Labeling of Phospholipids in the Surfactant and Subcellular Fractions of Rabbit Lung*

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The principal lung phospholipids were studied following pulse labeling of rabbit lung in vivo with the phospholipid precursors [3H]glycerol and [14C]palmitic acid. Alveolar wash, microsome, and lamellar body fractions subsequently were isolated. The phospholipids and the fatty acid compositions of each phospholipid in each lung fraction were quantified. The appearance of labeled phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine plus phosphatidylserine in each lung fraction was measured. Labeled precursors were incorporated into all phospholipid classes in the microsomal fraction simultaneously, and the specific activity of each phospholipid was maximal within 30 min of isotope administration. Maximal specific activities of all phospholipids were measured in the lamellar body fraction by 2 to 4 h, and in alveolar wash by about 9 h. Phosphatidylglycerol and phosphatidylcholine contained equal amounts of palmitic acid (50%) and had the same specific activities in the lung fractions when labeled with [14C]palmitic acid. The biological half-life of each phospholipid in each lung fraction also was determined for a period of 3 days following isotope administration. Phosphatidylglycerol and phosphatidylcholine were turned over more rapidly than were the other phospholipids. Relative biological half-life values for alveolar surfactant phospholipids labeled either with palmitic acid or glycerol were phosphatidylglycerol < phosphatidylcholine < phosphatidylserine < phosphatidylcholine plus phosphatidylserine. Phosphatidylglycerol is an unique phospholipid in surfactant, and thus, may be as good a biochemical marker for the study of surfactant metabolism as is disaturated phosphatidylcholine.

Surface active material isolated from mammalian lung is 80 to 90% lipid, and phospholipids represent more than 80% of the total lipids (1). However, the lipid composition of surfactant depends somewhat on animal source, method of isolation, and fractionation procedure (2, 3). The principal lung phospholipids were studied following pulse labeling of rabbit lung in vivo with the phospholipid precursors [3H]glycerol and [14C]palmitic acid. Alveolar wash, microsome, and lamellar body fractions subsequently were isolated. The phospholipids and the fatty acid compositions of each phospholipid in each lung fraction were quantified. The appearance of labeled phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine plus phosphatidylserine in each lung fraction was measured. Labeled precursors were incorporated into all phospholipid classes in the microsomal fraction simultaneously, and the specific activity of each phospholipid was maximal within 30 min of isotope administration. Maximal specific activities of all phospholipids were measured in the lamellar body fraction by 2 to 4 h, and in alveolar wash by about 9 h. Phosphatidylglycerol and phosphatidylcholine contained equal amounts of palmitic acid (50%) and had the same specific activities in the lung fractions when labeled with [14C]palmitic acid. The biological half-life of each phospholipid in each lung fraction also was determined for a period of 3 days following isotope administration. Phosphatidylglycerol and phosphatidylcholine were turned over more rapidly than were the other phospholipids. Relative biological half-life values for alveolar surfactant phospholipids labeled either with palmitic acid or glycerol were phosphatidylglycerol < phosphatidylcholine < phosphatidylserine < phosphatidylcholine plus phosphatidylserine. Phosphatidylglycerol is an unique phospholipid in surfactant, and thus, may be as good a biochemical marker for the study of surfactant metabolism as is disaturated phosphatidylcholine.

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lature and rinsed in saline. The resulting tissue, which was essentially free of blood and was called here lung parenchyma, was minced with scissors and homogenized with a motor-driven Teflon pestle homogenizer (Arthur Thomas Co.) in a controlled fashion (14). A fraction of the homogenate was extracted (15) to yield a lipid extract of the lung parenchyma.

