Synthesis of Phosphatidylcholine and Phosphatidylglycerol by Alveolar Type II Cells in Primary Culture*

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Saturated phosphatidylcholine and phosphatidylglycerol are important components of pulmonary surface active material. We studied the synthesis of these two phospholipid classes by alveolar type II cells in primary culture. During a 20-h incubation, type II cells incorporated a high percentage of glycerol, acetate, and palmitate into phosphatidylcholine (61.2, 76.4, and 76.8% of lipid radioactivity, respectively) and into phosphatidylglycerol (16.7, 5.8, and 6.6%). Acetate was incorporated principally by de novo synthesis of fatty acids rather than by chain elongation. We studied the pathways for synthesis of saturated phosphatidylcholine and phosphatidylglycerol with type II cells that had been in culture for 1 day. Palmitate was incorporated nearly equally into positions 1 and 2 of saturated phosphatidylglycerol, but predominantly (72%) into position 2 of saturated phosphatidylcholine. These data imply that saturated phosphatidylcholine is synthesized at least in part by acylation of 1-acyl-2-lysophosphatidylcholine. Alveolar type II cells also incorporated a mixture of saturated 1-[1,2-14C]palmitoyl-2-lyso phosphatidylcholine and 1-acyl-2-lysophosphatidyl-[1,2-14C]choline from the medium by direct acylation rather than by transacylation. As the duration of culture increased beyond 1 day, type II cells incorporated a lower percentage of palmitate into phosphatidylglycerol and saturated phosphatidylcholine.

**Experimental Procedures**

Preparation of Alveolar Type II Cells

Alveolar type II cells were prepared from specific pathogen-free Sprague-Dawley male rats that weighed from 180 to 300 g (11, 12). Type II cells were partially purified by dissociation of intact excised lung with crystalline trypsin and centrifugation of the resulting cell suspension over a discontinuous density gradient made with albumin. A higher concentration of trypsin (3 mg/ml) was used for the experiments in Table I and Figs. 1 and 2 and a lower concentration of trypsin (0.1 mg/ml) was used for all other experiments (12). The dispersed cells were further purified by differential adherence (11, 12). Cells were cultured in Dulbecco's modified Eagle's medium, 10% fetal calf serum, and antibiotics. For experiments in Table I and Figs. 1 and 2, we used 10 μg of gentamicin/ml and in all other experiments we used 50 μg of gentamicin/ml and 100 units of penicillin G/ml. Cells were incubated for 3 h in a T-75 culture flask, during which time few cells (mostly macrophages) attached to the plastic. The nonadherent cells were removed and, in most experiments, were placed in 35-mm tissue culture dishes with 2.5 ml of medium and 2.5 × 10^5 cells/dish; during the next 20 h of culture, type II cells adhered to the plastic. Nonadherent cells (mostly lymphocytes and nonviable cells) were removed and discarded. After the differential adherence, approximately 10^6 cells remained attached to each culture dish. Cells were quantitated by DNA determination (13); by this method, there is 8.3 μg of DNA/10^6 type II cells. The percentage of type II cells was determined from differential counts of cells stained with the modified Papanicolaou stain (14). Cells prepared with 3 mg/ml of trypsin and purified in culture yielded 83 ± 4% type II cells (mean ± S.D.; n = 6) and cells prepared with 0.1 mg/ml of trypsin and purified in culture yielded 94 ± 4% type II cells (n = 12). The total yield of purified adherent type II cells from six rat lungs dissociated with 0.1 mg/ml of trypsin was 10 to 15 × 10^6 cells.

Incorporation of Acetate, Palmitate, and Glycerol into Lipids

During the period of attachment of type II cells, the cells were incubated with radioactive acetate, palmitate, or glycerol as described in the legend to Table I.

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Metabolic Incubations

After 23 h in culture, the adherent cells were washed three times with defatted saline (sodium chloride 0.9% in 0.15 M NaCl) and incubated with a 1 ml aliquot of medium (Hanks' salts, 25 mM Hepes, pH 7.4). This medium is designated ME medium/Hepes. Fraction V bovine serum albumin and appropriate radioactive substrates were added to the ME medium/Hepes; the final concentrations of added substances are stated in the legends to the figures and tables.

