Surfactant Phosphatidylcholine Source, Fluxes, and Turnover Times in 3-day-old, 10-day-old, and Adult Rabbits*

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We have measured turnover times for alveolar surfactant phosphatidylcholine in rabbits at 3 and 10 days of age and in adult rabbits. To generate accurate estimates of the turnover time of surfactant phosphatidylcholine from lamellar bodies into the alveolar space, large numbers of rabbits at each age were injected with radiolabeled choline, palmatic acid, and phosphate. Phosphatidylcholine was isolated from lamellar body and surfactant fractions from each rabbit. Curves of specific activity versus time were analyzed using the Zilversmit equations for a two compartment precursor-product model. The curves for each labeled precursor at each age were consistent with the lamellar bodies being the sole precursors of surfactant phosphatidylcholine. The same equations were used to calculate turnover times for surfactant phosphatidylcholine; these were 8-10 h in 3- and 10-day-old rabbits but only 3 h in adult rabbits. From estimates of surfactant phosphatidylcholine pool sizes and turnover times, the flux of surfactant phosphatidylcholine was estimated to be 3.4 µmol/h in adult and 0.48 µmol/h in 3-day-old rabbits; however, the flux expressed/kg of body weight is 2.4 times larger in 3-day-old than in adult rabbits. Finally, the conservation of the radiolabeled precursors in phosphatidylcholine implies reutilization of phosphatidylcholine after synthesis and secretion.

Surfactant recovered by lavage of airways is about 80% phospholipid by weight (1). Phosphatidylcholine accounts for about 80% of the total phospholipid in surfactant (2, 3), and the major surface active component, dipalmitoylphosphatidylcholine, represents about 70% of the total phosphatidylcholine (4). Thus, most studies of surfactant metabolism have concentrated on the phosphatidylcholine component of surfactant. The biological half-lives of phosphatidylcholine in whole lung and in the alveolar space have been estimated by injecting various animal species with radioactive precursors of phosphatidylcholine (3, 5-9). These half-life values have been used to estimate secretion rates of phosphatidylcholine from lung to the alveoli and turnover times for alveolar phosphatidylcholine (5, 7). However, specific activity-time curves indicate that lung tissue is rapidly labeled with some precursors, but alveolar phosphatidylcholine is not, which prolongs the biological half-life of alveolar phosphatidylcholine (6). This lack of pulse labeling may lead to inaccuracies when biological half-life values are used for further calculations (10). Toshima et al. (5) and Thomas and Rhoades (7) calculated turnover times for alveolar phosphatidylcholine assuming that whole lung phosphatidylcholine is the precursor of alveolar space phosphatidylcholine. However, this assumption oversimplifies phosphatidylcholine metabolism in the lung.

While the lung contains many cell types, surfactant synthesis occurs in the microsomes of the Type II cell and subsequently is packaged into lamellar bodies for secretion (11, 12). Jobe et al. (3, 6, 13) injected adult and newborn rabbits with radioactive precursors of phosphatidylcholine. After an alveolar wash, they used the techniques described by Gil and Reiss (14) to isolate lamellar bodies and obtained curves of specific activity versus time for phosphatidylcholine in the lamellar bodies and in alveolar surfactant. Using the same techniques, we injected large numbers of 3-day-old, 10-day-old, and adult rabbits with labeled phosphatidylcholine precursors. We killed groups of rabbits at preset times chosen to better define the curves of specific activity versus time for phosphatidylcholine from whole lung, lamellar bodies, and alveolar surfactant. We then used the Zilversmit model (10) as adapted by Clements from the fetal-maternal research laboratories to determine the turnover time of alveolar phosphatidylcholine and to estimate the flux of phosphatidylcholine into the alveoli.