Micromosomal and lamellar body fractions were isolated from the homogenate by differential and sucrose density gradient centrifugation as described by Gil and Reiss (13) and modified for rabbit lung (14). The discontinuous gradients used for the isolation of lamellar bodies were made with 15 ml of 0.45 m sucrose in Tris buffer (14) layered over 10 ml of 0.30 m sucrose in Tris buffer. The lamellar body fractions were recovered after centrifugation of the gradients for 2 h at 25,000 rpm using a Spinco SW 27 rotor. This lamellar body fraction previously was shown to have the phospholipid composition (10-18), the phospholipid to protein ratio (13, 19), and the isopycnic density (1.065 to 1.07 g/ml at 20°C) characteristic of rabbit lamellar bodies (19). The lamellar bodies were not contaminated with enzymatic activity characteristic of microsomal, mitochondrial, or cell membrane elements (14). Previous studies from this laboratory have demonstrated the morphologic integrity and purity of lamellar body preparations (18, 20). A micromosomal fraction similarly prepared was not contaminated with mitochondrial enzymatic activity (14).

**Lipid Analysis**—All lipids were extracted by the method of Bligh and Dyer (15). A sequential two-step one-dimensional plating technique was used to recover sufficient amounts of the minor phospholipids from the large amounts of neutral lipids and other contaminants in the lipid extracts from the parenchyma and microsomes. The phospholipids were separated on hand-made silica gel H plates (20 x 20 cm) by streaking the lipid extracts for the initial separation using Solvent 1 (chloroform:methanol:acetic acid:water (65:25:8:4, v/v)). The plates were sprayed with methanol to identify two prominent bands, one containing phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylserine and the other containing phosphatidylinositol and phosphatidylinositol. The two bands were recovered from the silica by elution with chloroform:methanol (2:1) through a Teflon Millipore filter (14). The two phospholipid solutions were then taken to dryness in nitrogen and spotted separately in chloroform on a second silica gel H plate. This plate was developed with Solvent 2 (tetrahydrofurane:methylal:methanol:2 N ammonium hydroxide (80:15:8:8.4, v/v)). The clearly separated spots were located with methanol:chloroform:methanol:water:acetic acid:water (6:3:2:1:1:1, v/v). The plates were dried and developed with silica gel H plates developed in two dimensions using first solvent (12). The fatty acid methyl esters were separated by gas chromatography at 180°C using 15% diethyleneglycol succinate on a 10-ft column. The methyl esters were identified by comparison of retention times to those measured using standard methyl ester solutions (Supelco). The relative areas of the chromatograph was determined by digital integration and the fatty acid composition was determined.

Radioactivity appearing in the phospholipids was measured in Aquasol scintillation fluid (New England Nuclear), using phosphatidylcholine labeled with [H]choline or [14]Cpalmitate acid as standards for calculating channel overlap as before (14). Specific activity was expressed as counts per min per μmol of phospholipid phosphorus and was reproducible to ±6%.

Phosphatidylcholine was recovered from phosphatidylglycerol following treatment with phospholipase D (EC 3.1.4.4) from cabbage (Sigma type I) (23). The phosphatidylcholine was recovered from silica gel H plates after chromatography with chloroform:methanol:concentrated ammonium hydroxide:water (130:60:5.4, v/v) and quantified by phosphatidylcholine.

**RESULTS**

**Phospholipid Composition of Lung Fractions**—The phospholipids isolated from the four lung fractions were expressed as the percentage of phospholipid phosphate in Table I. The compositions of phospholipids from the lung parenchyma and microsomes were very similar, but quite different from the compositions of the phospholipids in lamellar bodies and the alveolar wash. The relative concentrations of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin decreased approximately 5-fold, 10-fold, and 20-fold, respectively, from parenchyma and microsomes to the surfactant-related fractions-lamellar bodies and alveolar wash. In contrast, the relative concentrations of phosphatidylglycerol and phosphatidylglycerol increased about 1.5- and 3.5-fold, respectively, from parenchyma to the surfactant-related fractions-lamellar bodies and alveolar wash. In contrast, the relative concentrations of phosphatidylglycerol and phosphatidylglycerol increased about 1.5- and 3.5-fold, respectively, from parenchyma to the surfactant-related fractions-lamellar bodies and alveolar wash. In contrast, the relative concentrations of phosphatidylglycerol and phosphatidylglycerol increased about 1.5- and 3.5-fold, respectively, from parenchyma to the surfactant-related fractions-lamellar bodies and alveolar wash. In contrast, the relative concentrations of phosphatidylglycerol and phosphatidylglycerol increased about 1.5- and 3.5-fold, respectively, from parenchyma to the surfactant-related fractions-lamellar bodies and alveolar wash.
dies in all four lung fractions. Phosphatidylserine represented about 15% of the phospholipid in the combined pool (Table I).

