The Labeling of Lung Phosphatidylcholine in Premature Rabbits

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Summary

Lung phosphatidylcholine metabolism was studied *in vivo* in premature rabbits delivered by cesarean section as early in gestation as compatible with prolonged viability (28.9 days). The newborn rabbits initially required supplemental oxygen and had respiratory distress. The amount of phosphatidylcholine isolated from the lung parenchyma changed little over the first 3 days of life, while phosphatidylcholine in the alveolar wash increased in 3 days from 0.05-3.1 $\mu\text{mol}/50\text{ g}$ animal. The phosphatidylcholine of the lungs of the premature rabbits was pulse labeled with isotopically labeled palmitic acid, choline, and phosphate given to the pregnant does 10 min before delivery of the newborns. After the initial incorporation period, the total amount of radioactive precursor (palmitic acid, choline, or ³²P) incorporated into lung phosphatidylcholine did not change for a period of 4 days. Labeled phosphatidylcholine was detected initially in alveolar wash 3 hr after administration of the three precursors and continued to accumulate for many hours. The biological half-life values for lung and alveolar phosphatidylcholine indicated that phosphatidylcholine was turning over very slowly. However, if the effect of dilution on the measured specific activity caused by phosphatidylcholine accumulation was considered, virtually no labeled alveolar or lung phosphatidylcholine disappeared during the 3-4 days of these observations. These results with premature newborn rabbits were similar to those for term newborn rabbits, but different from similar measurements made in the adult rabbit.

Speculation

Premature newborn rabbits are unable to deliver newly synthesized phosphatidylcholine rapidly to the alveolar space in the hours immediately after birth. These newborn animals seem to depend on stored phosphatidylcholine to maintain alveolar stability at birth. Continuous *de novo* synthesis then produces a slow accumulation of phosphatidylcholine in the alveolar space. If these results can be generalized to the premature human with respiratory distress syndrome, phosphatidylcholine synthesized shortly before or after birth would not be expected to mitigate alveolar collapse. A considerable period of respiratory support would be anticipated before large amounts of newly synthesized alveolar phosphatidylcholine would become available for the maintenance of alveolar stability. However, any phosphatidylcholine present may have a very long biological half-life as increasing amounts of alveolar phosphatidylcholine accumulate during the recovery period.

The newborn animal cannot survive the transition from intrauterine to extrauterine life in the absence of sufficient surfactant to establish and maintain a pulmonary gas exchange surface (1). The fully mature animal is well prepared for the transition, having accumulated large stores of surfactant in the lamellar bodies of the pulmonary Type II cells (20, 21). The premature animal, however, often has inadequate stores of surfactant (14) and/or

other as yet undefined biochemical manifestations of pulmonary immaturity, that prevent normal surfactant synthesis, secretion, and function. Phosphatidylcholine is the principal phospholipid in surfactant, comprising about 70% of the total phospholipid phosphorus recoverable from the alveolar space (22). Of the species of phosphatidylcholine present in surfactant, the majority are the highly surface active disaturated species (22). Fetal rabbit lung development in the days just before birth is characterized by the accumulation of both total phosphatidylcholine and disaturated phosphatidylcholine in the lung tissue and pulmonary fluid (14). However, the time required from synthesis to secretion of phosphatidylcholine at the alveolar surface of the premature rabbit is unknown. Also, the stability of phosphatidylcholine as estimated by a biological half-life has not been measured. This paper reports some measurements of the kinetics of lung phosphatidylcholine metabolism of premature newborn rabbits *in vivo*.

MATERIALS AND METHODS

ANIMALS

Litters of 17 pregnant does were studied at 28.9 days of the 31 day gestational period. Timing of gestation was arbitrarily begun 2 hr after the beginning of a 4-hr exposure period of the female to the male. The does were killed with 600 mg of pentobarbital (300 mg/ml) given rapidly iv, and the newborn rabbits were delivered by cesarean section within 2 min. Six newborns from different litters were killed at birth without being permitted to breathe (19). The other newborns were dried, and placed in a 37° incubator with an initial ambient FiO_2 of about 0.8 as measured with an IVAC oximeter. These newborns were handled as described by Barrett *et al.* (3). Six of the 17 litters had greater than 80% mortality within 2 hr of birth, and animals from these litters were not studied. These premature newborns expired after vigorous respiratory movements with minimally inflated lungs and gastric distention. The 11 litters extensively studied had mortalities of 0-21% within 3 hr of cesarean section. Animals studied within 3 hr of delivery were apparently healthy members of their respective litters. The 93 surviving newborns with a mean birth weight of $47.2 \pm 6.8\text{ g}$ (means $\pm$ SD) initially had tachypnea, cyanosis, and retractions; however, all animals not previously killed were weaned as rapidly as possible from oxygen and were pink in room air by 4 hr of life. The newborns kept for more than 6 hr were gavage fed 1-2 ml of SMA 20 cal infant formula every 4 hr. There was no late mortality in these animals.