Materials and Methods
Isotopes—[1-14C]Palmitic acid (52 Ci/mmol), [9,10-3H]palmitic acid (17.6 Ci/mmol), [methyl-3H]choline (80 Ci/mmol), [methyl-14C]choline (64.5 Ci/mmol), and carrier-free ortho[14C]phosphate were purchased from New England Nuclear. Stock solutions of 1 mCi/ml of [3H]palmitate or 100 µCi/ml of [14C]palmitate were prepared with 5% bovine serum albumin in saline (6). For the 3-day-old rabbits, a final isotope solution contained 7.5 µCi/ml of [14C]choline and 125 µCi/ml of [3H]palmitate. The rabbits were injected with 0.4 ml/100 g of body weight of isotope solution via an external jugular vein. The 10-day-old rabbits received, via an external jugular vein, 0.2 ml/100 g of body weight of an injection solution containing 15 µCi of [14C]choline and 500 µCi of [3H]palmitate/ml. Adult rabbits were injected via a marginal ear vein with 0.6 ml/kg of a solution containing 42 µCi/ml of [3H]choline, 50 µCi/ml of [14C]palmitate, and 42 µCi/ml of [3H]palmitate. Animals—Eight-four 3-day-old New Zealand White rabbits weighing (mean ± S.E.) 64.5 ± 1.2 g were removed from litters on the day of isotopic injection. The rabbits were fed 7 ml of infant formula (20 cal/oz) by oral gastric tube twice a day. Animals were anesthetized with intraperitoneal pentobarbital and killed by severing the abdominal aorta. The rabbits were studied as two groups of equal size 1 week apart. 4–5 rabbits were killed at one of 20 preset times to 96 h after isotope injection. One-half of the rabbits at each preset killing time were studied each week. This experimental design should decrease a systematic error which might result, for example, from...

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1 J. Clements, personal communication.
measuring the first half of the time points one week and the second half of the time points the next week. Fifty-four 10-day-old New Zealand White rabbits weighing 195.6 \( \pm 4.4 \) g were killed in groups of 4 or 5 animals at one of 11 preset time points over 12 h. Because of the short time course of this experiment, all rabbits were studied on a single day. The rabbits were not fed.

Eighty adult female New Zealand White rabbits weighing 1.05 \( \pm 0.1 \) kg were studied in two groups 1 week apart. Two rabbits at each of 20 preselected times following isotope injection were killed each week with intravenously injected pentobarbital and exsanguinated by cutting the abdominal aorta. The rabbits were allowed free access to water and rabbit chow during the 72-h experimental period. All rabbits at each age group were injected from a single batch of isotopic mixture. The killing times selected were chosen to accurately reflect the changes in the curves of specific activity versus time as suggested by previous data (6, 13).

Fraction Isolation—Immediately after killing, the chest was opened, the trachea cannulated, and the lungs were inflated with normal saline to total lung capacity. This saline was washed in and out three times and collected in a beaker. This same procedure was repeated four more times and the five washes were pooled. The total volume of the alveolar wash was recorded and the alveolar wash stored frozen at \(-20^\circ\) C. This procedure should remove \( >90\% \) of the surfactant recoverable by alveolar wash (4). The lungs were removed, weighed, degassed, and homogenized with a motor-driven Teflon pestle homogenizer in 8 ml of 0.32 M sucrose, 0.01 M Tris-HCl, 0.15 M NaCl, 0.001 M CaCl\(_2\), 0.001 M MgSO\(_4\), and 0.0001 M EDTA at pH 7.4, homogenized to as sucrose buffer (6). 0.5 ml of this homogenate was saved to measure the specific activity of total lung phosphatidylcholine.

Lamellar body fractions were then isolated at the interface between 0.45 M sucrose and 0.55 M sucrose buffers according to Gil and Reiss (14) as applied to rabbit lung (6). The lamellar bodies were recovered as a pellet after centrifugation at 20,000 \( \times g \) for 20 min. The isolation procedure for lamellar bodies from lung homogenate of 10-day-old rabbits was the same as for 3-day-old rabbits except that the lungs were homogenized in 16 ml of 0.32 M sucrose buffer.