**Appearance of Labeled Phospholipids in Lung Fractions.**—After the simultaneous injection of radiolabeled glycerol and palmitic acid, the appearance of the precursors in the phospholipids isolated from the lung and lung fractions was measured. The specific activities of the phospholipids in the lung parenchyma labeled with either glycerol or palmitic acid was maximal by ½ to 1 h after injection, indicating rapid incorporation and the ability to pulse-label the lung phospholipids. The incorporation of the labeled precursors into each phospholipid during the initial 3 h following the administration of isotope is shown in Table III. The ratios of specific activities of the other phospholipids to phosphatidylcholine should not be influenced greatly by the decay of radioactive phospholipids during the initial period. With the palmitic acid label, the specific activities of phosphatidylglycerol and phosphatidylethanolamine were the same. Since phosphatidylglycerol and phosphatidylethanolamine had similar amounts of esterified palmitic acid, both phospholipids incorporated palmitic acid with similar kinetics. Considerably less radioactive palmitic acid appeared in phosphatidylinositol or phosphatidylethanolamine plus phosphatidylserine. These phospholipids contain much less palmitic acid; yet a comparison of the ratio of the specific activity of phosphatidylglycerol to phosphatidylglycerol (0.25 and 0.17, respectively, Table II) indicated that these phospholipids incorporated radioactivity originating from palmitic acid at higher specific activity relative to that of the palmitic acid in phosphatidylcholine (Table III). The complication of varying fatty acid composition was avoided with the glycerol label, and considerably less labeled glycerol appeared in phosphatidylglycerol and phosphatidylethanolamine plus phosphatidylserine. Glycerol is a common precursor for all the phospholipids, thus, the lower specific activities of phosphatidylglycerol and phosphatidylethanolamine plus phosphatidylserine indicated less synthesis relative to phosphatidylcholine and phosphatidylglycerol.

The labeled glycerol could appear in phosphatidylglycerol as either the backbone diglyceride or as the free glycerol esterified to phosphate. The high specific activity ratios of phosphatidylglycerol to phosphatidylcholine (Table III) represented differential and rapid labeling of phosphatidylglycerol at the unacylated glycerol position. The ratios of specific activities of phosphatidate acid obtained by phospholipase D treatment of phosphatidylglycerol to phosphatidylglycerol were constant at 0.39 ± 0.05 (mean ± S.D.) for a period of at least 23 h after the injection of isotope, indicating that about

**TABLE II**

**Fatty acid composition of phospholipids from rabbit lung fractions**

The fatty acid compositions of the phospholipids from lung fractions were determined by gas chromatography of the fatty acid methyl esters. The results are presented as mean percent composition ± standard deviation of at least four analyses, except for single analyses where no standard error is given. —, trace amounts or not detected.

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylglycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Saturated</td>
<td>Phosphatidylglycerol</td>
<td>Phosphatidylethanolamine and phosphatidylserine</td>
</tr>
<tr>
<td>14:0</td>
<td>0.8 ± 0.6</td>
<td>1.4 ± 0.9</td>
</tr>
<tr>
<td>16:0</td>
<td>1.2 ± 0.7</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>16:1</td>
<td>1.8 ± 1.0</td>
<td>1.2 ± 1.1</td>
</tr>
<tr>
<td>16:2</td>
<td>17.9 ± 4.1</td>
<td>17.2 ± 2.2</td>
</tr>
<tr>
<td>16:8</td>
<td>14.0 ± 2.8</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td>16:17</td>
<td>12.7 ± 3.5</td>
<td>14.7 ± 2.9</td>
</tr>
<tr>
<td>18:2 + 20:1</td>
<td>0.7 ± 0.5</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>20:4</td>
<td>39.5 ± 11.0</td>
<td>38.3 ± 6.0</td>
</tr>
<tr>
<td>% Saturated</td>
<td>31%</td>
<td>30%</td>
</tr>
</tbody>
</table>