ISOTOPES

Palmitic acid (9,10-³H, 470 Ci/mmol and 1-¹⁴C, 52 mCi/mmol), choline chloride (methyl-³H, 42 Ci/mmol), and ³²P (carrier-free, as orthophosphate) were obtained from New England Nuclear, Boston, MA. The palmitic acid was converted to the sodium salt and stabilized with 6% bovine serum albumin (17). The final solutions containing 50 $\mu\text{Ci}/\text{ml}$ ¹⁴C-palmitic acid and 200 $\mu\text{Ci}/\text{ml}$

^3H -palmitic acid were stored at -20° until ready for use. All injections were given to the pregnant does slowly over 2 min via the marginal ear vein 10 min and/or 20 hr before delivery of the premature newborn rabbits. Each pregnant doe was injected with one or more isotopes; the amount of each isotope injected was 250 μCi of ^{14}C -palmitic acid or ^3H -choline and 2 mCi of ^3H -palmitic acid or ^{32}P -orthophosphate.

ALVEOLAR WASH

The newborn animals were anesthetized with ip pentobarbital and were exsanguinated by severing the abdominal aorta. The chest was opened and the trachea cannulated with the lungs *in situ*. The degree of inflation of the lungs at the time of the killing varied with age. The alveolar wash fraction was obtained by pooling five sequential washes of 2 ml of saline each (13). The approximately 8.5 ml resulting from pooled washes were extracted to yield a lipid extract of the alveolar wash. While further washings did not remove more surfactant phosphatidylcholine from animals killed at either birth or 3 days of life, air trapping may change the amounts of alveolar surfactant recovered. The lungs were removed from each animal, weighed, homogenized, and extracted to yield a lipid extract of the lung parenchyma.

LIPID ANALYSIS

All lipid extracts were obtained by the method of Bligh and Dyer (6). All purification of phosphatidylcholine was done in two dimensions on silica gel H using chloroform:methanol:acetic acid:water (65/25/8/4, v/v) as the first dimension solvent system and tetrahydrofuran:methylal:methanol:2N ammonium hydroxide (80/57/15.6/8.4, v/v) for the second dimension (16). The phosphatidylcholine was visualized with iodine and eluted from the silica by filtration through a Teflon millipore filter successively using methanol and chloroform-methanol (2:1) (17). The amount of lipid extract chromatographed from samples from lung parenchyma or alveolar wash usually contained 0.2–1 μM of phosphatidylcholine. The phosphatidylcholine recovered from the silica was assayed for phosphate by the method of Bartlett (4) and for radioactivity. Radioactivity was measured using Aquasol scintillation fluid (New England Nuclear), and ^3H , ^{14}C , and ^{32}P labeled phosphatidylcholine standards were used for calculating channel overlap (17).

OTHER DETERMINATIONS

Protein was measured in duplicate on two separate aliquots of lung homogenate according to Lowry *et al.* (23), with bovine serum albumin used for the standard curve. Duplicate samples of lung homogenate also were used to quantify DNA with diphenylamine reagent (8).

RESULTS

ANIMAL MODEL

The specific activity of the phosphatidylcholine in the lung parenchyma from animals of one litter was measured after injecting the pregnant doe with 2 mCi of ^3H -palmitic acid 10 min before delivery of the premature newborns by cesarean section. The rabbits were killed 2 hr later and the specific activity of the phosphatidylcholine was $13,300 \pm 1,570$ CPM/ μmol (mean $\pm$ SD) phosphatidylcholine phosphate, indicating that the phosphatidylcholine from the lungs of all the animals in the litter was labeled similarly. About 0.005% or 1 pmol of the palmitic acid administered to the mother appeared in the phosphatidylcholine of each newborn rabbit lung. Similar estimates for ^3H -choline or ^{32}P given to the mother showed about 0.007 and 0.001% recovery, respectively, of the isotopes in the phosphatidylcholine of each newborn lung.