The lungs were homogenized in 25 ml of 0.32 M sucrose buffer. The isolation procedure for lamellar bodies was the same as that for 3-day-old rabbits except that two lamellar body fractions were collected. The final step gradient contained 7 ml of 0.7 M sucrose buffer, 7 ml of 0.55 M sucrose buffer, and 7 ml of 0.45 M sucrose buffer. The band recovered between 0.45 M and 0.55 M sucrose was equivalent to the lamellar body fractions recovered from 3-day- and 10-day-old rabbits. A second band between 0.55 M and 0.7 M sucrose was recovered as a high density lamellar body fraction.

**Specific Activity Measurements**—Lipids in alveolar wash, lung, and lamellar body fractions were extracted according to Bligh and Dyer (4) as applied to rabbit lung (6). Phospholipids were isolated from phospholipid samples from 3-day-old and adult rabbits by one-dimensional thin layer chromatography as before (6). The phosphatidylcholine thus contained a small amount of phosphatidylinositol (<5% of the phosphatidylcholine by phosphate analysis) (3). Each sample was spotted quantitatively in duplicate. One spot was used for phosphate analysis (16) and the duplicate for radioactivity measurements. All radioactivity was measured in Aquasol-2 (New England Nuclear). Cross-channel contamination was estimated by adding either \( [\text{H}]\)oleune, [\( \text{C} \)]oleune, or inorganic \( [\text{P}] \) phosphate standards to several different samples and blanks followed by recounting these vials. No quenching was observed at the quantities of phosphatidylcholine used. All specific activities are expressed as counts/min/\( \)\( \mu \)mol of phosphatidylcholine. Phosphatidylcholine was isolated from samples from 10-day-old rabbits by two-dimensional thin layer chromatography (3). Aliquots of the phosphatidylcholine solution were spotted as phosphatidylcholine for specific activity and radioactivity determinations.

**Data Analysis of Specific Activity-Time Curves**—For 3-day-old and adult rabbits, specific activities for phosphatidylcholine from lamellar body and alveolar wash fractions were corrected for variations in lung specific activity (17). A semilog plot which best describes the data for lung specific activity versus time after injection from the peak of the radioactive label is used to plot the specific activity curve to the end of the experiment was determined by the method of least squares. The specific activities of the phospholipids in lamellar body and surfactant fractions were corrected by using the ratio of each experimental lung specific activity to the value calculated from the derived decay curve as a correction factor.

For data from 10-day-old rabbits, corrected specific activities were not used because the time course of the experiment was not sufficiently long to accurately determine equations for the decay of lung specific activities. Corrected specific activities (uncorrected for 10-day-old rabbits) were averaged for all rabbits killed at the same time. Any value falling more than 3 standard errors from the mean in any fraction was eliminated and the remainder were re-averaged. This eliminated no more than 1 rabbit/group and less than 10% of the rabbits at any age were discarded, leaving 3-5 rabbits in all groups.

The plot of these average specific activities versus time are referred to as specific activity-time curves.

**Mathematical Analysis**—From specific activity-time curves we calculated the turnover time for phosphatidylcholine in the alveolar space for each isotope for each age group of rabbits. This was done by applying the equations derived by Zilversmit (10) for the two-compartment model shown in Fig. 1. The assumptions for this analysis are 1) that alveolar wash and lamellar body pool sizes are stable; 2) that all material in the lamellar bodies flows into the alveolar space; and 3) that mixing within any pool is instantaneous. The radioactive label may be reutilized without affecting our calculations. (See the Appendix for derivation and equations.)

From the specific activities at each time point, the area between the lamellar body and alveolar wash curves was obtained from time 0 to each time point by a trapezoidal approximation. This area was plotted against the alveolar wash specific activity at each time \( t \). The slope of the best fit line for these points gives the turnover time for alveolar phosphatidylcholine. This line is determined by the method of least squares and is referred to as the area plot. For the \( ^{3}P \) label in adult rabbits, the area plot is shown only until the peak of the alveolar wash specific activity. Since there is very little downslope to the specific activity curves for lamellar bodies and alveolar wash, this part of the curve is inaccurate for this type of calculation.

