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Incorporation of [^{3}H]palmitate and [^{14}C]choline into disaturated phosphatidylcholines in rat alveolar macrophages

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We studied the synthesis of disaturated phosphatidylcholines in rat alveolar macrophages and, in some cases, compared it with that which occurs in isolated alveolar type II cells. Alveolar macrophages suspended in phosphate-buffered medium incorporate palmitate, choline and glycerol into disaturated phosphatidylcholines. The time-course for incorporation of palmitate into disaturated phosphatidylcholines is linear for 20–30 min and reaches a maximum in 2–3 h. Incorporation is dependent on extracellular palmitate with a V_{max} (at 1 mM) of 1.53 nmol palmitate incorporated into disaturated phosphatidylcholines per 5×10^5 cells per 2 h and a $K_{1/2}$ of 0.19 mM palmitate. Exposure of the cells to zymosan particles increases incorporation of palmitate disaturated phosphatidylcholines by almost 2-fold, while cholinergic and β-adrenergic agonists have no effect. On a per cell basis, alveolar macrophages incorporate only one-third to one-half as much palmitate into disaturated phosphatidylcholines as do type II cells isolated by centrifugal elutriation. The following results suggest there is extensive remodeling of disaturated phosphatidylcholines in alveolar macrophages: (1) palmitate- and choline-labeled disaturated phosphatidylcholines are catabolized by the cells; (2) the products of catabolism are palmitate and water-soluble choline products; (3) addition of unlabeled palmitate and choline to the medium enhances catabolism of the labeled phospholipid. Addition of oleate also enhances catabolism, suggesting that modification of phospholipids is not specific for the saturated variety. Some of the recently labeled disaturated phosphatidylcholines is released from alveolar macrophages into the extracellular space. Several possible functions of alveolar macrophage disaturated phosphatidylcholines are discussed.

Introduction

Pulmonary surfactant materials are a group of substances which serve to reduce surface tension at the air-liquid interface in the lungs and, thus, prevent alveolar collapse. The component of surfactant which is present in the greatest amount and which is the major surface active material is dipalmitoylphosphatidylcholine [1,2]. It is now generally accepted that the synthesis of pulmonary surfactant occurs primarily in alveolar type II cells. Some investigators have suggested that alveolar macrophages play some role in the removal, degradation, and/or reutilization of these materials [3–6]. However, alveolar macrophages have not been generally considered to be a site for significant amounts of surfactant synthesis.

It has been known for some time that di-

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palmitoylphosphatidylcholine is synthesized by alveolar macrophages [7–10]. In addition, several investigators have provided evidence for phospholipase A activity in alveolar macrophages and some have suggested a role for these cells in surfactant catabolism [7,9,11]. These processes have not been systematically investigated in these cells. In particular, their role in surfactant synthesis, clearance, and/or reutilization is not known. The objectives of this investigation were: (1) to characterize incorporation of[^{3}H]palmitate into disaturated phosphatidylcholines in alveolar macrophages, (2) to study factors which may affect this process, and (3) whenever possible, to compare disaturated phosphatidylcholine synthesis in these cells with that which occurs in alveolar type II cells isolated by centrifugal elutriation. A preliminary report of these results has appeared previously [12].

Methods

Isolation of alveolar macrophages. Alveolar macrophages were harvested from male Sprague-Dawley rats (200–300 g) according to the method of Myrvik et al. [13]. The animals were anesthetized with sodium pentobarbital (0.2 g/kg body weight) and exsanguinated by cutting the abdominal aorta. Pulmonary lavage was accomplished by washing the lungs from each animal 12 times in situ with a total volume of 80 ml of ice-cold, Ca^{2+}-free buffered medium (145 mM NaCl/5 mM KCl/9.35 mM Na_2HPO_4/1.9 mM NaH_2PO_4/and 5 mM glucose, pH 7.4). The cells were separated from the lavage fluid by centrifugation at 500 × *g* for 5 min. The alveolar macrophages from several animals were then pooled and washed twice by alternate centrifugation and resuspension in phosphate-buffered medium (same medium as above with 1.8 mM $CaCl_2$/1.0 mM $MgCl_2$). After the alveolar macrophages were washed, the cells were resuspended in phosphate-buffered medium for use in all experiments. The total number of cells in the suspension was determined by using a Coulter Model Z_B electronic cell counter (Coulter Instrument Co., Hialeah, FL). The cells used in these experiments were greater than 98% alveolar macrophages as identified by light microscopy. The contaminating cell types were leukocytes and erythrocytes. No alveolar type II cells were present in the lavage fluid.