The quantity of phosphatidylcholine in the alveolar wash and lung parenchyma of the premature newborn rabbits is presented

in Figure 1. The data were normalized to an average animal weight of 50 g because weight was the only number obtainable for all members of a litter at the same time. The quantity of phosphatidylcholine from the lung parenchyma did not increase significantly over the first 3 days of life. In contrast, the phosphatidylcholine from the alveolar washes increased from about 0.05 μmol in the animals not permitted to breathe at birth to 3.1 μmol by 3 days of age—a 60-fold increase. The phosphatidylcholine from the alveolar washes changed over 3 days from 0.65–25% of the total phosphatidylcholine isolated from the lung parenchyma plus the alveolar wash. While the sum of parenchymal and alveolar phosphatidylcholine increased in 3 days from 7.7 to about 12 μmol , this increase was primarily an increase in alveolar phosphatidylcholine.

When the total radioactivity of each precursor of phosphatidylcholine (^3H -choline, ^{14}C -palmitic acid, and ^{32}P) incorporated into phosphatidylcholine from parenchyma plus alveolar wash was expressed per 50 g animal, the radioactivity remained quite constant with time after the initial incorporation period (Fig. 2). Thus, while the absolute amount of phosphatidylcholine increased, the amount of isotope present in the phosphatidylcholine changed little with time. Measurements of lung protein and DNA were also used to normalize the total lung radioactivity (Fig. 2), and the curves appeared virtually identical to those generated using animal weight. Evidently, lung protein and DNA did not change to any large extent over the time course of these experiments.

LABELING OF PHOSPHATIDYLCHOLINE

Experiments were designed to measure the time required for labeled phosphatidylcholine to appear in the lung parenchyma

Fig. 1. The quantity of phosphatidylcholine in lung parenchyma and alveolar wash is shown. Samples from 72 and 68 animals from the same 8 litters were analyzed for the quantity of phosphatidylcholine in parenchyma and alveolar wash respectively. Phosphatidylcholine was isolated from the lipid extracts as described in *Methods*. The experimental points were grouped into increasingly long time intervals, with at least 5 data points per time interval. The mean and SD of the μmol of phosphatidylcholine/50 g animal weight have been plotted against the mean times. Each original data point also was used to determine a linear regression equation by the method of least squares, and the curves are shown with the grouped mean time interval data.

Fig. 2. The total isotope incorporation into premature rabbit lung is shown. A pregnant doe received 250 μCi ^3H -choline, 250 μCi ^{14}C -palmitic acid, and 2 mCi of ^{32}P orthophosphate 10 min before the cesarean delivery of a litter of 12 newborn rabbits (arrow indicates time of isotope injection). The newborns were weighed at 1 hr old and sequentially killed over a period of 94 hr. The radioactivity in the phosphatidylcholine of the parenchyma plus alveolar wash, and the lung protein and DNA were determined. The total radioactivity incorporated for each isotope has been expressed as a ratio of CPM per 50 g animal, lung protein, and lung DNA as indicated on the ordinate. $\blacktriangle$ — $\blacktriangle$ phosphatidylcholine labeled with ^{32}P ; $\triangle$ — $\triangle$ phosphatidylcholine labeled with ^3H -choline; $\bullet$ — $\bullet$ phosphatidylcholine labeled with ^{14}C -palmitic acid.

and alveolar wash of premature newborn rabbits. Figure 3 shows the changes in the specific activity of phosphatidylcholine over a 9-hr period for one litter of nine animals delivered 10 min after the mother was given simultaneously 250 μCi of ^{14}C -palmitic acid and 250 μCi of ^3H -choline. Palmitic acid was incorporated maximally into the phosphatidylcholine of the lung within 12 min of isotope administration and the specific activity of the phosphatidylcholine of the newborn lungs did not change over the 9-hr experimental period. Radioactive choline continued to appear in the lung phosphatidylcholine over a period of 3–4 hr. Thus, the palmitic acid was primarily incorporated into the fetal lung phosphatidylcholine *in utero* during the 10-min delay from the time of isotope administration to delivery, while the choline was incorporated into the lung phosphatidylcholine within the first few hours of the newborn period.