We quantified the pool size of phosphatidylcholine recovered by the alveolar wash procedure to determine the flux (flux = pool size/turnover time) (10). We have assumed that the alveolar wash procedure recovered the entire airway pool of phosphatidylcholine; a reasonable assumption based on information from several investigators (4, 7, 8). The accuracy of the pool size measurement does not affect the turnover time.

To determine the total area under each specific activity-time curve for each set of data, a computer-generated smooth fitting equation was obtained by successive least squares approximations. By extrapolating this equation to infinity, we were able to approximate the total area under the curve. This was not possible for 10-day-old rabbits since only the first part (upslope) of the curves was defined experimentally. All comparisons were made using the Student’s two-tailed \( t \) test.

**RESULTS**

**3-day-old Rabbits**—Fig. 2A shows the specific activities of \( [\text{H}]\)palmitic acid-labeled phosphatidylcholine in lamellar bodies and in alveolar wash over the 96-h study period. Fig. 2B shows the specific activity of \( [\text{C}]\)choline-labeled phosphatidylcholine in the same fractions. All four sets of data fit smooth curves with well defined upslopes to maximum specific activities and well defined downslopes. The decay rates defined by the downslopes of the alveolar wash curves give biological halflives of 50 h for \( [\text{H}]\)palmitate-labeled phosphatidylcholine and 80 h for \( [\text{C}]\)choline-labeled phosphatidylcholine. The inset in Fig. 2A shows the area plot with the best fit line giving a turnover time of 10.7 h for \( [\text{H}]\)palmitate-labeled phosphatidylcholine. The inset in Fig. 2B shows the
are shown in Fig. for [3H]choline, and 2.5 h for 32P. The biological half-life
ments-The average quantity of phosphatidylcholine in the
are the area plot and best fit line for [14C]choline-labeled phosphatidylcholine, giving a turnover time of 8.6 h.
10-day-old Rabbits—Fig. 3A shows the specific activity-time curves for phosphatidylcholine from lamellar bodies and alveolar wash from 10-day-old rabbits labeled with [3H]palmitate label. Fig. 3B shows the specific activity-time curves for the same fractions from 10-day-old rabbits with [14C]choline labeling the phosphatidylcholine. The turnover times defined by the area plots are ±6 h for palmitate-labeled phosphatidylcholine and 8.2 h for choline-labeled phosphatidylcholine.
Adult Rabbits—Specific activity-time curves for phosphatidylcholine from lamellar body and alveolar wash fractions are shown in Fig. 4, A and B for the [14C]palmitate and [3H] choline labels. Fig. 5 shows the data derived with 32P-labeled phosphatidylcholine. The turnover times determined by the slopes from the area plots are 3.0 h for [14C]palmitate, 3.8 h for [3H]choline, and 2.5 h for 32P. The biological half-life values and turnover times for all age groups are summarized in Table I.
The specific activities of low and high density lamellar body fractions recovered from adult rabbit lungs at each killing time and for each isotope were compared. There were no differences at any time for any isotope.

Flux and Area under Specific Activity Curve Measurements—The average quantity of phosphatidylcholine in the alveolar wash was measured for rabbits of all three age groups (Table II). These pool sizes were used to calculate the flux of phosphatidylcholine from lamellar bodies to the alveolar space for each age group. Dividing flux by average animal weight gives the flux/kg. An assumption for both the turnover and flux measurements is that pool sizes do not change. For all three age groups, the average micromoles of phosphatidylcholine recovered in the alveolar wash was plotted against the time of killing. At the three ages, the amount of phosphatidylcholine in the alveolar washes was statistically unchanged throughout the course of the experiments (data not shown).