**TABLE III**

**Specific activity of phospholipids relative to phosphatidylcholine in lung parenchyma ½ to 3 h after isotope injection**

The rabbits were injected with [14C]glycerol and [14H]glycerol as described under “Materials and Methods.” The phospholipids from the lung parenchyma were separated by thin layer chromatography and the specific activity of each phospholipid was determined as counts per min per pmol of phospholipid phosphate. The values are the ratios of the specific activities of the phospholipid to phosphatidylcholine for 3 h following the administration of isotope. PG, phosphatidylglycerol; PI, phosphatidylcholine; PE + PS, phosphatidylethanolamine + phosphatidylserine.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>0.5</td>
<td>1.11 ± 0.10</td>
<td>0.389 ± 0.041</td>
</tr>
<tr>
<td>1</td>
<td>1.06 ± 0.05</td>
<td>0.452 ± 0.024</td>
</tr>
<tr>
<td>2</td>
<td>1.11 ± 0.08</td>
<td>0.358 ± 0.024</td>
</tr>
<tr>
<td>3</td>
<td>0.94 ± 0.06</td>
<td>0.376 ± 0.027</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>1.05 ± 0.18</td>
<td>0.394 ± 0.041</td>
</tr>
</tbody>
</table>
68% of the glycerol radioactivity incorporated into the phosphatidylglycerol molecule appeared as the unacylated glycerol.

Radioactivity from both precursors appeared at maximal specific activity in the phospholipids isolated from microsomes within ½ h of the injection of isotope. Much shorter time periods would be required to accurately measure the kinetics of incorporation of the precursors into phospholipids of microsomal fractions.

The appearance in the lamellar body fraction of labeled phosphatidylcholine and phosphatidylglycerol was measured. Inadequate data were obtained for the other phospholipids because of the very small quantities present in this fraction (Table I). Within 2 h of the administration of the precursors, both phospholipids labeled with either \[^{3}H\]glycerol or \[^{14}C\]palmitic acid were at maximal specific activities. A decrease in the maximal specific activity of phosphatidylcholine or phosphatidylglycerol from the lamellar body fraction was not evident until 7 to 9 h after isotope injection.

The labeled phospholipids appeared in the alveolar wash in a relatively linear fashion for a period of at least 9 h (Fig. 1). Over the period of linear accumulation in the alveoli, the ratios of the specific activities of the labeled phospholipids to phosphatidylcholine were similar to ratios of the specific activities in the lung parenchyma (Table III). Again, the specific activities of phosphatidylcholine and phosphatidylglycerol labeled with palmitic acid were the same.

**Disappearance of Labeled Phospholipids from Lung Fractions**—The disappearance of the labeled phospholipids from each of the four lung fractions was measured over a period of 3 days. Figs. 2 to 5 present the decay data for the phospholipids labeled with \[^{14}C\]palmitic acid from lung parenchyma, microsomes, lamellar bodies, and the alveolar wash, respectively. Very similar curves were obtained when \[^{3}H\]glycerol was used as the labeled precursor of the phospholipids. The biological half-life values determined with both precursors are summarized in Table IV.

The decay of phosphatidylcholine from the parenchyma labeled with palmitic acid or glycerol was approximated by a two-component decay curve (Fig. 2, Table IV), while the data for the decay of the other phospholipids approximated single exponential decay curves.

A two-component exponential decay curve was fit to the data for the disappearance of phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol from the microsomes (Fig. 3). The initial decay rates were quite rapid, being 7, 4, and 7 h for phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol, respectively. The change in specific activity measured for phosphatidylethanolamine plus phosphatidylserine was small.

