction was purified from traces of PtdGro by an additional one-dimensional t.l.c. with chloroform/methanol/water (65:25:4, by vol.) as solvent. Neutral lipids were separated by one-dimensional t.l.c. with hexane/ether/acetic acid (70:30:4, by vol.) as solvent in order to obtain pure diradylglycerol, which was extracted with chloroform.

PtdCho and purified PtdEtn were incubated with phopholipase C, yielding the diradyl moieties. The diradylglycerols of PtdCho and PtdEtn as well as endogenous diradylglycerol were transformed to their $\alpha$-naphthylisocyanates as described by Rüstow *et al.* (1987). Separation of the diradyl derivatives into their subclasses was performed by t.l.c. developed consecutively with benzene/hexane/diethyl ether (48:53:2, by vol.) and toluene in the same direction. The subclasses were scraped off, and $C_{21:0}$ fatty acid as internal standard was added to the silica gel, which

**Table 2. Acylation of 1-[$^{14}$C]palmitoyl-lyso-PtdCho and 1-[$^{14}$C]palmitoyl-lyso-PtdEtn by membranes of pulmonary type II cells**

Values from three independent experiments are given as nmol of PtdCho or PtdEtn synthesized/60 min per mg of protein, with S.D. in parentheses.

| Addition | Lyso-PtdCho | Lyso-PtdEtn |
|---|---|---|
| None | 22.4 (5.2) | 11.5 (0.8) |
| CoA | 58.7 (11.0) | 30.1 (8.3) |
| Preincubation for CoA depletion* | 22.0 (3.2) | 12.1 (1.6) |
| [1-$^{14}$C]Palmitic acid† | < 1 | < 1 |
| +CoA† | < 1 | < 1 |
| +ATP† | < 1 | < 1 |
| +CoA+ATP† | 30.3 (2.1) | 27.4 (1.4) |
| Preincubation for acyl-CoA depletion‡ | 23.9 (1.0) | 9.8 (2.5) |
| Type II cell cytosol | 20.7 (4.0) | 13.4 (1.9) |

* Type II cell membranes were depleted of endogenous CoA by preincubation with 1 mM-GTP, 2 mM-succinate and 0.1–0.3 unit of succinyl-CoA synthetase at 37 °C for 20 min.

† When the incorporation of [1-$^{14}$C]palmitic acid had to be determined, labelled lysophospholipid was replaced by non-labelled at the same concentration.

‡ Type II cells were preincubated with 1.5 mM-Gro-3-*P*, 3 mM-$MgCl_2$ and 10 mM-cysteine at 37 °C for 20 min.

was then extracted with chloroform. Samples were taken to measure radioactivity and to determine the fatty acyl pattern (for methodology see Rüstow *et al.*, 1987). Molecular species of the diacyl subclass were analysed by h.p.l.c. with distearoylglycerol as internal standard. Individual peaks were collected into scintillation vials, the solvent was evaporated and toluene-based scintillation fluid was added. The radioactivity was measured in a Philips liquid-scintillation counter PW 4700 equipped with automatic compensation for quenching.

## RESULTS

Table 1 shows the subclass patterns of endogenous and newly formed PtdCho and PtdEtn. As in other cells, the endogenous level of ether lipids, particularly the plasmalogens, was enriched in the PtdEtn fraction. [$^3$H]Glycerol was incorporated into the ether classes of diradylglycerol to a similar proportion as into the ether classes of PtdEtn, whereas the labelling of the ether classes of PtdCho was decreased. With prolongation of the incubation time from 10 to 90 min, the proportion of label incorporated into the diacyl subclasses increased in all three lipids. Also in Table 1 the endogenous content and [$^3$H]glycerol labelling of the dipalmitoyl species of diacylglycerol, PtdCho and PtdEtn are shown (for a complete species pattern of type II cell PtdCho, see Schlame *et al.*, 1988). Although both species initially are synthesized to the same extent, after prolonged incubation, however, the proportion of labelled dipalmitoyl-PtdCho increased, whereas that of dipalmitoyl PtdEtn did not. The decrease in the latter proportion actually measured was not statistically significant. We assume that short-time incubation represents primarily synthesis *de novo* and that longer incubation reflects both acyl remodelling and synthesis *de novo*.