We compared the total area under each specific activity-time curve for lamellar bodies in Figs. 2 and 4 with the area under the respective specific activity-time curve for the alveolar wash from time 0 to \( \infty \) using the computer-generated equations for the smooth curves drawn in each figure. The areas under the paired specific activity-time curves for phosphatidylcholine from airways and lamellar bodies were similar, with a mean per cent area difference of 6.6%.

Total Phosphatidylcholine-Associated Radioactivity—In 3-day-old and adult rabbits, the total (alveolar plus lung) [3H] counts/min and total [14C] counts/min recovered in phosphatidylcholine were averaged at each time. Total counts/min were calculated from phosphatidylcholine recovered from aliquots of the lung homogenate and the alveolar wash. A line determined by the method of least squares was fit to these averages. The slope of this line gives the half-life for total counts/min in phosphatidylcholine. The half-lives for total [3H]palmitate-labeled phosphatidylcholine and total [14C]choline-labeled phosphatidylcholine in 3-day-old rabbit lung are 97 h and 125 h, respectively. These half-lives are not statistically different (\( p > 0.05 \)). For adult rabbits, the half-lives of
FIG. 4. Specific activity-time curves and area plots for adult rabbits. A, [14C]palmitate label. Symbols and curves as in Fig. 2A. The slope of the area plot in the inset is 3.0 h (r = 0.92). B, [3H]choline label: Symbols and curves as in Fig. 2A. The slope of the area plot shown in the inset is 3.8 h (r = 0.96).

TABLE I
Values derived from labeling curves

For each isotope, the areas under the computer-generated specific activity time curves for lamellar bodies and alveolar wash from 0 to \( \infty \) are calculated. The difference between these curves is divided by the larger of the two, then multiplied by 100, giving a percent area difference between 0 and 100. A value close to 0 suggests a precursor-product relationship.

<table>
<thead>
<tr>
<th>Age of rabbits</th>
<th>Precursor</th>
<th>Biological half-life</th>
<th>Turnover time</th>
<th>Area difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-day</td>
<td>[3H]palmitate</td>
<td>50</td>
<td>10.7</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>[14C]choline</td>
<td>80</td>
<td>8.6</td>
<td>8.8</td>
</tr>
<tr>
<td>10-day</td>
<td>[3H]palmitate</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[14C]choline</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>[3H]palmitate</td>
<td>20</td>
<td>3.8</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>[14C]choline</td>
<td>45</td>
<td>3.0</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>[3H]choline</td>
<td>25</td>
<td>2.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

TABLE II
Flux of surfactant phosphatidylcholine from lamellar bodies into the alveolar space

<table>
<thead>
<tr>
<th>Age of rabbits</th>
<th>Alveolar wash pool size</th>
<th>Flux*</th>
<th>Flux/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-day</td>
<td>4.67 ± 0.16 (n = 84)</td>
<td>0.48</td>
<td>7.5</td>
</tr>
<tr>
<td>10-day</td>
<td>4.11 ± 0.21 (n = 54)</td>
<td>0.46</td>
<td>2.4</td>
</tr>
<tr>
<td>Adult</td>
<td>10.50 ± 0.46 (n = 80)</td>
<td>3.40</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Flux was calculated from the average pool size and average turnover time for each age group for palmitate and choline labels. Because of the type of calculation involved in arriving at a turnover time and flux (see the Appendix) we have not defined a standard error for these measurements.

Discussion

Evidence has accumulated indicating that surfactant is synthesized in the lung Type II cell and packaged in lamellar bodies for secretion to the alveoli. There has been no demonstration of a precursor-product relationship between lamellar bodies and airway surfactant. If such a precursor-product relationship exists for the complicated mixture known as surfactant, then it must hold for any single component of surfactant. The major component of surfactant is phosphatidylcholine. Surfactant phosphatidylcholine is a mixture of saturated phosphatidylcholine and unsaturated phosphatidylcholine species. However, the fatty acid compositions of the phosphatidylcholines and percentage of saturated phosphatidylcholines recovered from surfactant and lamellar bodies are identical (3).