Lipid Analyses

Extraction and Separation—After the incubation period, medium was removed and the cells were washed three times with ME medium/Hepes. The cells were extracted six times with ethanol (15), lipids (0.6 to 1.0 mg) isolated from dog lung were added as carriers, and the ethanol was evaporated under a stream of nitrogen. The residue was extracted with chloroform:methanol (2:1), and the solution was partitioned into organic and aqueous phases by the method of Folch et al. (16). The aqueous phase was formed by the addition of 100 mM KCl to which we added sodium acetate (1 mM) or glycerol (1 mM) for the experiments in which radioactive acetate or glycerol was used as precursor. Individual phospholipids were separated by two-dimensional thin layer chromatography (TLC) on Silica Gel G plates impregnated with boric acid (17). The spots were identified by brief exposure to iodine vapor: phosphatidylcholine and phosphatidylglycerol were eluted from the silica with chloroform:methanol:water:acetic acid (50:50:2:1) followed by chloroform:methanol:water (30:60:10) (18).

To isolate both the saturated and the unsaturated species of phosphatidylcholines (Tables II and IV), we reacted the total phosphatidylcholine fraction with osmium tetroxide, and separated the mixture with sodium acetate (1 mM) for the experiments in which radioactive acetate or glycerol was used as precursor. Individual phosphatidylcholines were synthesized by type A2 phospholipase A2 (Crotalus adamanteus). We separated lysophosphatidylcholine by TLC with a solvent system of 25% diethyl ether in hexane:diethyl ether:acetic acid (50:50:1). We found that the separation of diglycerides, acidiglycerides, fatty acids, monoglycerides, and phosphatidylcholines and monoglycerides was improved by spraying the Silica Gel plate briefly with water before applying the samples. For each analysis, the amount of radioactivity in saturated diglyceride acetates derived from phosphatidylcholine ranged from 1600 to 5900 cpn for 14C and from 1940 to 6380 cpn for 3H. For saturated diglyceride acetates derived from phosphatidylglycerol, the amount of radioactivity ranged from 580 to 1890 cpn for 14C and from 740 to 3440 cpn for 3H. The percentage of hydrolysis of diglyceride acetates ranged from 29 to 45%. The recovery of total radioactivity in these experiments ranged from 97 to 100%. Monoglyceride acetates contained nearly twice as much radioactivity as monoglycerides. Fatty acids liberated by pancreatic lipase during these limited hydrolyses were assumed to come from position 1, whereas the monoglycerides and monoacylglycerides were assumed to contain long chain fatty acids only in position 2 (23).

Schmidt Decarboxylation Reaction—We saponified the lipids synthesized by type II cells from [1-14C]acetate. The fatty acids were extracted, purified by TLC, and decarboxylated according to a modification of the Schmidt procedure (24). In each decarboxylation procedure, we ran [1-14C]palmitate as a control; the percentage of radioactivity recovered as CO2 ranged from 75 to 88%. We calculated the percentage of radioactivity in position C-1 of the fatty acids synthesized from [1-14C]acetate by the formula:

\[
dpm \text{ of CO}_2 \text{ from sample} \times 100 \%
\]

\[
dpm \text{ of unreacted fraction of radioactivity} \times 100 \%
\]

Preparation of Radiolabeled LysoPhosphatidylcholine—Saturated 1-9,10-3H]palmitoyl-2-lysophosphatidylcholine and 1-acyl-2-lyso phosphatidylcholine-[1-14C]choline were prepared biosynthetically by incubation of type II cells with either [9,10-3H]palmitate or [1-14C]choline. The saturated phosphatidylcholines were isolated by the osmium tetroxide method (19), combined, and hydrolyzed by phospholipase A2 (C. adamanteus). We separated lysophosphatidylcholine by TLC with a solvent system of chloroform:methanol:water:acetic acid (100:60:7:14), eluted the lysophosphatidylcholine, and diglyceride acetates from the TLC plates ranged from 85 to 95%. We measured radioactivity by liquid scintillation counting in a dioxane:naphthalenewater system (21). Counts were corrected for quenching and for spillover of the 14C into the 3H channel with the external standard channels ratio and quench curves generated in our laboratory.