Incubation of cells and preparation of cells for analysis. Incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines was determined by incubating alveolar macrophages in medium containing various concentrations of palmitic acid with trace amounts of [^{3}H]palmitate and measuring the amount of radioactivity in the disaturated phosphatidylcholines which were produced. After isolation of the alveolar macrophages as described above, the cells were suspended in phosphate-buffered medium containing an appropriate amount of palmitate. The palmitic acid (Sigma Chemical Co., St. Louis, MO) was complexed with bovine serum albumin in a molar ratio of 5.3 : 1 (fatty acid/bovine serum albumin) as described by Hendry and Possmayer [14] prior to addition to the incubation medium. In most experiments the number of cells used was $1 \cdot 10^6$ cells per ml. At the start of the experiment, 10^{-4} μmol of [9,10-^{3}H]palmitic acid (specific activity, 11.8 Ci/mmol; New England Nuclear Corp.; Boston, MA) was added to each sample and the cell suspensions were incubated at 37°C for varying lengths of time. Following the incubation period, a 0.5 ml aliquot of the cell suspension was mixed well with 10 ml of chloroform/methanol (2 : 1, v/v). 1 mg of mixed lipids isolated from rat lungs was added to each sample of cell suspension and these samples were saved for analysis. In some experiments incorporation of choline or glycerol into disaturated phosphatidylcholines was measured by adding 1 μCi [*methyl*-^{3}H]choline chloride (specific activity, 80 Ci/mmol; New England Nuclear) or 1 μCi [1,3-^{14}C]glycerol, (specific activity, 55.1 mCi/mmol; New England Nuclear) to the incubation medium.

In one series of experiments we studied the effects of foreign particles and chemical mediators on disaturated phosphatidylcholine synthesis. Zymosan, an extract from the cell walls of *Saccharomyces cerevisiae* yeast (Sigma), was used as the foreign particle. It was prepared by boiling 50 mg in 3 ml of phosphate-buffered medium for 15 min. Then the zymosan was separated from the medium by centrifugation, and a stock suspension was prepared by resuspending the pellet in fresh phosphate-buffered medium. This procedure

seemed to prevent the zymosan particles from sticking together. Acetyl-β-methylcholine chloride and N^6,$O^{2'}$-dibutyryladenosine 3′ : 5′-cyclic monophosphate were obtained from Sigma. Terbutaline sulfate was obtained from Astra Pharmaceutical Products, Inc. (Worcester, MA). For these experiments the cells were incubated in phosphate-buffered medium (containing 0.1 mM palmitate) containing either zymosan (final conc. 5 mg/ml) or drug (final conc. 10^{-4} M).

Isolation of disaturated phosphatidylcholines and determination of radioactivity. Disaturated phosphatidylcholines were isolated from the samples, which were prepared as described above, according to the method of Mason et al. [15]. Briefly, total lipids were extracted with chloroform/methanol (2 : 1, v/v), the solvent was evaporated, and the lipids were reacted with osmium tetroxide dissolved in carbon tetrachloride. Following evaporation of the CCl_4, the samples were dissolved in chloroform/methanol (20 : 1, v/v) and placed on a column of neutral alumina (100–200 mesh, Bio-rad Laboratories, Richmond, CA). The disaturated phosphatidylcholines were eluted from the columns with chloroform/methanol/7 M ammonium hydroxide (70 : 30 : 2, v/v). After evaporation of the solvent, 10 ml of Aquasol (New England Nuclear) was added to each sample of disaturated phosphatidylcholines and the samples were counted in the tritium channel of a liquid scintillation spectrometer (Model 3380, Packard Instrument Co., Downers Grove, IL). With this isolation procedure we were able to obtain greater than 98% recovery of [^{14}C]dipalmitoylphosphatidylcholine. Furthermore, we established that the amount of [^{3}H]palmitate recovered in the disaturated phosphatidylcholine fraction which was not incorporated into disaturated phosphatidylcholines was less than 0.5% of that added initially. The results of our experiments are expressed as nmol of palmitate or choline incorporated into disaturated phosphatidylcholines. In some experiments we identified phospholipids by using thin-layer chromatography [16].