If an isotope pulse labeled the alveolar wash pool and was not reutilized, then the biological half-life determined with that isotope would equal the turnover time multiplied by 0.69. Furthermore, the biological half-lives for two different isotopes fulfilling these criteria and labeling the same molecular species should be the same. However, in previous measurements in rats, rabbits, and lambs, the biological half-lives of airway associated-phosphatidylcholine labeled with intravenously administered radioactive palmitic acid, choline, or phosphate have been different (3, 6, 18, 19). The approach we used to demonstrate a precursor-product relationship and to calculate a turnover time does not rely on pulse labeling nor does it depend on lack of reutilization of surfactant (both of which would prolong the biological half-life).

Several criteria must be fulfilled to document a precursor-product relationship for the two-compartment model proposed (10) (see the Appendix). 1) The specific activity in the precursor pool must rise and fall before that in the product pool. This is true for phosphatidylcholine labeled with radioactive palmitate, choline, and phosphate. 2) The two curves should cross at the peak specific activity of the product pool. The point of intersection in our study varies somewhat but is near the predicted location in all figures. 3) The areas under the lamellar body and alveolar wash specific activity-time curves from time 0 to \( \infty \) for a given label should be identical. The areas under the curves agreed closely (Table I) giving a
percentage of area difference near 0. 4) The area plot (see "Materials and Methods") should give a straight line. The area under the alveolar wash specific activity-time curve is subtracted from the area under the lamellar body specific activity-time curve such that, after the intersection, further contributions to the area plot are negative. If a variation from a precursor-product relationship exists, then the upslope and downslope of the area plot will not superimpose. The correlation coefficient of the data for the area plot is an indication of the tendency of the curve to depart from a precursor-product relationship. The correlation coefficients of the area plots all exceed 0.92, indicating a precursor-product relationship.

The 3-day-old rabbits showed no significant weight gain over the course of the experiment. If the rabbits had been cared for by their mothers, they would have gained about 45 g and the lamellar body and alveolar wash phosphatidylcholine pool sizes may have increased (20). Thus, the lack of growth, while not normal, resulted in a stable alveolar wash phosphatidylcholine pool size. If this lack of growth had altered surfactant metabolism, then the early time points on the area plot would have varied from the remainder of the points resulting in a deviation from a straight line. As seen in Fig. 2, this did not occur. The recovery of phosphatidylcholine from the lamellar bodies of 3-day-old rabbits did not change during the course of the experiment, suggesting that no change occurred in the lamellar body pool size. Since a precursor-product relationship existed for 3-day-old and adult rabbits, we measured specific activities only through the upslope of the alveolar wash specific activity-time curve for 10-day-old rabbits. The 12-h experimental period was selected so that we could assume no changes in phosphatidylcholine pool sizes for the calculation of turnover times. The striking result is the change in turnover time of surfactant phosphatidylcholine from about 9 h for 3-day- and 10-day-old rabbits to about 3 h for adult rabbits.

Values reported by other investigators for turnover times of alveolar wash phosphatidylcholine have varied considerably (5, 7). Magoon et al. (21) and Young et al. (22) have measured alveolar wash phosphatidylcholine turnover times using a similar method. Young et al. (22) used adult rats for their measurements and found a turnover time of about 10 h. Magoon et al. (21) studied 20 adult male rabbits which were larger than ours (average weight about 2 kg), and used slightly different fraction isolation and mathematical analysis techniques. Their results indicate a possible variation from a lamellar body-alveolar wash precursor-product relationship. While we used four times more rabbits of a different size and sex, the explanation for differences in the results is not apparent.2