Degradation of Saturated Phosphatidylcholine with Phospholipase A2—An aliquot of saturated phosphatidylcholine and 60 μg of egg phosphatidylcholine were evaporated to dryness and dissolved in 2 ml of diethyl ether:ethanol, 19:1 by volume (18, 22). Fifty microliters of phospholipase A2 (Crotalus adamanteus, 1 mg/ml in 5 mM Hepes and 5 mM CaCl2, pH 7.4) was added, and the reaction mixture was incubated for 4 h at 37°C. The reaction mixture was evaporated to dryness and redissolved in chloroform:methanol (1:1). Oleic acid, egg phosphatidylcholine, and lysophosphatidylcholine were then added as carrier lipids. The reaction products were separated by TLC, localized by iodine vapor, and scraped into counting vials for determination of radioactivity (18). In these experiments (Table II), from 93 to 98% of the phosphatidylcholine was hydrolyzed and recovery of total radioactivity was 89 to 96%. In analyzing the data, we assumed that the radioactivity found in the fatty acid fraction came exclusively from position 2 of phosphatidylcholine and the radioactivity in the lysophosphatidylcholine fraction came from position 1.

Degradation of Saturated Diglyceride Acetates with Pancreatic Lipase—Analyses were performed according to the method of Rennkonen (23). The reaction mixture consisted of an aliquot of diglyceride acetate, 200 μg of triolein, 425 μl of 1 M Tris (pH 8.0, prepared in water saturated with diethyl ether), 25 μl of 20% CaCl2, 10 μl of 1% sodium deoxycholate, and 1 ml of pancreatic lipase (6 units/ml in 1 M Tris, pH 8.0). These reagents were incubated for 10 min at 40°C. The reaction was stopped by the addition of 200 μl of 6 N HCl. We added 1 ml of methanol and extracted the reaction products three times with diethyl ether. The diethyl ether was removed and backwashed with water. The reaction products were and were then reisolated by TLC in a solvent system of hexane:diethyl ether:acetic acid (50:50:1). We found that the separation of diglycerides, acidiglycerides, fatty acids, monoglycerides, and phosphatidylcholines and monoglycerides was improved by spraying the Silica Gel plate briefly with water before applying the samples. For each analysis, the amount of radioactivity in saturated diglyceride acetates derived from phosphatidylcholine ranged from 1600 to 5900 cpn for 14C and from 1940 to 6380 cpn for 3H. For saturated diglyceride acetates derived from phosphatidylglycerol, the amount of radioactivity ranged from 580 to 1890 cpn for 14C and from 740 to 3440 cpn for 3H. The percentage of hydrolysis of diglyceride acetates ranged from 29 to 45%. The recovery of total radioactivity in these families ranged from 97 to 100%. Monoglyceride acetates contained nearly twice as much radioactivity as monoglycerides. Fatty acids liberated by pancreatic lipase during these limited hydrolyses were assumed to come from position 1, whereas the monoglycerides and monoacylglycerides were assumed to contain long chain fatty acids only in position 2 (23).

Other Methods

Palmitate was bound to defatted bovine serum albumin according to the method of Specor et al. (25).

Materials

Materials and animals for isolating type II cells were obtained from the same suppliers as described previously (13). Culture media and fetal calf serum were obtained from the Cell Culture Facility of the University of California, San Francisco. Plastic culture dishes were from Corning Glass Works, Corning, N. Y. [U-14C]glycerol, sodium [1-14C]acetate, [1-14C]palmitic acid, and 25 μg of [9,10-3H]palmitic acid, and [1-14C]choline were purchased from New England Nuclear Corp., Boston, Mass. [1-3H]Glycerol was obtained from the Radiochemical Centre, Amersham, England. Fraction V bovine serum albumin was obtained from Miles Laboratories, Kankakee, Ill. Defatted bovine serum albumin, oleic acid, triolein, phospholipase C, and pancreatic lipase were obtained from Sigma Chemical Co., St Louis, Mo. Phospholipase A2 (C. adamanteus) was purchased from Miami Serpentarium, Miami, Fla. Phosphatidylglycerol was obtained from Avanti, Birmingham, Ala. Silica G and H thin layer plates were obtained from Analtech, Inc., Newark, Del. All the organic solvents were reagent
RESULTS

