LUNG PHOSPHOLIPIDS I. IN VIVO STUDIES OF THE INCORPORATION OF ^{32}P, [METHYL-^{14}C] CHOLINE, 1-^{14}C-PALMITIC ACID AND 1-^{14}C-OLEIC ACID INTO PHOSPHATIDYLETHANOLAMINE, PHOSPHATIDYL-N,N-DIMETHYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE

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SUMMARY: ^{32}P and 1-^{14}C-palmitate were rapidly incorporated into lung phosphatidyl-N,N-dimethylethanolamine (PDME). The half-life of the PDME based on the decay of 1-^{14}C-Palmitate was about 5 hrs. A greater half-life (>20 hrs.) occurred with PDME labelled with 1-^{14}C-oleate. No evidence was found for the conversion of phosphatidylcholine to PDME or the recycling of phosphatidylcholine N-methyl groups to PDME. In light also of the known negligible contribution of methionine methyl transfer in the biosynthesis of dipalmitoylphosphatidylcholine, which is synthesized mainly by the CDP-choline mechanism, lung PDME should not be viewed primarly as an intermediate in the successive N-methylation of phosphatidylethanolamine. Rather, it appears to be an important biosynthetic product.

Numerous studies have indicated that an important metabolic function of pulmonary parenchyma in mammals is the synthesis and secretion of surface-active lipoprotein (surfactant) essential for alveolar patency during respiration (1-9). A quantitatively important component of the surfactant is dipalmitoyl-phosphatidylcholine (dipalmitoyl-PC) (10). A rapid rate of phospholipid synthesis in lung appears to be vital for continuous replenishment of surfactant at the alveolar lining (11-14).

The cytidinediphospho (CDP)-choline mechanism (15) is the dominant de novo pathway for the synthesis of PC in adult lung. A minor de novo pathway is the N-methylation of phosphatidylethanolamine (PE) (16-18) by the stepwise transfer of three methyl groups from S-adenosylmethionine (19-20). The presence of phosphatidyl-N,N-dimethylethanolamine (PDME) in lung is evidence that the latter pathway does occur. The ratios of PE:PDME:PC in extracts of whole rat lung are

1.5:1:10(21). The PDME and PC in alveolar* lipids both are predominatly esterified with palmitate and both markedly lower surface tension at an air-liquid interface (22).

This communication reports the in vivo time-course of uptake of radioactive precursors into lung PE, PDME and PC in order to further elucidate the pathways for the biosynthesis and degradation of surfactant phospholipids.

MATERIALS AND METHODS

^{32}P-Na_2HPO_4(50.6mCi/mmole), $^{14}CH_3$-choline (61mCi/mmole), 1-^{14}C-palmitic acid (55.2mCi/mmole) and 1-^{14}C-oleic acid (62mCi/mmole) were purchased from Amersham/Searle Corp. Male Sprague-Dawley rats (250-350g) were maintained on standard lab chow. All injections were made into the tail vein. Fatty acids were administered bound to fatty acid free bovine albumin (Pentex Chemical Co.).

Experiments were performed at least twice for each isotope. For most time periods, the pooled tissue of two rats was used. Rats were decapitated 5 min. to 20 hr. after administration of the isotopic compound. Lungs were excised and washed with chilled saline and then homogenized in 20 ml of 2:1 (v/v) $CHCl_3$:CH_3OH in a VirTis "45" homogenizer. The homogenate was added to 100 ml more of the $CHCl_3$:CH_3OH containing 0.1% butylated hydroxytoluene (antioxidant) and lipids were extracted and washed according to the method of Folch et. al (23). Following evaporation of the solvent the lipid residue was dissolved in a small volume of the $CHCl_3$-CH_3OH and stored overnight under N_2 at -20°C. An aliquot was used for the determination of total phospholipid phosphorous (24). Phospholipids were separated on thin-layers of silica gel H containing 1mM-Na_2CO_3 using $CHCl_3$:CH_3OH:CH_3COOH:H_2O (100:60:16:8) (25). Separation of PDME from PE was made on Adsorbosil-5 (Applied Science Lab.) thin-layer plates using $CHCl_3$:CH_3OH:CH_3COOH:H_2O (95:35:4:3) as the solvent. Lipid standards were purchased from Applied Science Lab. and Mann Research Lab. Synthetic L-dipalmitoyl-α glycerylphosphoryl-N,N-dimethylethanolamine was used as the chromatographic