The difference in the proportion of label incorporated into dipalmitoyl-PtdCho or -PtdEtn after prolongation of the incubation time might result from a preferred degradation of the PtdEtn species by phospholipase $A_2$. However, 1,2-di[1-$^{14}$C]-palmitoyl-PtdEtn and 1-palmitoyl-2-[1-$^{14}$C]linoleoyl-PtdEtn were hydrolysed at the same rate in type II cell homogenate (results not shown). Another possibility to explain this difference was that the PtdEtn species is a palmitoyl donor in the transacylation of lyso-PtdCho. But no acyl transfer could be measured from 1,2-di[1-$^{14}$C]palmitoyl-PtdEtn and 1-palmitoyl-2-[1-$^{14}$C]-linoleoyl-PtdEtn to lyso-PtdCho when these substrates were incubated with type II cell membranes (results not shown).

**Table 3. Comparison of the species patterns of PtdCho synthesized *de novo* in type II cells and by transacylation of the palmitoyl-lyso-PtdCho in type II cell membranes**

Values from three independent experiments are given as percentages of total label incorporated into the palmitic acid-containing species, with S.D. in parentheses. '*De novo*' refers to labelled species formed by incubation of type II cells with [1(3)-$^3$H]glycerol for 10 min; 'Transacylation' refers to acylation of 1-[1-$^{14}$C]palmitoyl-lyso-PtdCho with or without added CoA by type II cell membranes for 2 or 60 min. * These fractions contained more than one molecular species.

| Fraction of molecular species | *De novo* | Transacylation +CoA 2 min | Transacylation +CoA 60 min | Transacylation −CoA 2 min | Transacylation −CoA 60 min |
|---|---|---|---|---|---|
| $C_{16:0}$-$C_{20:5}$, $C_{18:2}$-$C_{22:6}$, $C_{18:2}$-$C_{20:4}$* | 7.3 (0.6) | 5.2 (1.4) | 10.7 (0.6) | 5.7 (1.8) | 13.1 (2.3) |
| $C_{16:0}$-$C_{16:1}$, $C_{18:2}$-$C_{18:2}$* | 15.8 (2.4) | 12.2 (3.7) | 11.4 (1.4) | 12.4 (1.5) | 9.2 (1.6) |
| $C_{16:0}$-$C_{22:6}$, $C_{16:0}$-$C_{20:4}$* | 14.2 (1.1) | 30.4 (2.6) | 27.6 (3.8) | 33.3 (6.0) | 40.8 (2.9) |
| $C_{16:0}$-$C_{18:2}$ | 8.8 (0.1) | 8.6 (1.0) | 13.5 (1.1) | 7.6 (0.9) | 12.0 (1.3) |
| $C_{16:0}$-$C_{22:5}$, $C_{18:1}$-$C_{18:2}$* | 8.7 (1.1) | 1.1 (0.2) | 5.3 (1.0) | 1.2 (0.1) | 4.9 (0.9) |
| $C_{16:0}$-$C_{20:3}$ | 4.0 (0.5) | 4.9 (1.5) | 3.3 (0.8) | 5.4 (2.0) | 1.1 (0.2) |
| $C_{16:0}$-$C_{16:0}$ | 16.0 (1.5) | 33.4 (1.8) | 26.8 (3.5) | 32.1 (2.3) | 10.2 (2.1) |
| $C_{16:0}$-$C_{18:1}$ | 25.3 (0.7) | 2.4 (0.2) | 4.5 (0.7) | 1.8 (0.8) | 6.4 (0.9) |

**Table 4. Comparison of the species patterns of PtdEtn synthesized *de novo* in type II cells and by transacylation of the 1-[$^{14}$C]palmitoyl-lyso-PtdEtn in type II cell membranes**

Values from three independent experiments are given as percentages of total label incorporated into the palmitic acid-containing species, with S.D. in parentheses. '*De novo*' refers to incorporation of labelled glycerol by type II cells incubated for 10 min; 'Transacylation' refers to acylation of 1-[1-$^{14}$C]palmitoyl-lyso-PtdEtn with or without addition of CoA by type II cell membranes for 2 or 60 min. * These fractions contained more than one molecular species.