Synthesis of the Phospholipids of Surface Active Material—We tested the ability of type II cells in primary culture to synthesize the lipids of surface active material by incubating the cells for 20 h with radioactive acetate, palmitate, or glycerol. The results, which have been published in preliminary form in recent symposia (12, 26), are shown in Table 1. The pattern of distribution of radioactivity in the various phospholipid classes is noteworthy for two reasons. First, the types of lipids synthesized from these precursors are very similar to the phospholipid composition of surface active material and alveolar type II cells from rats (12). Second, the pattern of distribution was the same when either acetate or palmitate was used as the precursor, a result which suggests that acetate is incorporated into fatty acids by de novo synthesis rather than by chain elongation. We determined whether acetate was incorporated into fatty acids by de novo synthesis or chain elongation by incubating cells with [1-14C]acetate, isolating the fatty acids synthesized from [1-14C]acetate, and comparing the amount of radioactivity in the carboxyl carbon (obtained by the Schmidt decarboxylation reaction) to the amount of radioactivity in the remaining portion of the fatty acids. If acetate were incorporated into fatty acids only by de novo synthesis and if palmitate were the sole product, 12.5% of the radioactivity would be found in the carboxyl carbon. If acetate were incorporated by chain elongation and if only 1 acetate were added/fatty acid, all of the radioactivity would be found in the carboxyl carbon. We found 14.5 ± 0.5% (mean ± S.E.; n = 4) of the radioactivity in the carboxyl carbon. Therefore, type II cells incorporate acetate into fatty acids by de novo synthesis under the conditions of these experiments. Because type II cells maintained in culture for more than 2 days become larger and the cellular cytoplasmic inclusions become smaller and less distinct (12, 27), we wanted to determine whether type II cells kept in culture retained their ability to produce the lipid components of surface active material. We maintained cells in culture for 1, 2, 3, and 6 days and then incubated the cells for 2 h with [1-14C]palmitate. The results are shown in Figs. 1 and 2. Incorporation of palmitate into total lipid and into saturated phosphatidylcholine/µg of DNA increased with time in culture (Fig. 1). This is consistent with the observation that type II cells become larger. However, because the percentage of palmitate incorporated into saturated phosphatidylcholine and phosphatidylglycerol decreased with the duration of time in culture (Fig. 2), we concluded that as time in culture increased, there was relatively less synthesis of the lipids of surface active material and more synthesis of other cellular phospholipids. We therefore performed the rest of the studies of lipid biosynthesis on Day 1 of culture.

Studies of the Synthesis of Saturated Phosphatidylcholine and Saturated Phosphatidylglycerol—We wanted to determine the relative importance of various pathways for synthesizing saturated phosphatidylcholine, i.e. de novo synthesis (via saturated phosphatidic acid and saturated diglyceride), deacylation-reacylation, and deacylation-transacylation (4).

We first incubated type II cells with [1-14C]palmitate for short periods of time and determined the percentage of palmitate that was incorporated into positions 1 and 2 of saturated phosphatidylcholine. As shown in Table II, the percent-

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### Table I

Incorporation of acetate, palmitate, and glycerol into different lipids

| Lipid                     | Acetate | Palmitate | Glycerol |
|---------------------------|---------|-----------|----------|
| Lyso phosphatidylcholine  | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.5 ± 0.1 |
| Phosphatidylserine        | 1.4 ± 0.5 | 1.3 ± 0.4 | 2.3 ± 0.7 |
| Phosphatidylinositol      | 2.3 ± 0.4 | 1.6 ± 0.4 | 3.5 ± 0.4 |
| Sphingomyelin             | 2.4 ± 0.3 | 1.9 ± 0.8 | 0.5 ± 0.2 |
| Phosphatidylethanolamine  | 5.2 ± 1.3 | 7.6 ± 1.8 | 61.2 ± 1.4 |
| Diphosphatidylglycerol    | 6.6 ± 0.6 | 16.7 ± 2.2 |
| Phosphatidylglycerol      | 17.5 ± 6.3 | 26.7 ± 2.2 |
| Neutral lipids            | 0.2 ± 0.2 | 0.3 ± 0.1 | 2.9 ± 0.9 |
| Other                     | 0.2 ± 0.2 | 0.2 ± 0.2 | 1.0 ± 0.4 |
| Number of experiments     | 4        | 4         | 4        |
| Recovery of radioactivity (%) | 95.6 ± 5.3 | 95.4 ± 2.1 | 95.6 ± 2.3 |