*Alveolar lipids are represented by those lipids found in the fluid collected from endobronchial saline lavage after subsequent centrifugation to remove debris.

standard for PDME. All chromatograms were sprayed with 0.02% aqueous Rhodamine 6G and viewed under u.v. light. Successive 4 mm zones were automatically scraped into scintillation vials with the Snyder-Kimble scraper (Analabs, Inc.) and counted in the solvent used by Wood and Healy (26) in a Beckman LS-250 liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Time-course of incorporation of ^{32}P into lung PE and PDME is shown in Fig. 1A. At 1 hr. nearly all radioactivity is in PDME. Between 1 and 8 hr. incorporation into PDME was essentially constant, though it was much slower than the initial uptake observed between 5 min. and 1 hr. Between 8 and 20 hr. the level of activity of PDME decreased, while that of PE continued to increase. If the conversion of PE to PDME occurs *via* a two-step methylation of PE, Fig. 1A suggests that a pool of PE in the lung is rapidly N-methylated. In a study using ^{32}P incubated with lung slices Weinhold and Villee (27) reported that ^{32}P uptake into PE was slow. An unknown material, which had the same chromatographic characteristics as PDME shown herein, incorporated isotope very rapidly.

1-^{14}C-Palmitate is incorporated into PE and PDME very rapidly, as shown in Fig. 1B. Maximum level of radioactivity of either phospholipid was attained within 5 min. Noteworthy is the fact that by 20 hrs. nearly all the activity in PDME was gone. The half-life of palmitate in PDME in the present study is estimated (semi-log plot of cpm vs time) to be about 5 hrs. The overall turnover of palmitate in PE is much slower as judged by the relatively high ^{14}C-PE to ^{14}C-PDME ratio after 20 hrs. Similarly, the incubation of lung slices with 1-^{14}C-palmitate was shown by Naimark and Klass (21) to yield relatively high specific activity in both PE and PDME. Scarpelli (9) showed that activity was found in alveolar phospholipids within 5 min. after injection of labelled palmitate. In Scarpelli's study (9) activity was preferentially incorporated into those phospholipids which migrated with "PE", presumably PE plus PDME as judged by the trailing peak overlapping PE. The "PE"

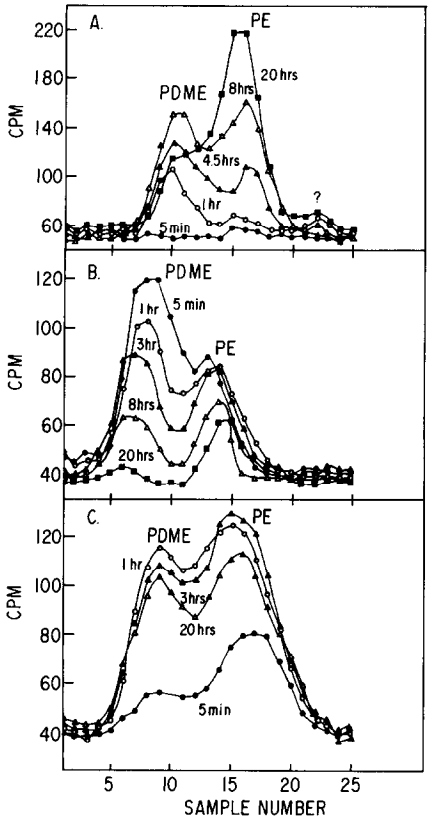

Fig. 1. Time-course distribution in vivo of ^{32}P ^{14}C activity into lung phosphatidyl-N,N-dimethylethanolamine (PDME) and phosphatidylethanolamine (PE). A, ^{32}P; B, 1-^{14}C-palmitic acid; C, 1-^{14}C-oleic acid. For each time period 30 μg. (A,B) or 60 μg. (C) of total lung phospholipid phosphorous was applied to thin-layer chromatograms. In A, ^{32}P was also present in an unidentified component, possibly phosphatidic acid, with a slightly greater R_f than PE