| Fraction of molecular species | *De novo* | Transacylation | | | |
|---|---|---|---|---|---|
| | | +CoA | | −CoA | |
| | | 2 min | 60 min | 2 min | 60 min |
| $C_{16:0}$-$C_{20:5}$, $C_{18:2}$-$C_{22:6}$, $C_{18:2}$-$C_{20:4}$* | 6.4 (1.0) | 3.4 (0.7) | 6.5 (0.8) | 4.9 (1.0) | 6.7 (2.0) |
| $C_{16:0}$-$C_{16:1}$, $C_{18:2}$-$C_{18:2}$* | | 3.1 (1.0) | 7.4 (3.4) | 3.2 (0.7) | 4.2 (0.6) |
| $C_{16:0}$-$C_{22:6}$, $C_{16:0}$-$C_{20:4}$* | 27.7 (3.1) | 20.4 (3.5) | 53.8 (6.6) | 10.2 (2.1) | 67.0 (4.0) |
| $C_{16:0}$-$C_{18:2}$ | 11.2 (3.0) | 3.3 (1.2) | 7.4 (1.9) | 2.1 (0.7) | 6.8 (1.0) |
| $C_{16:0}$-$C_{22:5}$, $C_{18:1}$-$C_{18:2}$* | 8.4 (1.8) | 2.0 (0.7) | 5.6 (1.1) | 2.5 (0.8) | 5.8 (0.2) |
| $C_{16:0}$-$C_{20:3}$ | 3.0 (1.0) | 4.8 (0.1) | 3.5 (1.0) | 8.3 (1.2) | 1.8 (0.5) |
| $C_{16:0}$-$C_{16:0}$ | 16.6 (0.8) | 60.1 (3.9) | 12.0 (2.0) | 71.1 (3.0) | 5.8 (2.4) |
| $C_{16:0}$-$C_{18:1}$ | 23.3 (0.6) | 5.9 (1.2) | 5.0 (1.9) | 5.1 (1.6) | 3.4 (0.3) |

However, when endogenous lipids of type II cell membranes served as acyl donors, added [1-$^{14}$C]palmitoyl-2-lyso-PtdCho and -lyso-PtdEtn were acylated, yielding labelled PtdCho or PtdEtn respectively. Table 2 shows that the acylation of both lysophospholipids was increased by addition of CoA, but was not changed by preincubations depleting type II cell membranes by endogenous acyl-CoA or endogenous CoA respectively. Also, the addition of type II cell cytosol did not affect the formation of labelled PtdCho and PtdEtn, and labelled palmitic acid was only incorporated if ATP and CoA were added together for activation of fatty acids. PtdEtn formed by the acylation of labelled lyso-PtdEtn was not converted into PtdCho by $Ca^{2+}$-dependent base exchange, measured as described by Kanfer (1980). Methylation of labelled PtdEtn to PtdCho was not detected, either (results not shown).

In Tables 3 and 4 the distributions of label into the palmitic acid-containing species of PtdCho and PtdEtn formed by the incorporation of labelled glycerol in type II cells (synthesis *de novo*) were compared with the patterns formed by the acylation of [1-$^{14}$C]palmitoyl-lysophospholipids in type II cell membranes (transacylation). Type II cell membranes were found to acylate 1-[1-$^{14}$C]palmitoyl-lyso-PtdCho and -lyso-PtdEtn with a high selectivity for palmitate and arachidonate and a strong discrimination against oleate (Tables 3 and 4). The proportion of labelled dipalmitoyl-PtdEtn formed by cofactor-independent and CoA-mediated transacylation decreased on prolonged incubation from 71 to 5.8% or from 60 to 12% respectively. The proportion of dipalmitoyl-PtdCho formed by cofactor-independent transacylation decreased also from 32% after 2 min to 10% after 60 min incubation. In contrast, the proportion of labelled dipalmitoyl-PtdCho formed by CoA-mediated transacylation decreased only from 33.4 to 26.8% in the same time interval.

Fig. 1 shows the acylation of labelled palmitoyl-lyso-PtdCho and -lyso-PtdEtn by type II cell membranes as function of time. From the percentages of the label proportions of the dipalmitoyl species given in Tables 3 and 4 and the nmol of total PtdCho and PtdEtn formed after 2 and 60 min incubation (Fig. 1), the nmol of dipalmitoyl species of both phospholipids can be calculated.