Since lamellar bodies appear to undergo a maturational process (12, 23), we decided to study lamellar bodies isolated at two different densities from the sucrose gradients. We used the strict criteria of Gil and Reiss (14) for the isolation of lamellar bodies between 0.45 and 0.55 M sucrose solutions. Others have isolated lamellar bodies at various densities between 0.35 and 0.8 M sucrose (24–26). "Lamellar bodies" isolated from 0.55–0.7 M sucrose have a similar phospholipid composition as those isolated between 0.45 and 0.55 M sucrose, but they might have different precursor-labeling characteristics (23). We found that the specific activities of phosphatidylcholine from the 0.55–0.7 M sucrose fractions from adult rabbits were not significantly different from the phosphatidylcholine in the 0.45–0.55 M sucrose fractions.

2 J. A. Clements, M. W. Magoon, and J. Goerke, personal communication.

The differences in biological half-lives we found within age groups depending on the radiolabeled precursor used is not surprising. Individual components of phosphatidylcholine have their own precursor pools and routes of incorporation (27), and there is no reason to expect that the kinetics of metabolism of the precursors should be identical. For example, the precursors are cleared from blood and appear in lung phosphatidylcholine at different rates (28). Furthermore, different parts of a phospholipid molecule might be utilized to different degrees which would also affect the biological half-life. When turnover times for phosphatidylcholine are calculated from the lamellar body and alveolar wash specific activity-time curves, the values show good agreement between the different labeled precursors. We would expect to find a poor correlation coefficient for the area plot if lamellar bodies were precursors for phosphatidylcholine in areas of the lung other than the alveolar wash. Hence, lamellar bodies secrete phosphatidylcholine only into the pool of phosphatidylcholine recovered by alveolar wash.

Our data also provide information about lamellar body internal dynamics. The synthesis of saturated phosphatidylcholine from de novo synthesized unsaturated phosphatidylcholine is probably catalyzed within Type II pneumocytes by lysophosphatidylcholine acyltransferase (29). Surfactant and lamellar body phospholipids other than phosphatidylcholine contain palmitic acid. If after being incorporated into a phosphatidylcholine molecule in the lamellar bodies as isolated here, deacylation-reacylation reactions shifted palmitate to other phospholipids from phosphatidylcholine, then the precursor-product relationship with this precursor would be lost. Thus, this kind of transfer could only occur if it were bidirectional with no net palmitate flux, implying that the specific activity of palmitate in different phospholipids in lamellar bodies would have to be similar. However, the specific activities of the palmitic acid in the different phospholipids in lamellar bodies and alveolar wash are different (3).

The major surface-active component of surfactant is saturated phosphatidylcholine. Our results are valid for this subclass of phosphatidylcholine as well. The previous argument does not preclude transfer of palmitate between phosphatidylcholine molecules. To loose the precursor-product relationship between saturated phosphatidylcholine in lamellar bodies and the alveolar wash, there would have to be primary incorporation of palmitate into saturated phosphatidylcholine. There would then be a subsequent deacylation-reacylation reaction transferring a palmitate from saturated phosphatidylcholine to a phosphatidylcholine with no palmitate. But the equally rapid rate of incorporation of radioactive palmitate into monopalmitoyl and saturated phosphatidylcholine determined by Jobe (28) suggests that monopalmitoyl phosphatidylcholine is not formed from the saturated species. Thus, if any transfer of palmitate does take place between phosphatidylcholine molecules, the chance of a labeled palmitate going in either direction would be the same, so the precursor-product relationship would still hold for saturated phosphatidylcholine.