Phosphorus determinations. The amount of phosphorus in lipids extracted from various samples of pulmonary lavage fluid and alveolar macrophages was determined by the method of Bartlett [17]. All steps were carried out using disposable glassware. Briefly, following extraction of the lipids with chloroform/methanol (2 : 1, v/v), each sample was evaporated to dryness and 1.65 M H_2SO_4 (1.5 ml) was added. The samples were heated at 150–160°C for 3 h, 30% H_2O_2 (three drops) was added, and then the samples were maintained at 150°C for an additional 90 min. Ammonium molybdate (4.6 ml of a 0.22% solution) and Fiske-SubbaRow reagent (0.2 ml) were added to each sample and they were heated at 100°C for 7 min. Phosphorus was determined by reading the optical densities at 830 nm against standards. In order to obtain phospholipid content, lipid phosphorus values were multiplied by 25 [18].

Results

Time-course and substrate dependence

Time-courses for incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines in alveolar macrophages were determined at two different levels of extracellular palmitate. The results are shown in Fig. 1. At both low (0.1 mM) and high (1.0 mM) external levels of palmitate, incorporation increases rapidly for approx. 30 min and then

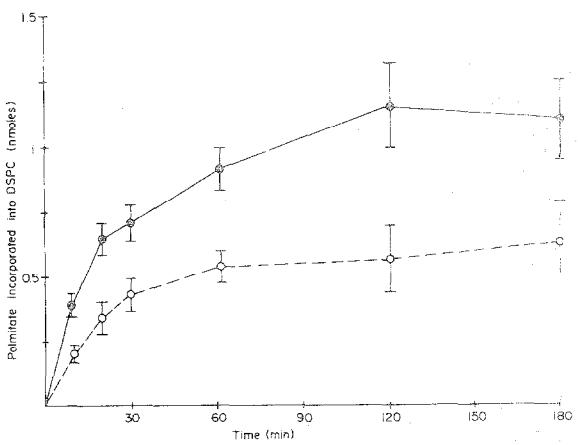

Fig. 1. Time-courses for incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines (DSPC) in alveolar macrophages suspended in phosphate-buffered medium and two different concentrations of palmitate (1.0 mM palmitate (●———●); 0.1 mM palmitate (○--○). Incorporation was measured in samples (0.5 ml total volume) which contained $5 \cdot 10^5$ cells. The points are mean values for five experiments and the bars represent the standard errors of the means.

increases more slowly. After 2–3 h of incubation, incorporation seems to be maximal. These results differ from those obtained with isolated alveolar type II cells [19] in which incorporation occurs to a greater extent but where the time required for maximal incorporation is longer. For example, at 1.0 mM extracellular palmitate, the process is not complete for 4–5 hours in type II cells.

The dependence of incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines on the external palmitate concentration is shown in Fig. 2. The measurements were made following an incubation period of 2 h. The relationship between extracellular palmitate and incorporation displays saturation kinetics. Maximal incorporation and the $K_{1/2}$ value (the external palmitate level at which one-half maximal incorporation occurs), obtained from a double-reciprocal plot, are 1.53 nmol/$5 \cdot 10^5$ cells per 2 h and 0.19 mM, respectively. When these experiments were performed with type II cells [19], the curve was the same general shape but the $K_{1/2}$ value was less, i.e., 0.10 mM.

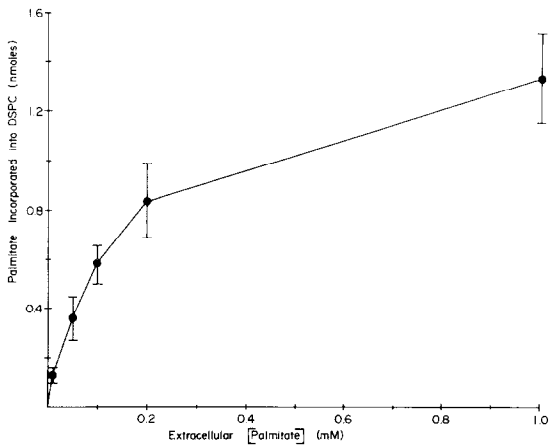

Fig. 2. Incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines (DSPC) in alveolar macrophages as a function of the extracellular palmitate concentration. The extracellular fluid consisted of phosphate-buffered medium and varying amounts of palmitate. The measurements were made from samples of cell suspension (0.5 ml total volume) which contained $5 \cdot 10^5$ cells after an incubation period of 2 h. The points are mean values for six experiments and the bars represent the standard errors of the means.