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fraction of Scarpelli turned over at a rate similar to that of (^{14}C-palmitate)-PDME in the present study. The work of others showed that in agreement with the appearance of labelled PDME in the alveolar lining (9), incubation of the alveolar lavage fraction with $^{14}CH_3$-S-adenosyl-L-methionine and co-factors yielded isotope-labelled phospholipids (7). The rapid preferential uptake of palmitate into PE and PDME reported in Fig. 1B is suggestive of a pool of PE that is rapidly acylated. Although these experiments are complicated by the heteogeneity of phospholipid pools, 1-^{14}C-palmitate could be delineating a pool of a disaturated-PE which is N-methylated to form a corresponding acyl species of PDME.

Uptake of 1-^{14}C-oleate was different than that seen with ^{32}P or 1-^{14}C-palmitate (Fig. 1C). At each time, more activity was present in PE than in PDME and little decay of radioactivity occurred with each of the compounds in 20 hrs.

The time-course of incorporation of four precursors into PC is shown in Table 1. 1-^{14}C-Palmitate and $^{14}CH_3$-choline are rapidly turned over in PC. No $^{14}CH_3$-choline activity was observed in PE and PDME within 20 hrs. The latter finding supports an earlier report (28) which showed that recycling of PC methyl groups to PDME and direct conversion of PC to PDME is of little quantitative significance in the lung.

When the *in vivo* incorporation of $^{14}CH_3$-L-methionine into the major molecular species of lung PC was measured, the dipalmitoyl-PC had the lowest specific

Table 1

Incorporation *in vivo* of ^{32}P, [methyl-^{14}C] choline, 1-^{14}C-palmitate and 1-^{14}C-oleate into lung phosphatidylcholine.

Values are expressed in cpm/μg phosphatidylcholine phosphorous. Rats were given an intravenous injection of 100μCi ^{32}Pi, 20μCi ^{14}C-palmitate, 20μCi ^{14}C-oleate or 20μCi of ^{14}C-choline. For the isotope and time period in this table corresponding with that of Fig. 1, the same source of pooled lung tissue was used.

Time After Administration	PRECURSOR			
	^{32}P	$^{14}CH_3$-Choline	1-^{14}C-Palmitate	1-^{14}C-Oleate
5 min.	n.d.*	444	197	42
1 hr.	54	1313	253	81
3 "	—	1649	228	84
4.5 "	141	—	—	—
8 "	184	—	138	—
20 "	190	831	53	67

* not detectable

activity (29). The quantitative significance of a saturated fatty acid species of PDME as a precursor to dipalmitoyl-PC, therefore, is doubtful. However, a dipalmitoyl-PDME might be able to serve as an "acyl donor" for the synthesis of dipalmitoyl-PC or of other lipids. Moderately high levels of phospholipase-A(EC3.1.1.4) (30) and a lysophosphatide-acylating system (31-32) are found in the lung. In vivo evidence for phospholipase A activity was found in the present study; a chromatogram of lung phospholipids in rats sacrificed 5 min. after in vivo labelling with $^{14}CH_3$-choline yielded significant isotope uptake into lysophosphatidylcholine. A disaturated-PDME may be degraded at the alveolar lining where phospholipases in alveolar macrophages (33) could conceivably account for the relatively rapid turnover of surfactant phospholipids.

Investigation of the role of the acellular alveolar lining and of macrophages could add a further understanding of the synthesis and degradation of the surfactant phospholipids.

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