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![Fig. 1. Incorporation of palmitate into total lipid and saturated phosphatidylcholine (DSPC). Day 0 designates the day on which type II cells were isolated and placed in culture in 35-mm tissue culture dishes. Dishes were removed at 23 h for various analyses, this time is designated as Day 1 of culture. Three dishes were used for palmitate incorporation, two for DNA determination, and one for Papanicolaou stain. The rest of the cultures were re-fed with Dulbecco's modified Eagle's medium and 10% fetal calf serum on Days 1 and 2. For palmitate incorporation, the adherent cells were washed and incubated with 1.6 ml of medium/h of medium/Hepes, 3 mg/ml of bovine serum albumin (34 µmol of mixed fatty acids/liter), and albumin-bound [1-14C]palmitate (4 µmol/liter, 0.2 µCi) for 2 h at 37°C. The cells were washed and the lipids were extracted and processed as described in the text. The mean ± S.E. for triplicate incubations in one of two experiments is shown.](image-url)
Phosphatidylcholine and Phosphatidylglycerol Synthesis

Fig. 2. Percentage of palmitate incorporation. For explanation, see legend to Fig. 1.

TABLE II
Incorporation of palmitate into phosphatidylcholine

Type II cells were cultured in 35-mm culture dishes and were purified by differential adherence as described in the text. They were preincubated for 15 min at 37°C in ME medium/Hepes which contained 3 mg/ml of Fraction V bovine serum albumin. The medium was removed and replaced with warm medium (1.6 ml) which consisted of ME medium/Hepes, 3 mg/ml of bovine serum albumin (34 μmol of mixed fatty acids/liter), and albumin-bound [1-14C]palmitate (20 μmol/liter, 0.9 μCi). The calculated total fatty acid to albumin molar ratio was 1.2. After the designated periods of incubation, duplicate dishes were washed, the cells were extracted, and the lipids were processed as described in the text. The experiment, there was 9.24 μg of DNA/culture dish (the equivalent of 1.1 × 10^6 cells/dish). Based on the assumptions that all fatty acids are esterified at an equal rate (28) and that the intracellular specific activity of fatty acids is the same as the specific activity of the fatty acids in the medium, the calculated synthesis of total phosphatidylcholine was 2.54 nmol/10^6 cells h⁻¹. The means of duplicate samples are given.

| Time (min) | Unsaturated PC | Saturated PC | Saturated PC ( incorporation into total PC) | Saturated PC (radioactivity in position 2) |
|-----------|----------------|--------------|---------------------------------------------|-------------------------------------------|
| 10        | 3,200          | 2,210        | 74                                          | 85                                        |
| 30        | 13,100         | 24,000       | 65                                          | 78                                        |
| 60        | 27,000         | 40,800       | 60                                          | 72                                        |

- PC, phosphatidylcholine.

age of incorporation into position 2 was significantly greater than into position 1.

We next showed that the distribution of palmitate incorporated into positions 1 and 2 of saturated phosphatidylglycerol was quite different from the distribution in saturated phosphatidylcholine. We incubated type II cells for 20 h with [1,3-3H]glycerol (to achieve equilibrium labeling (29) of the phospholipid backbone) and then incubated the cells for 30 min with [1-14C]palmitate. We isolated phosphatidylcholine and phosphatidylglycerol, converted these phospholipids to diglyceride acetates, isolated the saturated diglyceride acetates, and partially degraded the diglyceride acetates with pancreatic lipase to ascertain the distribution of radioactive palmitate in positions 1 and 2. The results are shown in Table III. Palmitate was incorporated predominantly into position 2 of saturated phosphatidylcholine, whereas it was incorporated nearly equally into positions 1 and 2 of saturated phosphatidylglycerol (Table IIIA).