Magnitude of incorporation

The amount of palmitate incorporated into disaturated phosphatidylcholines in alveolar macrophages was measured at various times and compared with that which occurs in type II cells isolated by centrifugal elutriation [19]. The results are shown in Table I. These experiments were done with an extracellular concentration of 1 mM palmitate, i.e., a level which produces maximal incorporation in both cell types. There is not much difference between the two cell types in the amount of incorporation which occurs during the first 30 min of incubation; i.e., the initial rates of incorporation are similar in the two cell types. However, in type II cells incorporation is maintained for a longer period of time. Therefore, over longer incubation periods, the type II cells incorporate more palmitate into disaturated phosphatidylcholines. After 5 h of incubation, a time during which maximal synthesis occurs in both cell types, the amount of incorporation in type II cells is 2–3-times greater than that in alveolar macrophages on a per cell basis.

We also measured the incorporation of choline (0.05 nM) and glycerol (0.1 mM) into disaturated phosphatidylcholines. These data are not presented. In alveolar macrophages the amount of palmitate incorporated into disaturated phosphatidylcholines is approx. 10-fold and 500-fold greater than the incorporation of choline and

TABLE I

INCORPORATION OF [^{3}H]PALMITATE INTO DISATURATED PHOSPHATIDYLCHOLINES (DSPC) IN ALVEOLAR MACROPHAGES AND TYPE II CELLS

Experiments were performed with the cells suspended in phosphate-buffered medium containing 1.0 mM palmitate. Type II cells were isolated via centrifugal elutriation [19]. Measurements were made from samples of cell suspension (0.5 ml total volume) which contained $5 \cdot 10^5$ cells. The numbers shown are mean values for five experiments ± S.E.

Time (h)	Palmitate incorporated into DSPC (nmol/$5 \cdot 10^5$ cells)	
	Alveolar macrophages	Type II cells
0.5	0.71 (±0.07)	1.00 (±0.13)
1.0	0.92 (±0.08)	1.54 (±0.15)
2.0	1.16 (±0.16)	2.32 (±0.27)
5.0	1.33 (±0.18)	3.50 (±0.33)

glycerol, respectively. These relative magnitudes of incorporation are similar to those which occur in alveolar type II cells maintained in primary cell culture [20]. The fact that glycerol is incorporated into disaturated phosphatidylcholines suggests that de novo synthesis of disaturated phosphatidylcholines occurs in alveolar macrophages. However, these data do not rule out the possibility that unsaturated phosphatidylcholines are synthesized from glycerol and then rapidly converted to disaturated phosphatidylcholines.

Factors which may affect incorporation of palmitate into disaturated phosphatidylcholines

We determined the effects of exposure to foreign particles and chemical mediators on incorporation of palmitate into disaturated phosphatidylcholines. The results are shown in Table II. In order to determine the effects of foreign particles on disaturated phosphatidylcholine synthesis in alveolar macrophages, the cells were exposed to zymosan. The amount of zymosan used causes a maximal release of superoxide anion from the cells (data not reported here). Exposure to these particles produces a 90% stimulation of palmitate incorporation into disaturated phosphatidylcholines. This result has been reported previously by Elsbach [8]. The effects of cholinergic and β-adrenergic stimulation on disaturated phosphatidylcholine synthesis were also studied. Neither methacholine, terbutaline, nor dibutyryl cyclic AMP (10^{-4} M) has any effect on this process. Therefore, these results indicate that zymosan stimulates incorporation of palmitate into disaturated phosphatidylcholines, while cholinergic and β-adrenergic agonists have no effect.

TABLE II

EFFECTS OF EXPOSURE TO FOREIGN PARTICLES AND CHEMICAL MEDIATORS ON INCORPORATION OF [^{3}H]PALMITATE INTO DISATURATED PHOSPHATIDYLCHOLINES (DSPC) IN ALVEOLAR MACROPHAGES

Experiments were performed with the cells suspended in phosphate-buffered medium containing 0.1 mM palmitate. The amounts of materials listed in the left hand column are: zymosan, 5 mg/ml; methacholine, terbutaline and dibutyryl cyclic AMP, 0.1 mM. Measurements were made from samples of cell suspension (0.5 ml total volume) which contained $5 \cdot 10^5$ cells after an incubation period of 2 h. The control value is 0.53 ($\pm$0.08) nmol of palmitate incorporated into disaturated phosphatidylcholines. The numbers shown are mean values for five experiments $\pm$S.E.