The disappearance of labeled phosphatidylcholine and phosphatidylglycerol from the lamellar bodies was represented by single-component exponential curves (Fig. 4). The half-life values for phosphatidylcholine labeled with \[^{14}C\]palmitic acid and \[^{3}H\]glycerol were the same, but longer than corresponding half-life values for phosphatidylglycerol (Table IV). The similarity of the half-life values with both isotopes indicated that phosphatidylcholine and phosphatidylglycerol labeled with glycerol or palmitic acid possibly were turned into...
TABLE IV

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Lung fraction (phospholipid precursor)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung parenchyma</td>
</tr>
<tr>
<td></td>
<td>Glycerol Palmitate</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>8 (0.873) 10 (0.947)</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>30 (0.890) 34 (0.936)</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>11 (0.968) 18 (0.987)</td>
</tr>
<tr>
<td>Phosphatidylethanolamine + phosphatidylerine</td>
<td>80 (0.786) 124 (0.671)</td>
</tr>
</tbody>
</table>

Biological half-life values of phospholipids in lung fractions (hours)
The biological half-life values for the phospholipids labeled with \[^{14}C\]palmitic acid were calculated from the regression equations of the data presented in Figs. 3 to 6. Half-life values similarly were determined for the phospholipids labeled with \[^{3}H\]glycerol. The longer half-life values where two values are given indicate the slow decay components if the decay curves had two slopes, as in Figs. 3 and 4. Data were inadequate to determine half-life values. The r values for the regression equations are given in parentheses to the right of each half-life value.

**DISCUSSION**
Previously no studies have measured labeled precursor incorporation into a number of lung phospholipids simultaneously in vivo. Although uncertainty remains as to the function of the phospholipids other than dipalmitoylphosphatidylcholine over in the lamellar body fraction as intact molecules, since the labeled acyl groups and the labeled backbone glycerol of the phospholipids had similar half-life values. The data for phosphatidylinositol and phosphatidylethanolamine plus phosphatidylerine were not reliable because of the very small amounts of these phospholipids in the lamellar body fraction.

The decay curves for the phospholipids from the alveolar wash labeled with \[^{14}C\]palmitic acid are shown in Fig. 5. The data points were approximated by simple exponential decay curves. The relative order of biological half-lives was phosphatidylglycerol < phosphatidylcholine < phosphatidylinositol < phosphatidylethanolamine plus phosphatidylerine. The ratios of the specific activities of phosphatidic acid derived from phosphatidylglycerol to phosphatidylglycerol labeled with \[^{3}H\]glycerol did not change and was 0.42 ± 0.06 (S.D.), indicating that there was not preferential decay of either of the glycerol labels.

![Figure 3](https://example.com/fig3.png)

**FIG. 3.** Disappearance of phospholipids labeled with \[^{14}C\]palmitic acid from rabbit lung microsomes. All data were obtained and presented as in Fig. 2, except the sources of the phospholipids were the microsomal fractions isolated by ultracentrifugation. The extrapolated specific activity values at \(t = 0\) were as follows: phosphatidylcholine (PC), 6554 cpm/\(\mu\)mol; phosphatidylglycerol (PG), 8177 cpm/\(\mu\)mol; phosphatidylinositol (PI), 1649 cpm/\(\mu\)mol; phosphatidylethanolamine + phosphatidylerine (PE + PS), 1900 cpm/\(\mu\)mol.