Because the distribution of palmitate (Table IIIA) was calculated from a limited hydrolysis with pancreatic lipase as contrasted with the complete degradation of saturated phosphatidylcholine with phospholipase A₂ (Table II), we also compared the 14C/3H (fatty acid/backbone) ratio in monoglyceride acetates and diglyceride acetates as an internal check on the direct measurement of the 14C. The 3H was derived from [1,3-3H]glycerol and the 14C from [1,3-3H]glycerol. The comparison of the 14C/3H ratio is independent of the extent of the hydrolysis of the diglyceride acetates by pancreatic lipase and requires only physical separation of the two compounds. In the conversion of diglyceride acetates to monoglyceride acetates by pancreatic lipase, the fatty acid in position 1 is removed. This analysis was complicated, however, by the fact that 10 to 19% of the 3H radioactivity was found in the fatty acid portion of the phosphatidylcholine and, therefore, the exact distribution of palmitate was not calculated from these data. Nevertheless, it was instructive to compare the 14C/3H ratios of the diglyceride acetates with those of the monoglyceride acetates (or monoglycerides) produced by pancreatic lipase (Table IIIIB). The ratio of 14C/3H of monoglyceride acetate/diglyceride acetate was 0.57 for those species derived

TABLE III
Incorporation of [1-14C]palmitate into saturated phosphatidylcholine and phosphatidylglycerol in type II cells prelabeled with [1,3-3H]glycerol

Type II cells were cultured from 3 to 23 h with [1,3-3H]glycerol in 35-mm plastic culture dishes. The medium consisted of Dulbecco's modified Eagle's medium, 10% fetal calf serum, gentamicin (50 μg/ml), penicillin (100 units/ml), and [1,3-3H]glycerol (2 μmol/liter, 100 μCi/2.5 ml). The cells were washed and incubated for 30 min with albumin-bound [1-14C]palmitate (20 μmol/liter, 0.9 μCi/1.6 ml) and bovine serum albumin (3 mg/ml) in ME medium/Hepes. The medium was removed and the cells were washed. The lipids were then extracted and analyzed as described in the text. The means ± S.E. of the results from three experiments are given.

| Species | Saturated phosphatidylcholine | Saturated phosphatidylglycerol |
|---------|------------------------------|-------------------------------|
| A. Monoglycerides + monoglyceride acetates | 2.60 ± 0.01 | 1.09 ± 0.15 |
| Fatty acids | Calculated percentage of 14C in position 2 | 72.3 ± 0.1% | 31.7 ± 3.5% |
| B. 14C/3H monoglyceride acetates | 0.83 ± 0.03 | 0.57 ± 0.01 |
| 14C/3H diglyceride acetates | 0.83 ± 0.03 | 0.57 ± 0.01 |

TABLE IV
Incorporation of [1,3-3H]glycerol into different species of phosphatidylcholine and phosphatidylglycerol

Type II cells were isolated and cultured as described in the legend to Table III. Species of phosphatidylcholine and phosphatidylglycerol were separated as diglyceride acetates by argentation thin layer chromatography as described in the text. The means ± S.E. of three experiments are given. The percentages are calculated from the tritium recovered in each fraction.

| Species | Phosphatidylcholine | Phosphatidylglycerol |
|---------|-------------------|---------------------|
| Saturates | 55.1 ± 1.6 | 42.5 ± 1.3 |
| Monones | 33.0 ± 1.3 | 36.8 ± 1.1 |
| Dienes | 5.3 ± 0.4 | 6.6 ± 0.4 |
| Polynes | 4.6 ± 0.2 | 12.0 ± 1.8 |
from saturated phosphatidylglycerol, suggesting that half of the $^{14}C$ (palmitate) was in position 2; in contrast, the ratio was 0.83 for the species derived from saturated phosphatidycholine, suggesting that there was more palmitate in position 2 than in position 1.

The 20-h incubation with $[1,3-^{3}H]glycerol$ should allow sufficient time for equilibrium labeling of the various species of phosphatidylcholine and phosphatidylglycerol (29). We analyzed the distribution of radioactivity in $^{3}H$ in different species of diglyceride acetates derived from phosphatidylcholine and phosphatidylglycerol (Table IV). The actual percentages of the different species may be slightly different from the distribution of radioactivity shown in Table IV, since 10 to 19% of the radioactivity in the diglyceride acetates was in the fatty acid portion of the molecules. However, since both the total and the saturated diglyceride acetates had the same percentage of $^{3}H$ in the fatty acid and glycerol portions of the molecules, it is unlikely that the percentages of saturated species are overestimated. We did not determine the chemical composition of the different species because we did not have a sufficient number of cells and we had to add carrier lipids for our analyses.