Treatment	Palmitate incorporated into DSPC (% control)
Control	100
Zymosan	188 ($\pm$15)
Methacholine	118 ($\pm$14)
Terbutaline	97 ($\pm$18)
Dibutyryl cyclic AMP	101 ($\pm$9)

Fate of intracellular labeled disaturated phosphatidylcholines

In this paper we have shown that at high exter-

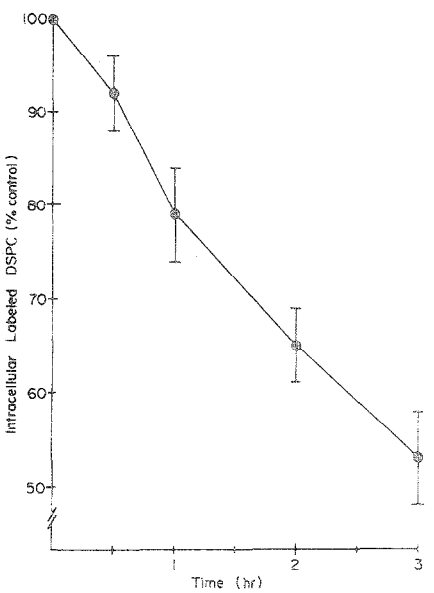

Fig. 3. Time-course for the disappearance of intracellular labeled disaturated phosphatidylcholines (DSPC). Alveolar macrophages were incubated in phosphate-buffered medium containing [^{3}H]palmitate (total palmitate 1 mM) for 2 h. Cells were then spun down, washed three times and resuspended in palmitate-free medium. The amounts of labeled disaturated phosphatidylcholines in the intra- and extracellular compartments were measured over a 3-h incubation period. The labeled disaturated phosphatidylcholines in the medium were 15% of the total at zero time and declined during the incubation period. The intracellular disaturated phosphatidylcholines shown above are expressed as a percent of that present at zero time. Measurements were made from samples of cell suspension (0.5 ml) containing $5 \cdot 10^5$ cells. The points are mean values for five experiments and the bars represent the standard errors of the means.

nal levels of palmitate (1 mM) incorporation is not complete for 2–3 h. The following experiments were performed in order to determine the fate of [^{3}H]palmitate-labeled disaturated phosphatidylcholines after 2 h of incorporation in vitro. The results are shown in Fig. 3. Alveolar macrophages were incubated with [^{3}H]palmitate for 2 h, centrifuged, washed free of palmitate, and resuspended in palmitate-free medium. The amount of [^{3}H]palmitate in disaturated phosphatidylcholines was measured in the intra- and extracellular compartments over an additional 3-h period. The labeled disaturated phosphatidylcholines in the medium were 15% of the total at zero time and declined during the incubation period. The intracellular labeled disaturated phosphatidylcholines diminish in an almost linear fashion with time, such that only 50–60% of the initial amount is present after 3 h of incubation in palmitate-free medium. The disappearance of disaturated phosphatidylcholines is greatly slowed if the cells are maintained at 2°C (Table III), suggesting that an enzymatic process(es) is involved.

In a separate set of experiments we followed the disappearance of disaturated phosphatidylcholines labeled with both [^{3}H]palmitate and [^{14}C]choline. The labeling occurred during a 2-h period of incorporation and the results are shown in Table III. The choline-labeled disaturated phosphatidylcholines become diminished to the same extent as that labeled with palmitate. Addition of unlabeled palmitate to he medium enhances the disappearance of both palmitate- and choline-labeled disaturated phosphatidylcholines, probably due to enhanced exchange (deacylation-reacylation). A similar result is obtained if oleate is included in the incubation medium (data not presented). Addition of unlabeled choline to the medium produces a slight increase in the rate of disappearance of choline-labeled disaturated phosphatidylcholines but has no effect on the palmitate-labeled phospholipid.