![Figure 4](https://example.com/fig4.png)

**FIG. 4.** Disappearance of phosphatidylcholine (PC) and phosphatidylglycerol (PG) labeled with \[^{14}C\]palmitic acid and \[^{3}H\]glycerol from the lamellar bodies. All data were obtained and presented as in Fig. 2; however, the sources of the phospholipids were the lamellar body fractions of rabbit lungs. Results for both labeled palmitic acid and glycerol are presented and the appropriate isotopically labeled precursor is indicated on the figure. The extrapolated specific activity values at \(t = 0\) were as follows: phosphatidylcholine labeled with \[^{3}H\]glycerol, 3043 cpm/\(\mu\)mol; phosphatidylcholine labeled with \[^{14}C\]palmitic acid, 5725 cpm/\(\mu\)mol; phosphatidylglycerol labeled with \[^{3}H\]glycerol, 7686 cpm/\(\mu\)mol; phosphatidylglycerol labeled with \[^{14}C\]palmitic acid, 4576 cpm/\(\mu\)mol.

![Figure 5](https://example.com/fig5.png)

**FIG. 5.** Disappearance of phospholipids labeled with \[^{14}C\]palmitic acid from rabbit alveolar wash. All data were obtained and presented as in Fig. 2, except the source of the phospholipids was the alveolar wash. The extrapolated specific activity values at \(t = 0\) were as follows: phosphatidylcholine (PC), 7365 cpm/\(\mu\)mol; phosphatidylglycerol (PG), 6771 cpm/\(\mu\)mol; phosphatidylinositol (PI), 2023 cpm/\(\mu\)mol; phosphatidylethanolamine plus phosphatidylerine (PE + PS), 1282 cpm/\(\mu\)mol.
tidylcholine in surfactant (10), compositional analysis of surfactant phospholipids demonstrates that monoenoic phosphatidylcholine species plus the "minor" phospholipids comprise 47% of purified dog surfactant phospholipids (1). The data for rabbit surfactant are similar (17). These other phospholipids must be considered as an integral part of surfactant.

The lung is composed of at least 40 cell types and only the type II pneumocyte synthesizes surfactant phospholipids for storage and secretion (24). Ideally these studies should be done in a pure culture of metabolically "normal" type II cells, and methods for isolation of lung cell types are improving (25). However, in vivo studies remain essential to establish the physiologic norm for later comparison with any in vitro model systems.

Macklin first attributed the metabolism of surfactant to the type II pneumocyte (26), and Askin and Kuhn demonstrated by autoradiographic electron microscopy preferential labeling of the phospholipids of the type II cells following pulse labeling with palmitic acid (27). These results were confirmed using labeled palmitic acid to study the labeling pattern of lung cells 1 to 10 min after isotope injection (28). Chevalier and Collet followed the incorporation and subcellular localization within the type II cell of [3H]choline and [3H]glycerol in mice (29).

Shortly after choline injection, the tritium label was localized almost exclusively over the endoplasmic reticulum of the type II pneumocyte. Later the label moved via the golgi to the lamellar bodies and the alveolar space. This anatomical description of sequential subcellular localization of pulmonary phosphatidylcholine stimulated the study of subcellular fractions pulse-labeled with phospholipid precursors reported here.

The results for phosphatidylcholine labeled with [14C]palmitic acid were similar to those previously obtained with a similar group of animals (14); however, glycerol had not been studied as a precursor of lung phosphatidylcholine in rabbits. The two labeled precursors become incorporated into phosphatidylcholine and the other phospholipids by very different pathways. The glycerol label appears as the backbone diglyceride of all of the phospholipids. Palmitic acid will become incorporated either into phosphatidic acid or by the several proposed deacylation-reacylation reactions. The labeled palmitic acid seems to be incorporated without modification into rabbit lung phosphatidylcholine (12). In spite of the different pathways of incorporation, the specific activities of phosphatidylcholine labeled with either glycerol or palmitic acid increased in parallel with time in the lipid extracts of the microsomes, lamellar bodies, and alveolar wash (Fig. 1). The biological half-life values for phosphatidylcholine labeled with either glycerol or palmitic acid measured in each of the lung fractions were essentially equivalent (Table IV). These biological half-life values indicate that the phosphatidylcholine molecule may turn over as a unit; however, half-life values measured with [3H]choline or [3H]glycerol are much longer (14). Prolonged initial incorporation or reutilization of the choline or phosphate precursors may explain the longer half-life values.