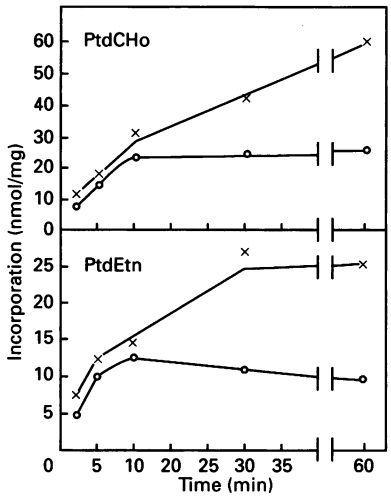


**Fig. 1. Incorporation of 1-[$^{14}$C]palmitoyl-lyso-PtdCho and -lyso-PtdEtn by type II cell membranes as function of time**

Type II cell membranes were incubated without (○) or with (×) addition of CoA (final concn. 15 $\mu$M) as described in the Materials and methods section. Values are given as means of two independent experiments.

Dipalmitoyl-PtdCho formed by cofactor-independent transacylation was nearly constant. It decreased only from 2.4 nmol/mg of protein after 2 min to 2.2 nmol/mg of protein after 60 min incubation. However, dipalmitoyl-PtdEtn formed by this pathway decreased strongly from 3.5 nmol/mg of protein after 2 min to 0.5 nmol/mg of protein after 60 min incubation. Dipalmitoyl-PtdEtn formed by CoA-mediated transacylation

also decreased (from 4.5 to 3.0 nmol/mg of protein). In contrast, dipalmitoyl-PtdCho formed by this transacylation increased greatly, from 3.7 nmol/mg of protein after 2 min to 15.8 nmol/mg of protein after 60 min incubation.

## DISCUSSION

The formation of glycerolipids in pulmonary type II cells starts with the stepwise acylation of Gro-3-*P* and DHAP. There are contradictory reports about the relative contribution of both pathways in lung (Fisher *et al.*, 1976; Schlossman & Bell, 1977; Wykle *et al.*, 1977; Akino & Abe, 1977; Mason, 1978). DHAP acylation starts in most tissues in peroxisomes and is primarily included in the ether-lipid synthesis. How much labelled glycerol is incorporated into ether lipids of type II cells is not known. Pulmonary type II cells, however, are rich in peroxisomes (Schneeberger, 1972; Hirai *et al.*,1983) and therefore a high rate of ether lipid formation can be expected. Although a direct comparison of diacyl- and ether-lipid synthesis with [1(3)-$^3$H]glycerol as precursor is not possible, since one *sn*-1 H atom is lost during the ether-bond formation (Friedberg & Heifetz, 1973), a high synthesis rate of ether lipids in pulmonary type II cells can be derived from the data of Table 1. Therefore the relative contributions of Gro-3-*P* and DHAP acylation for the synthesis of dipalmitoyl-PtdCho cannot be calculated correctly from the incorporation of these precursors into the total phospholipid fraction. The formation of ether lipids via the DHAP path should be taken into account. The large amount of glycerol-labelled alkenylacylglycerol, determined after incubation for 10 min, indicates either a rapid degradation of choline and/or ethanolamine plasmalogens by phospholipase C and/or by the combined action of phospholipase D and phosphatidate phosphatase in pulmonary type II cells.

The incorporation of labelled glycerol into dipalmitoyl-PtdCho and -PtdEtn was nearly identical after short-time incubation. On the one hand, dipalmitoyl-PtdEtn was not formed from endogenous diacylglycerol (Post *et al.*, 1983, Rüstow & Kunze, 1984). Labelled glycerol, on the other hand, was incorporated into dipalmitoyl-PtdEtn. These results suggest that endogenous and newly formed diacylglycerols represent different substrate pools. Glycerol-labelled dipalmitoyl-PtdEtn did not change significantly with prolongation of the incubation time, whereas dipalmitoyl-PtdCho increased from about 11 to 18%.

The reason for this enrichment of glycerol-labelled dipalmitoyl-PtdCho seems to be the additional formation of this species, e.g. by remodelling. Remodelling includes the action of phospholipase $A_2$ and the reacylation of the lyso-compound thus produced by lyso-PtdCho acyltransferase, which can utilize palmitoyl-CoA as a substrate (Crecelius & Longmore, 1984*a*). Studies in whole lung tissue suggested an interesting alternative for the cleavage of unsaturated acyl residues by phospholipase $A_2$. Stymne & Stobart (1985) reported a lyso-PtdCho acyltransferase activity in lung microsomes operating in a backward direction. This reaction is stimulated by free CoA, and the resulting lyso-PtdCho may then be reacylated by the lyso-PtdCho acyltransferase operating in the forward direction.