Experiments were performed to identify the products of catabolism of disaturated phosphatidylcholines and the results are shown in Table IV. Virtually all of the [^{3}H]palmitate label in disaturated phosphatidylcholines appears as free

TABLE III

CATABOLISM OF INTRACELLULAR LABELED DISATURATED PHOSPHATIDYLCHOLINES (DSPC) IN ALVEOLAR MACROPHAGES

Cells were incubated in phosphate-buffered medium containing 1 mM palmitate and 0.05 mM choline for 2 h. In one group of cells, the radioactive label was [^{3}H]palmitate and in the other the label was [^{14}C]choline. Alveolar macrophages were then spun down, washed three times and resuspended in phosphate-buffered medium with and without palmitate and choline (no radioactive label was present). The intracellular labeled disaturated phosphatidylcholines which were present after a 3 h incubation are shown above as a percent of that present at zero time. The numbers shown are mean values for five experiments ±S.E.

Treatment	Intracellular labeled DSPC after 3-h incubation (% zero time)	
	Palmitate label	Choline label
Palmitate-free medium (37°C)	52 (±5)	55 (±1)
1 mM palmitate (37°C)	21 (±2)	22 (±2)
0.05 mM choline (37°C)	55 (±5)	34 (±2)
Palmitate-free medium (2°C)	92 (±3)	–

TABLE IV

PRODUCTS OF CATABOLISM OF INTRACELLULAR LABELED DISATURATED PHOSPHATIDYLCHOLINES (DSPC) IN ALVEOLAR MACROPHAGES

Cells were incubated in phosphate-buffered medium containing 1 mM palmitate and 0.05 mM choline for 2 h. In one group of cells, the radioactive label was [^{3}H]palmitate and in the other the label was [^{14}C]choline. Alveolar macrophages were then spun down, washed three times, resuspended in phosphate-buffered medium and incubated at 37°C for 3 h. During the 3 h incubation, intracellular labeled disaturated phosphatidylcholines disappeared; 46(±2)% and 51(±3)% of the palmitate and choline label disappeared, respectively. Possible products of catabolism were isolated by thin-layer chromatography. The counts appearing in the products are expressed as percent of the counts disappearing from disaturated phosphatidylcholines. LPC, lysophosphatidylcholine. The numbers shown are mean values for five experiments ±S.E.

Product	$\frac{\text{Counts in product}}{\text{counts lost from DSPC}} \times 100$
[^{3}H]palmitate label:	
Palmitate	100 (±2)
LPC	0.5 (±0.3)
[^{14}C]choline label:	
Water-soluble products	99 (±2)
LPC	0.2 (±0.1)

palmitate after catabolism. Similarly, all of the [^{14}C]choline label appears in the water-soluble fraction following catabolism. No lysophosphatidylcholine is formed in either case. These results suggest that both phospholipase A_1 and phospholipase A_2 are involved. All of these data taken together suggest that a considerable amount of exchange of both the palmitate and choline labels occurs.

Extracellular release of disaturated phosphatidylcholines

In order to determine if any of the disaturated phosphatidylcholines are released from alveolar macrophages, we measured the amount of labeled disaturated phosphatidylcholine which appears in the extracellular fluid during incorporation of palmitate into the phospholipid. The results are shown in Fig. 4. The total amount of labeled disaturated phosphatidylcholines, i.e., that present in both the intra- and extracellular compartments, is given as the solid line. Note that the amount of labeled disaturated phosphatidylcholines increases for about 3 h. We have already demonstrated that both synthesis and catabolism of disaturated phosphatidylcholines occur in these cells. The fact that the amount of labeled disaturated phosphatidylcholine increases during the first 3 h suggests that over this time period synthesis exceeds catabolism. This is probably due to the high specific activity of the label in the free palmitate fraction forcing the reactions in the direction of synthesis. After 3 h, the total amount of labeled disaturated phosphatidylcholines remains constant. At this point, incorporation of labeled palmitate into disaturated phosphatidylcholines is probably equal to catabolism of the labeled phospholipid.

The labeled disaturated phosphatidylcholines in the extracellular fluid are shown as the dashed line. Some of the disaturated phosphatidylcholines appear to be released from the cells very early during incorporation, i.e., during the first 30 min. Then, the extracellular disaturated phosphatidylcholines remain rather constant up to 3 h. After 3 h, when the total amount of labeled disaturated phosphatidylcholines remains constant, there seems to be a steady release of disaturated phosphatidylcholines from the cells. In other experiments we measured the rate of oxygen consumption by alveolar macrophages prior to the 5-h incubation period. We obtained a value of 55 ($\pm$4) nmol/10^7 cells per min. This rate of oxygen consumption did not change significantly during the incubation period and addition of sodium succinate to the cells did not cause any increase in oxygen consumption. These results indicate that the release of disaturated phosphatidylcholines which occurs is not due to impaired cell viability or leakiness of the cell membranes.