The specific activity of phosphatidylcholine isolated from the alveolar wash which was labeled with palmitic acid and choline was measured simultaneously for the same litter of 9 animals (Fig. 3). Phosphatidylcholine labeled with either precursor was not detectable in the alveolar wash until 3 hr after isotope administration, and the curves for the appearance of phosphatidylcholine labeled with the two precursors were indistinguishable. The reproducibility of the 3-hr lag period from the time of administration of precursor to the detection of labeled phosphatidylcholine in the alveolar wash was verified by repeating the same experiment with six litters and by using ^{32}P as well as choline and palmitic acid as phospholipid precursors (Fig. 4). The 3-hr lag period was clearly demonstrated and was independent of the labeled precursor studied. The radioactive phosphatidylcholine continued to accumulate in the alveolar space for a number of hours.

BIOLOGICAL HALF-LIFE OF PHOSPHATIDYLCHOLINE

The biological half-life is a measurement of the relative stability of a molecule *in vivo*. The decay curves for the phosphatidylcholine from lung parenchyma labeled with palmitic acid, choline, and ^{32}P given 10 min before delivery and palmitic acid given 20 hr before delivery are shown in Figure 5. The half-life values determined from the curves as summarized in Table 1 emphasize the very slow apparent decay, irrespective of the phosphatidylcholine precursor chosen. Similar data for the alveolar wash phosphatidylcholine are shown in Figure 6, with the calculated half-life values also collected in Table 1. The limitations of these measurements are discussed later in this paper.

DISCUSSION

The animals used for these experiments were premature rabbits at the earliest gestational age consistent with survival. Six of 17 litters expired very rapidly in spite of vigorous ventilatory effort, while the 11 litters actually studied had respiratory distress and initially required supplemental oxygen. However, these animals recovered rapidly and were maintained for as long as 4 days after birth. The quantity of phosphatidylcholine in the pulmonary fluid recoverable from the rabbits of 28.9 days gestation who were not permitted to breathe was 0.05 $\mu\text{mol}/50$ g rabbit, while 30.2 day term newborns previously studied and similarly treated had about 0.5 μmol phosphatidylcholine/50 g animal (17). In spite of the initial small amounts of alveolar phosphatidylcholine, the newborns rapidly recovered from their initial respiratory distress. Within about 8 hr, the alveolar wash of premature and term newborns contained similar amounts of phosphatidylcholine.

There was considerable heterogeneity in the litters studied, probably representing the biologic variability inherent in the timing of lung maturation—a phenomenon clearly evident in the human (12). For this reason, the results for individual litters (Figs. 2, 3) and for data pooled from a number of litters (Figs. 1, 4–6) were presented. The variability in the determination of specific

Fig. 3. The labeling of phosphatidylcholine in lung parenchyma and alveolar wash is shown. A pregnant doe was given 250 μCi of ^3H -choline and 250 μCi of ^{14}C -palmitic acid 10 min before delivery by cesarean section of a litter of 9 rabbits. The specific activity of the phosphatidylcholine from the parenchyma and alveolar wash was determined after sequential killing of the newborns and is expressed as CPM/ μmol phosphatidylcholine phosphate $\times 10^3$. $\bullet$ — $\bullet$ phosphatidylcholine from parenchyma labeled with ^{14}C -palmitic acid; $\blacktriangle$ — $\blacktriangle$ phosphatidylcholine from parenchyma labeled with ^3H -choline; $\circ$ — $\circ$ phosphatidylcholine from alveolar wash labeled with ^{14}C -palmitic acid; $\triangle$ — $\triangle$ phosphatidylcholine from alveolar wash labeled with ^3H -choline.

Fig. 4. The labeling of phosphatidylcholine in alveolar wash is shown. The specific activity for phosphatidylcholine from alveolar wash samples was determined for animals from six litters. Because the maximal specific activity of phosphatidylcholine achieved in the parenchyma varied somewhat from litter to litter, the data were expressed as the ratio of the measured specific activity of the phosphatidylcholine from the alveolar wash (SA_{AW}) from a given litter divided by the maximal specific activity achieved in the lung parenchymal phosphatidylcholine of that litter (SA_{LP-MAX}). The number of animals studied were 13 for ^{32}P labeled phosphatidylcholine, 42 for 3H -choline labeled phosphatidylcholine, and 46 for ^{14}C -palmitic acid labeled phosphatidylcholine.

activity or in the quantity of phosphatidylcholine in an extract was $\pm 10\%$, so the data reflect not only heterogeneity among litters, but also heterogeneity among members of the same litter.