To test for the presence of transacylase pathway in type II cells, we incubated cells for 1 h with a mixture of $[9,10^{-14}C]palmitoyl-2-lysophosphatidylcholine$ and $1-acyl-2-lysol-lysophosphatidyl-1,2-^{14}C]choline$. The results are shown in Table V. Lysophosphatidylcholine was readily incorporated into phosphatidylcholine, but there was no change in the $^{14}C$/$^{3}H$ ratio (palmitate/choline) of the phosphatidylcholine, suggesting that exogenous lysophosphatidylcholine was incorporated by direct acylation rather than by transacylation.

**Discussion**

There is now substantial evidence (6, 12, 14, 30-39) that type II cells can make and secrete the lipid components of surface active material. What is not known is what factors regulate lipid synthesis and by what pathways the saturated species of phospholipids are synthesized. There are four main pathways that have been considered possible for the synthesis of saturated phosphatidylcholine. These are 1) $de novo$ synthesis from saturated diglycerides; 2) serial methylation of phosphatidylethanolamine; and two pathways for converting unsaturated phosphatidylcholines into saturated phosphatidylcholine, i.e. 3) deacylation-reacylation and 4) deacylation-transacylation. A detailed discussion of the evidence in support of or against each pathway can be found in recent reviews (4-6). We will focus on the results of this report and of others who used isolated type II cells. Kikkawa et al. (11) showed that serial methylation of phosphatidylethanolamine is not a quantitatively important reaction in the synthesis of phosphatidylcholine by isolated type II cells. We have tried to compare the relative contribution of the three other possible routes of phosphatidylcholine synthesis. Our data support previous observations of others (20, 39) which indicate that the deacylation-reacylation pathway is quantitatively important.

The assumptions and limitations inherent in our data should be considered before the data are discussed. First, the cell isolation procedure may have altered metabolic pathways in our cells. We dissociated lungs with trypsin; Finkelstein and Mavis (40) reported that trypsin can alter certain enzymes of lipid synthesis in type II cells. Second, we performed most of our studies after 1 day of culture. We found both morphologic and biochemical evidence of change during short term culture and it is possible that the cells we studied after 1 day in culture are different from freshly isolated type II cells (12). Third, because the fatty acids used in culture medium can affect which species of phospholipid are synthesized in $vitro$ (35, 41, 42), we chose to perform the long (20-h) incubations (Table I) in the presence of 10% fetal calf serum and the short incubations in the presence of the mixed fatty acids found in bovine serum albumin. Fourth, we used methods that isolate total saturated species of phosphatidylcholine and phosphatidylglycerol. We have assumed that the saturated species of phosphatidic acid, diglyceride, phosphatidylcholine, and phosphatidylglycerol are all predominantly dipalmitoyl species (31, 43, 44). In subsequent experiments, we isolated type II cells with elastase (37) and analyzed the fatty acid composition of positions 1 and 2 of the saturated phosphatidylcholine and phosphatidylglycerol. For saturated phosphatidylcholine, position 1 contained 9% myristate, 87% palmitate, and 3% stearate; position 2 contained 13% myristate, 84% palmitate, and 2% stearate (n = 3). For saturated phosphatidylglycerol, position 1 contained 2% myristate, 90% palmitate, and 8% stearate; position 2 contained 8% myristate, 87% palmitate, and 4% stearate (n = 3). Hence, both saturated phosphatidylcholine and phosphatidylglycerol are predominantly dipalmitoyl species.

With these limitations in mind, we can discuss the three observations that, when they are considered together, indicate that the deacylation-reacylation pathway is quantitatively important for the synthesis of saturated phosphatidylcholine. 1) Palmitate is incorporated preferentially into position 2 of saturated phosphatidylcholine. 2) Palmitate is incorporated equally into positions 1 and 2 of saturated phosphatidylglycerol. 3) Type II cells readily acylate lysophosphatidylcholine to form saturated phosphatidylcholine.