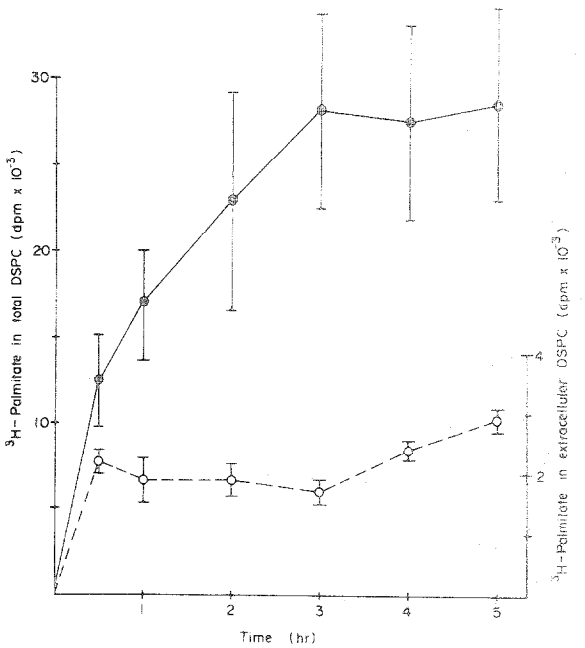

Fig. 4. Total (intra- and extracellular) and extracellular labeled disaturated phosphatidylcholines (DSPC) during incorporation of [^{3}H]palmitate into disaturated phosphatidylcholines in alveolar macrophages. Experiments were performed with cells suspended in phosphate-buffered medium containing 1.0 mM palmitate. Measurements of total disaturated phosphatidylcholines (●) were made from samples of cell suspension (0.5 ml total volume) which contained $5 \cdot 10^5$ cells. Extracellular disaturated phosphatidylcholines (○) were measured from 0.5 ml samples of the medium following centrifugation to remove cells. The points are mean values for five experiments and the bars represent the standard errors of the means.

Discussion

The results presented in this paper demonstrate that various substrates are incorporated into disaturated phosphatidylcholines in rat alveolar mac-

rophages. Palmitate, choline and glycerol are all incorporated into disaturated phosphatidylcholines. Several other investigators have studied disaturated diphosphatidylcholine synthesis in rabbit alveolar macrophages and reported some similar results. Mason et al. [9] reported incorporation of choline, palmitate and glycerol into disaturated phosphatidylcholines in these cells. Kikkawa et al. [10] studied incorporation of choline and glycerol into disaturated phosphatidylcholines. These investigators concluded that the majority of disaturated phosphatidylcholine synthesis occurs via the cytidine diphosphate-choline pathway. Elsbach [7] observed that lysophosphatidylcholine is converted into disaturated phosphatidylcholines in alveolar macrophages. It has been reported that 19–29% of the total phosphatidylcholines synthesized by these cells is disaturated phosphatidylcholine [9,10]. All of these data, including those reported by us, indicate that synthesis of disaturated phosphatidylcholines occurs in alveolar macrophages.

Until now, the incorporation of palmitate into disaturated phosphatidylcholines in alveolar macrophages has not been characterized. Some of our results provide such information. Palmitate incorporation becomes maximal after 2–3 h of incubation in vitro and the relationship between incorporation and extracellular palmitate displays saturation kinetics. Cholinergic and β-adrenergic agonists have no effect on this process. Palmitate incorporation into disaturated phosphatidylcholines is stimulated by exposure of the cells to zymosan particles (Table II and ref. 8). One possible explanation for this is that the disaturated phosphatidylcholines formed may be used in phagocytotic membranes.

Incorporation of palmitate into disaturated phosphatidylcholines in alveolar macrophages can be compared with that which occurs in type II cells isolated via centrifugal elutriation [19]. The time-courses for the incorporation of palmitate into disaturated phosphatidylcholines are somewhat different in the two cell types. For example, at 1.0 mM external palmitate, a maximal level, alveolar macrophages maintain a maximal rate of synthesis for less than 30 min. However, type II cells maintain a maximal rate of synthesis for 2–3 h. Incorporation plateaus in 5 h in type II cells and in 2-3 h for alveolar macrophages. Palmitate incorporation displays saturation kinetics in both cell types with a $K_{1/2}$ of 0.19 mM in macrophages and 0.10 mM in type II cells. On a per cell basis, incorporation of palmitate into disaturated phosphatidylcholines in alveolar macrophages is only one-third to one-half of the activity which occurs in type II cells. A similar result was obtained by Kikkawa et al. [10]. They reported three times as much incorporation of choline into disaturated phosphatidylcholines in rabbit type II cells maintained in primary cell culture than in rabbit alveolar macrophages. Our results also indicate that β-adrenergic and cholinergic agonists do not stimulate incorporation of palmitate in macrophages, a result similar to that reported by us in type II cells [19].