Previous studies with autoradiographic techniques using adult mice showed that the Type II cell is the principal source of surfactant phosphatidylcholine (2, 7). Biochemical and autoradiography techniques after the pulse labeling of mice with 3H palmitic acid demonstrated that the Type II pneumocyte was the only cell type to incorporate the label appreciably (10). Thus, the metabolism of lung phosphatidylcholine using *in vivo* pulse labels should reflect primarily the metabolism of phosphatidylcholine in the Type II pneumocyte.

Chevalier and Collet (9) studied the synthesis and appearance of phosphatidylcholine in the alveolar space using mouse lungs labeled *in vivo* with 3H -choline. With electron microscopic autoradiography, they found 3H -choline in the endoplasmic reticulum of the Type II cell at 5 min, and in lamellar bodies and the alveolar space by 2 hr. These anatomic descriptions represent a framework for the interpretation of experiments designed to label phosphatidylcholine with various precursors and to follow the radioactive phosphatidylcholine through lung fractions to the alveolar space (16-19).

Three different radioactive precursors of phosphatidylcholine were studied. Each precursor enters the phosphatidylcholine molecule by a different incorporation pathway (27). Choline becomes incorporated into phosphatidylcholine after phosphorylation and

conversion to cytidine diphosphocholine. ^{32}P enters cellular high energy phosphate pools and appears in phosphatidylcholine via the α -phosphate of ATP. Palmitic acid enters phosphatidylcholine either by acylation of the diglyceride precursor pool or by transacylation reactions that use either phosphatidylcholine or lysophosphatidylcholine as precursors.

These experiments rely on the previously described technique of labeling fetal phospholipids by injecting the labeled precursors of phospholipids into the maternal circulation (11, 17). Elphick *et al.* (11) demonstrated at 28 days gestational age, labeled palmitic acid administered to pregnant does appeared maximally in the fetal circulation within 3 min and had a half-life in the fetal circulation of 30-60 sec. The labeled fatty acids preferentially were incorporated into fetal phospholipids (15). Biezenski (5) has shown that esterified fatty acids and phospholipids do not cross

Fig. 5. The decay of labeled phosphatidylcholine in lung parenchyma is shown. The specific activity of phosphatidylcholine was determined for a number of litters of premature rabbits. The experimental values were normalized to the extrapolated specific activity of phosphatidylcholine at zero time ($SA_{t=0}$) which was defined as 1.0. The normalized experimental points were transformed to a linear form ($\log_{10}$) and the curves shown were calculated by the method of least squares. The four part figure is labeled according to the three different isotopes used and time before cesarean section of injection of the ^{14}C -palmitic acid precursor into the pregnant does (-10 min or -20 hr). Choline and ^{32}P were injected 10 min before the delivery of the newborns.

Table 1. Biologic half-life (hr) of phosphatidylcholine¹

	Parenchyma	Alveolar
Precursor of phosphatidylcholine:		
10 min before delivery		
^{14}C -palmitic acid	97	150
3H -choline	>500	397
^{32}P -orthophosphate	360	395
20 hr before delivery		
^{14}C -palmitic acid	100	270

¹ Half-life values were calculated from the regression equations of the curves presented in Figures 5 and 6.

Fig. 6. The decay of labeled phosphatidylcholine from the alveolar wash is shown. The specific activity was determined and presented as in Figure 5. The four sections of the figure are labeled according to isotope used and time of injection of ^{14}C -palmitic acid (-10 min or -20 hr) before delivery of the newborn rabbits. Choline and ^{32}P were injected 10 min before the delivery of the newborns.

the rabbit placenta directly. We also demonstrated that palmitic acid was cleared rapidly from newborn rabbit serum, and that the radioactivity appearing in phosphatidylcholine was palmitic acid (17). Because the palmitic acid was rapidly cleared from fetal serum and reached maximal specific activity in the newborn lung within 12 min of maternal administration, palmitic acid was assumed to pulse label the premature lung. Choline was not as good a pulse label because incorporation continued for several hours.