After short incubations with radioactive palmitate, position 2 of saturated phosphatidylcholine has approximately three times the radioactivity of position 1 (Tables II and III). The most likely explanation for this observation is that palmitate is incorporated at least in part by a deacylation-reacylation mechanism. One would expect that palmitate would be incorporated equally into positions 1 and 2 by $de novo$ synthesis or by the deacylation transacylation pathway. It is, however, also possible to imagine that either of these two pathways could produce asymmetric incorporation, for example if there were a large pool of 1-acyl-2-lysophosphatidic acid or if there were different pools of acceptor and donor lysophosphatidylcholine for the transacylase (lysophosphatidylcholine acyltransferase) (45). Palmitate is also incorporated predominantly into position 2 of saturated phosphatidylcholine in...
urethane adenoma (20), in whole lung (18, 20), and in surface acute material (49).

We have assumed that saturated phosphatidic acid and saturated diglyceride, the precursor molecules for de novo synthesis, have palmitate equally distributed in positions 1 and 2. Because we isolated too few type II cells to measure these intermediates directly, we tested this assumption indirectly by determining the distribution in saturated phosphatidylglycerol, reasoning that it might reflect the distribution in saturated phosphatidic acid. The rationale for this approach is that 1) in lung, phosphatidylglycerol is thought to be synthesized de novo (47); 2) type II cells readily synthesize saturated phosphatidylglycerol; and 3) the de novo synthesis of phosphatidylcholine and phosphatidylglycerol probably occurs on the endoplasmic reticulum (47, 49), implying that the same pool of phosphatidic acid might be used for the de novo synthesis of both phosphatidylcholine and phosphatidylglycerol. By this reasoning, the finding that radioactive palmitate was equally distributed in positions 1 and 2 of saturated phosphatidylglycerol (Table IIIA) suggests that, during the incubation, saturated phosphatidic acid also had palmitate equally distributed in positions 1 and 2 of saturated diglyceride (by lung in vivo (49) and saturated phosphatidic acid (by urethane adenoma in vitro (50)). Since saturated phosphatidylcholine had three times as much radioactive palmitate in position 2 as in position 1, it seems likely that saturated phosphatidylcholine was made to a large extent by a mechanism other than de novo synthesis.

We used a mixture of saturated radiolabeled lysophosphatidylcholine to evaluate the synthesis of saturated phosphatidylcholine by the transacylation pathway with intact type II cells. Although we did not determine the fatty acid composition of the radioactive saturated lysophosphatidylcholine, it should be the same as that of position 1 of saturated phosphatidylcholine in type II cells, namely 9% myristate, 87% palmitate, and 3% stearate. We, like Smith and Kikkawa (34), found that lysophosphatidylcholine was rapidly incorporated into saturated phosphatidylcholine. However, because there was no increase in the H/13C (palmitate/choline) ratio of the saturated phosphatidylcholine synthesized from the mixture of labeled saturated lysophosphatidylcholines, we did not demonstrate transacylation. Our results are in contrast to the work of Akino et al. (51, 52) and Hallman and Rajvanshi (53) who used doubly labeled lysophosphatidylcholine to demonstrate the presence of transacylation for the synthesis of saturated phosphatidylcholine in whole lung and in lung slices. There are several explanations for these differences. First, pulmonary cells other than type II cells may have accounted for the results in experiments with whole lung (39). Second, our procedure for preparing type II cells might result in a loss of lysophosholipid:lysolecinith acyltransferase activity (40). Third, we used intact cells in our experiments; lysophosphatidylcholine may have been acylated in the cellular plasma membrane (54) and therefore may not have entered the cells to be available to lysophosholipid:lysolecinith acyltransferase which is located in the cytosol (45, 55).

Recently, Batenburg et al. (39) measured the activity of lysophosholipid:lysolecinith acyltransferase and lysophosholipid:lysolecinith acyltransferase in sonicates of type II cells and whole lung. They found that the specific activity of lysophosholipid:lysolecinith acyltransferase was much greater in type II cells than in whole lung and that the enzyme from type II cells showed a preference for palmitoyl coenzyme A over oleoyl coenzyme A. In contrast, the specific activity of lysophosholipid:lysolecinith acyltransferase was the same in type II cells and in whole lung.

Data with intact lung, adenomas of type II cells, and isolated type II cells suggest that the deacylation-reacylation pathway is important, but clearly additional studies of the enzymes involved and direct measurement of the intracellular intermediates are needed before one will be able to describe quantitatively the pathways and regulation of the synthesis of saturated phosphatidylcholine in type II cells.

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