It would seem that the incorporation of both labeled palmitate and labeled choline into disaturated phosphatidylcholines is a result of the exchange of the labeled for unlabeled substrate. The evidence for this is three-fold: (1) both palmitate- and choline-labeled disaturated phosphatidylcholine appear to be catabolized in alveolar macrophages, (2) the products of catabolism are palmitate and water-soluble choline products, suggesting both phospholipase A_1 and phospholipase A_2 activities, (3) addition of unlabeled palmitate and choline to the medium enhances catabolism. Some of these results differ from those obtained with alveolar type II cells [19]. Although catabolism of recently-synthesized disaturated phosphatidylcholines also occurs in type II cells, the destruction of disaturated phosphatidylcholines is slowed by extracellular palmitate. The modification of lecithine in alveolar macrophages does not appear to be specific for the saturated variety, since addition of oleate to the medium also enhances catabolism of palmitate-labeled disaturated phosphatidylcholines. All of these data together suggest that extensive exchange of disaturated phosphatidylcholine palmitate and/or choline occurs in rat alveolar macrophages.

Some disaturated phosphatidylcholines appear to be released from alveolar macrophages into the extracellular space (Fig. 4). There is a steady release from the cells after 3 h of incorporation of palmitate into disaturated phosphatidylcholines. In contrast, we have found that there is no steady

release of newly-synthesized disaturated phosphatidylcholines from alveolar type II cells isolated by centrifugal elutriation within 7 h of the onset of synthesis (unpublished data). This is probably due to the fact that some time is requireed for packaging the material into lamellar bodies in type II cells [21], since it is well established that these cells release disaturated phosphatidylcholines. The form in which disaturated phosphatidylcholines are released from alveolar macrophages is not known. Note that some disaturated phosphatidylcholines are released from the cells within the first 30 min of incubation and then release plateaus for a while. A similar result was obtained with our type II cell preparation. The reason(s) for this remains unknown.

Why are disaturated phosphatidylcholines extensively modified in alveolar macrophages? How are the disaturated phosphatidylcholines used? Although the answers to these questions remain unknown, there are several possibilities. It is possible that the lecithins are simply used for normal cell functions. Since these cells are phagocytic, the disaturated phosphatidylcholines may also be used in phagocytic membranes. Another possibility is that the phospholipid is used as part of the pulmonary surfactant materials. The fact that the disaturated phosphatidylcholines are released from the cells supports this possibility. However, the ability of alveolar macrophages to synthesize other components of surfactant, including the 36 kDa apoprotein, has not been demonstrated.

If some of the disaturated phosphatidylcholines are used as surfactant, the contribution made to production of total surfactant disaturated phosphatidylcholines in the lungs by alveolar macrophages is probably not a major one. In the rat lung, 14.5% of the cells are type II and only 3.2% are alveolar macrophages [22]. Since type II cells incorporate 2–3-times more palmitate into disaturated phosphatidylcholines than do macrophages on a per cell basis, type II cells account for about 10–20-times more disaturated phosphatidylcholine synthesis in the lungs than do macrophages. However, in human lungs the number of alveolar macrophages may be somewhat larger in some individuals [23]. Perhaps in these instances, the amount of lung disaturated phosphatidylcholines from alveolar macrophages may be greater.

In summary, our data indicate that disaturated phosphatidylcholine synthesis occurs in alveolar macrophages. We have presented experiments to characterize the incorporation of palmitate into disaturated phosphatidylcholines in these cells and compared the results with those obtained in isolated alveolar type II cells. There appears to be a considerable amount of exchange of the palmitate and choline portions of the phospholipid molecule. The results also suggest that disaturated phosphatidylcholines are released from the cells into the extracellular space. Several possible functions of alveolar macrophage disaturated phosphatidylcholines have been discussed.

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