It is also interesting to consider the flux of phosphatidylcholine from lamellar bodies into the alveolar space. Adult rabbits, not surprisingly, secrete more phosphatidylcholine than younger rabbits. When this flux is expressed per kg of body weight, the 3-day-old rabbits secrete more phosphatidylcholine. By the age of 10 days, the absolute secretion rate has not changed but the flux/kg of body weight approximates that of the adult rabbits. Furthermore, once a labeled precursor enters phosphatidylcholine it remains within the confines of the total lung compartment as part of phosphatidylcholine much longer in 3-day-old than in adult rabbits.
Total lung phosphatidylcholine (excluding alveolar wash phosphatidylcholine) is almost pulse labeled with palmitate (28). If large amounts of radiolabeled palmitate do not enter lung phosphatidylcholine from extra-lung sources or from other pools within the lung, then phosphatidylcholine, once secreted into the alveolar space, must be reutilized. This is demonstrated, for example, by data from the 3-day-old rabbits. The lung phosphatidylcholine pool size in these animals is 15.1 ± 0.24 μmol. The flux of phosphatidylcholine into the alveolar space is 0.48 μmol/h. Not all of the lung phosphatidylcholine is related to surfactant (30). Even if we assume that 50% is, then without reutilization it can be shown that the half-life of lung phosphatidylcholine would be about 17 h. In fact, the half-life of lung phosphatidylcholine labeled with palmitate is 60 h (lung half-life data not shown). Hence, phosphatidylcholine or its component parts finds its way back into lung tissue after secretion into the alveolar space. This concept of reutilization has been suggested previously by Oyarzun et al. (31).

**Acknowledgments**—We wish to thank Drs. Clements, Magoon, and Goerke for sharing their preliminary data with us and for extensive discussions about our experiments and data analysis.

**APPENDIX**

This analysis is adapted from Žilversmit et al. (10). According to the model in Fig. 1:

\[ Q_i = \text{micromoles of phosphatidylcholine in pool } i. \]
\[ q_i(t) = \text{phosphatidylcholine counts per min in pool } i \text{ at time } t. \]
\[ k_{ij} = \text{specific activity of phosphatidylcholine in pool } i \text{ at time } t. \]
\[ k_{ij} = \text{total flux of phosphatidylcholine from pool } i \text{ into pool } j. \]
\[ i \text{ and } j = \text{LB, AW, or out where “out” implies only that the material leaves the pool and does not specify where it goes.} \]

\[ \frac{d}{dt} q_{AW}(t) = k_{LB-AW} [q_{LB}](t) - k_{AW-in} [q_{AW}](t) \quad (1) \]

By assumption \( Q_i \) = constant for \( i = \text{LB and AW} \). Then

\[ k_{LB-AW} = k_{AW-in} = k \quad (2) \]

\[ \frac{d}{dt} q_{AW}(t) = k ([q_{LB}](t) - [q_{AW}](t)) \quad (3) \]

\[ \frac{d}{dt} [q_{AW}](t) = k ([q_{LB}](t) - [q_{AW}](t)) \quad (4) \]

\[ \frac{Q_{AW}}{k} \int_{t_1}^{t_2} d [q_{AW}](t) = \int_{t_1}^{t_2} ([q_{LB}](t) - [q_{AW}](t)) dt \quad (5) \]

Let \( t_1 = 0 \) and \( t_2 = \tau \). Then

\[ \frac{Q_{AW}}{k} [q_{AW}](\tau) = \int_{0}^{\tau} ([q_{LB}](t) - [q_{AW}](t)) dt \quad (6) \]

The left side of Equation 6 is the specific activity of the surfactant in the alveolar space at time \( \tau \) after injection multiplied by the turnover time \( (T_i) \). \( (T_i) \text{ is defined as the time required to fill an empty pool.} \) The right side of Equation 6 is the area between the lamellar body specific activity time curve and the alveolar space specific activity time curve from \( t = 0 \) to \( t = \tau \). Plotting the right side of Equation 6 for each time \( \tau \) on the y axis and the left side for each time \( \tau \) on the x axis gives a line whose slope is \( Q_{AW}/k = T_i \). Measuring \( Q_{AW} \) then allows a calculation of \( k \) (flux). Since the calculations do not contain explicit information on the flow of material into lamellar bodies, it does not matter whether this comes from reutilized or newly synthesized material.

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