Type II cell membranes acylate 1-[1-$^{14}$C]palmitoyl-lyso-PtdCho and -lyso-PtdEtn, yielding palmitoyl-labelled PtdCho or PtdEtn respectively. Preincubation of type II cell membranes, depleted of endogenous CoA and endogenous acyl-CoA, did not change the formation of PtdCho or PtdEtn. Palmitic acid was incorporated only when ATP and CoA were available for fatty acid activation (Table 2). In contrast with the results of Nijssen & van den Bosch (1986*a*), who used microsomes and cytosol from total lung, addition of type II cell cytosol to type II cell membranes did not affect the quantity of PtdCho and PtdEtn formed by acylation of the corresponding lyso compounds (Table 2). On the contrary, the addition of CoA increased the rate of acylation of both acyl-lysophospholipids. From these results we assume that in type II cells there operates a cofactor-independent acylation, representing the transacylation as defined by Kramer & Deykin (1983). The CoA-mediated transacylation which occurs also in type II cells represents acyl exchange caused by the backward reaction of the acyl-CoA:lyso-PtdCho or -PtdEtn acyltransferase (Stymne & Stobart, 1985).

The species patterns of PtdCho and PtdEtn formed by the cofactor-independent transacylation of the corresponding lysophospholipids showed a high selectivity for palmitate and arachidonate and a strong discrimination against oleate (Tables 3 and 4). Longer incubation (60 min) resulted in a drastic decrease in the proportion of labelled dipalmitoyl-PtdCho and -PtdEtn, with a corresponding increase in the polyunsaturated-fatty-acid-containing species. CoA-mediated transacylation also showed a high selectivity for palmitate and arachidonate and a strong discrimination against oleate. However, longer incubation caused in this case a different change in the species patterns of PtdCho and PtdEtn. Dipalmitoyl-PtdEtn decreased greatly with prolonged incubation time and represents a minor component only, whereas dipalmitoyl-PtdCho decreased only slowly and remained a major component. The decrease in the relative proportion of dipalmitoyl-PtdCho and -PtdEtn with prolonged incubation time might be caused by a preferential degradation or by depletion of the acyl-donor pool for palmitate so that mainly polyunsaturated species will be formed. In the latter case the nmol of dipalmitoyl species formed should be the same after 2 or 60 min incubation. Calculation of the nmol of dipalmitoyl-PtdEtn formed by both transacylation activities shows that they decrease strongly with prolonged incubation time. These results support the idea that the dipalmitoyl species of PtdEtn formed are preferentially degraded. This idea corresponds to the very low level of the dipalmitoyl species measured in the PtdEtn fraction of type II cells (Table 1). In contrast, the nmol of dipalmitoyl-PtdCho formed by cofactor-independent transacylation did not change significantly, and the amount of dipalmitoyl-PtdCho formed by CoA-mediated transacylation increased from 3.7 to 15.8 nmol/mg of protein in the same time interval. This means that CoA-mediated transacylation may be included in the formation of the high level of palmitoyl-PtdCho in pulmonary type II cells.

The very high proportion of dipalmitoyl-PtdEtn formed by both transacylation activities after short-time incubation is quite unexpected. It might be possible that this formation represents an effect *in vitro* caused by the relatively high concentration of the lyso compound. The reason for the different fates of dipalmitoyl-PtdEtn and -PtdCho formed by transacylations is unknown. We measured, as mentioned above, on the one hand degradation (dipalmitoyl-PtdEtn) in each case and on the other hand a very small degradation or a strong increase (dipalmitoyl-PtdCho) with prolonged incubation time. These results correspond to the content of this species measured in the endogenous PtdEtn and PtdCho fraction of type II cells (Table 1).

However, our results also demonstrate that neither the species patterns synthesized *de novo* nor those formed by CoA-mediated and cofactor-independent transacylation resemble the species patterns of endogenous PtdCho and PtdEtn of type II cells. Therefore it has to be assumed that in the maturation of the species patterns of type II cell lipids different pathways are simultaneously active. Consequently their balanced participation in forming dipalmitoyl-PtdCho cannot be measured directly. The analysis of individual pathways tells us whether they should be included in this process. Quantification of the participation of

individual pathways in the formation of the endogenous species patterns, if at all possible, can only be a rough approximation.

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