The present experiments were undertaken to see if the characteristics of *de novo* synthesis of phosphatidylcholine, as measured by the pulse label technique, varied with the gestational age of newborn rabbits. Term rabbit lung rapidly incorporated palmitic acid into phosphatidylcholine while choline incorporation occurred over a period of 6 hr (17), results very similar to those demonstrated in Figure 3 for the premature rabbit. Similarly, a 3-hr lag between the time of isotope administration and the detection of labeled phosphatidylcholine at the alveolar space was identical in term and premature animals. This lag was independent of the precursor of phosphatidylcholine chosen, indicating that phosphatidylcholine synthesized by *de novo* pathways was "processed" from synthesis to the alveolar space in a manner indistinguishable from phosphatidylcholine labeled with palmitic acid, probably by acyl exchange reactions (27). The slow accumulation of labeled phosphatidylcholine at the alveolar space was similar in both premature and term animals. The time before labeled phosphatidylcholine was detected in the alveolar wash of adult rabbits was $\frac{1}{2}$ –1 hr and the maximal specific activity of alveolar phosphatidylcholine was achieved by about 6 hr (16). Thus, the timing of the appearance of labeled phosphatidylcholine in alveolar wash was similar for premature and term newborn animals, but distinct from the adult rabbit.

In contrast to the results reported here, Scarpelli (24) measured the appearance of labeled phosphatidylcholine in the tracheal fluid of fetal lambs in acute fetal lamb preparations after the

administration of labeled palmitic acid. Thirty min after injection of isotope, fetal tracheal fluid phosphatidylcholine was labeled. The difference in timing may represent species differences in pulmonary phospholipid metabolism, inasmuch as a 3-hr delay was demonstrated from precursor administration to appearance of labeled phosphatidylcholine in the alveolar fluid of *in utero* rabbits (19).

The biologic half-life measurements can be compared to similar measurements made elsewhere. Half-life values for phosphatidylcholine from lung and alveolar wash have been measured numerous times in adult rats (26, 27) and recently in adult (16, 18) and term newborn rabbits (17). However, as discussed (17), the assumption of a constant pool size of phosphatidylcholine is not valid for the newborn. The quantity of phosphatidylcholine in the lung parenchyma plus the alveolar wash increased over the time course of the decay measurements (Fig. 1). If no phosphatidylcholine degradation occurred, this increase represented a rate of synthesis of about $0.07 \mu\text{M}$ phosphatidylcholine/50 g animal per hour. The actual decay rate of phosphatidylcholine in the newborn lung was very slow because, as demonstrated in Figure 2, the total radioactivity within the lung changed very little over a period of 4 days. Thus, the measured decay rates are principally a reflection of dilution of the phosphatidylcholine present at birth by phosphatidylcholine synthesized after birth. The half-life values (Table 1) were comparable to similar values obtained for term newborn animals (17). A mechanism that artificially could prolong the measured half-life values would be the continued entrance of significant amounts of radioactive precursors or prelabeled phosphatidylcholine from another body source into lung phosphatidylcholine pools. This problem was discussed for the phosphatidylcholine of adult lung (26); however, the possibility cannot be totally discounted. Yet, it seemed unlikely that three different precursors of phosphatidylcholine would maintain equally stable body pools such that the precursors would enter the lung in amounts just sufficient to balance degradation over a period of 4 days. Similarly, the phosphatidylcholine labeled with palmitic acid and synthesized 20 hr before birth had a measured decay rate very similar to that of the palmitic acid labeled phosphatidylcholine synthesized at birth.

Phosphatidylcholine disappearance from the tracheal-bronchial tree of *in utero* animals may be different from the decay kinetics measured here for newborn animals. Scarpelli *et al.* (25) measured the half-life of labeled phosphatidylcholine in the pulmonary fluid of fetal sheep *in utero*. The half-life was very rapid, being 15–57 min. The labeled phosphatidylcholine disappeared with the simultaneous appearance of labeled fatty acids in the blood of the fetal animals.

The phosphatidylcholine from adult rabbit lung and alveolar wash displayed quite different decay kinetics (16, 18). The half-life for phosphatidylcholine isolated from the alveolar wash was measured as 16, 30, and 80 hr with the palmitic acid, choline, and ^{32}P labels, respectively. Similar half-life values have been reported for phosphatidylcholine from adult rat lung and alveolar washes (26).

Apparently the metabolism of phosphatidylcholine in the term and viable premature newborn rabbit is very similar in kinetic time measurements. The difference is in the quantity of phosphatidylcholine available at birth at the alveolar space. Even with 10 times less phosphatidylcholine in the tracheal-bronchial tree present at birth between 28.9–30.2 days of gestation, the premature animal cannot secrete phosphatidylcholine synthesized around the time of birth to the alveolar space any more rapidly than can the term newborn. The slow secretion of phosphatidylcholine by newborn animals will require extensive studies of the biochemistry of lamellar body growth, development, and secretion.

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