The two-component exponential decay curves described for the phosphatidylcholine in the lung parenchyma and microsome fraction have been discussed (14). Since the lung is a heterogeneous mixture of cell types, the different decay rates may represent phosphatidylcholine metabolism in different cell populations. The two decay rates do not represent simply the metabolism of disaturated versus unsaturated phosphatidylcholine species, since disaturated and total phosphatidylcholines from parenchyma and alveolar wash have similar half-life values (14).

Phosphatidylglycerol is present in most mammalian tissues in only trace amounts; however, lung tissue contains appreciable amounts and the alveolar wash contains even more (17, 20). Phosphatidylglycerol has been proposed as a stabilizer of the surfactant lipoprotein complex and as essential for the surface-active characteristics of the lung (30). Developmental studies demonstrate that the appearance of phosphatidylglycerol coincides with mature lung function in the human neonate (16). In vivo studies in rats indicate that phosphatidylglycerol is synthesized primarily in lung microsomes, as are the other phospholipids (22). The phosphatidylglycerol synthesized by mitochondria may be utilized directly as a precursor of cardiolipin (22). Rat lung perfusion studies with labeled glucose, acetate, or lactate demonstrated that phosphatidylglycerol had a higher specific activity than did the other lung phospholipids. However, exogenous labeled palmitic acid preferentially labeled phosphatidylcholine (31). In the rabbit in vivo at times shortly after [3H]palmitic acid injection, labeled palmitic acid appeared at equal specific activities in phosphatidylglycerol and phosphatidylcholine (Table III). Since palmitic acid represents 50 and 48% of the fatty acid residues for parenchymal phosphatidylcholine and phosphatidylglycerol, respectively, the incorporation of labeled palmitic acid was equivalent for the two glycerolipids.

The biological half-life values for phosphatidylglycerol in each of the cell fractions may be more representative of surfactant metabolism than are the half-life values of the phospholipids more integral to general cellular metabolism. The disappearance of labeled phosphatidylglycerol from the microsomal phospholipids was faster than was the disappearance of the other phospholipids (Fig. 2, Table IV). Decay curves for parenchymal phosphatidylglycerol labeled with either [3H]glycerol or [3H]palmitic acid were simple exponential curves with short half-life values relative to those for the other phospholipids. The half-life values for labeled phosphatidylglycerol in the lamellar bodies or alveolar wash were similarly shorter than those measured for the other phospholipids. Taken together the data indicated that phosphatidylglycerol was an actively metabolized phospholipid with probably no pulmonary function other than as a component of surfactant. Phosphatidylglycerol may be the unique phospholipid in surfactant, and thus may represent as good a biochemical marker for the study of surfactant metabolism as is dipalmitoylphosphatidylcholine.

Phosphatidylinositol remains a little studied pulmonary phospholipid. Phosphatidylinositol demonstrated a small increase in the percentage of saturated fatty acid residues in alveolar versus microsomal fractions (Table III), but the phospholipid was unique in that the percentage of composition in the lung fractions studied was relatively constant at 3 to 5% (Table I). Labeled phosphatidylinositol had longer measured half-life values in the lung fractions than did phosphatidylcholine or phosphatidylglycerol. Developmental data indicating that phosphatidylinositol is involved in the maturation of pulmonary surfactant in preparation for birth is the only strong indication that this glycerolipid is important to surfactant function (16).

Phosphatidylethanolamine and phosphatidylserine have been studied together because both phospholipids represent highly unsaturated phospholipid classes (Table II) that are preferentially excluded from surfactant. These phospholipids are not surface active (11) and are generally considered as integral membrane components. They are much less metabolically active than are the other phospholipids studied, having lower initial specific activities and longer half-life values. The data implies that phosphatidylethanolamine and phosphatidylserine are metabolized in lung similarly to the liver metabolism of these compounds (32).
REFERENCES

Labeling of phospholipids in the surfactant and subcellular fractions of rabbit lung.

A Jobe, E Kirkpatrick and L Gluck