Developmental Changes in Enzyme Activities in Fetal and Neonatal Rabbit Lung.

Cytidylyltransferase, Cholinephosphotransferase, Phospholipases A₁ and A₂, β -Galactosidase, and β -Glucuronidase

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Summary

Lungs were obtained from rabbit fetuses (on each day from d 24 to d 30 of gestation), neonates and adults, and were fractionated for enzyme assays. The developmental profile of cytidylyltransferase shows a decrease in specific activity from d 25 to d 29 ($P < 0.05$) then a sharp rise from d 30 to adult values in d 0 neonates ($P < 0.05$). Cholinephosphotransferase specific activity changes little from d 25 to birth, apart from a non-significant peak on d 29. There is a sharp rise from neonatal d 0 to adult values on d 1 ($P < 0.01$). The specific activity of microsomal phospholipase A₂ declines from d 25 to reach adult values in the neonate ($P = 0.05$). In contrast, the specific activity of lysosomal phospholipase A₂ rises from d 24–28 then falls in the neonate ($P < 0.05$). Adult values are higher than those in the fetus and neonate. Three other lysosomal enzyme specific activities rise to d 28 then decline: phospholipase A₁, β -galactosidase, and β -glucuronidase. The results demonstrate that the level of microsomal phospholipase A₂ does not control the extent of remodeling of phosphatidylcholine for surfactant production. Lysosomal phospholipase A₂ only increases in parallel with the other lysosomal enzymes, indicating an increase in the number of lysosomes in the lung.

In his review of the metabolism of phospholipids in the lung, van Golde (57) pointed out that the pathways of *de novo* synthesis of phosphatidylcholine yield predominantly unsaturated molecular species, and could not supply all the saturated (dipalmitoyl) phosphatidylcholine needed for the production of pulmonary surfactant. Sufficient dipalmitoyl phosphatidylcholine could be obtained by "remodelling" the unsaturated material. In such a process, the action of phospholipase A₂ (EC 3.1.1.4) would remove the unsaturated fatty acid from the 2-position of phosphatidylcholine, yielding mainly 1-palmitoyl-2-lysophosphatidylcholine, which could then be reacylated with a palmitoyl group to form a molecule of dipalmitoyl phosphatidylcholine. Phospholipase A₂ might, therefore, show changes in activity during lung development that would be indicative of its importance in the biosynthesis of dipalmitoyl phosphatidylcholine in fetal and neonatal lungs. It might also be possible that this enzyme could be a site for therapeutic intervention to raise the level of enzyme activity in cases of respiratory distress syndrome of the newborn, in which the rate of synthesis of surfactant is too low to support normal respiration. Alternatively, excessive levels of phospholipase A₂ activity might result in undesirable surfactant breakdown.

This study looks at the activity of phospholipase A₂ in developing rabbit lung. The alkaline-optimum enzyme of the microsomal fraction and the acid-optimum enzyme of the lysosomal

fraction were assayed separately. In order to provide developmental patterns for comparison, assays were also made of two other enzymes of phosphatidylcholine biosynthesis. These were CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15), usually referred to below simply as cytidylyltransferase, which catalyses the transfer of a cytidylyl group from CTP on to the phosphate of phosphocholine, forming CDPcholine, and CDPcholine:1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2), referred to as cholinephosphotransferase, which transfers the phosphocholine moiety from CDPcholine on to the free hydroxyl group of a 1,2-diacylglycerol, converting it to a molecule of phosphatidylcholine. For further comparison, three other lysosomal enzymes, namely phospholipase A₁ (EC 3.1.1.32), β -galactosidase (EC 3.2.1.23), and β -glucuronidase (EC 3.2.1.31), were also assayed. The results are displayed as specific activities in the specified fractions of lung homogenate, and also as total activity in the fraction to demonstrate that large changes in the amounts of other proteins are not responsible for the changes in specific activity.

MATERIALS AND METHODS

Lung fractionation. The procedures used were modified from our previous work (24, 25), in which fractions were prepared from rabbit lung homogenates by differential sedimentation and characterised with marker enzymes. New Zealand White rabbits of 25, 26, 27, 28, 29, and 30 d gestation (accurate to within 2 h) were obtained from Pemberley Rabbits, Cambridge, United Kingdom. The does were killed by cervical dislocation or intravenous barbiturate. The uterus was removed and the lungs of each fetus were excised and placed together (one litter) in a weighed beaker of ice cold 0.25 M sucrose in 50 mM Tris·HCl (pH 7). In some cases, when the doe had been killed by cervical dislocation, the maternal lungs were also removed into a separate beaker of ice cold medium. Some data are also included from Old English rabbits of 24 d gestation, the lungs from three litters being collected together. Newborn New Zealand White rabbits were killed by decapitation, and the lungs removed and placed together (one litter) in ice cold medium. D 0 represents newborns less than 6-h-old; d 1 represents animals 18–24-h-old.

The beaker plus medium plus lungs were reweighed to determine lung wet weight for the litter. Lungs were rinsed with ice cold medium, chopped with scissors, rinsed again, then homogenised in ice cold 0.25 M sucrose, 50 mM Tris·HCl (pH 7) (10 ml/g with a minimum of 90 ml) for 2 x 30 s with a Silverson homogeniser (Silverson Machines Ltd., Chesham, Bucks., United Kingdom). Homogenates of adult lung were filtered through four layers of muslin. The homogenate was centrifuged at 5000 g for

20 min, and the resulting supernatant was recentrifuged at 13,000 *g* for 20 min. This pellet was resuspended in 1 mM Na₂EDTA, 1 mM NaHCO₃, frozen and thawed five times, and dialysed overnight against 1 mM Na₂EDTA and 1 mM NaHCO₃. After centrifugation at 100,000 *g* for 120 min, the resulting supernatant, fraction LS, contained the soluble proteins from lung lysosomes. The supernatant from the 13,000 *g*, 20-min centrifugation was recentrifuged at 100,000 *g* for 120 min. This pellet was resuspended in 50 mM Tris·HCl (pH 7.5), 250 mM NaCl, by two passes of a Dounce homogeniser, to yield fraction M, containing lung microsomes. The corresponding supernatant, fraction S, contained lung cytosol.

Small samples of chopped lung were taken for histologic examination. Samples of lung fractions were dialysed against distilled water, then assayed for protein by the method of Lowry *et al.* (33) as modified by Campbell and Sargent (10), using as reference a Protein Standard Solution (Sigma London Chemical Co. Ltd., Poole, Dorset, United Kingdom).

Cytidyltransferase assay. The assay conditions were based on those of Sundler (53), and Rooney *et al.* (45). In a final volume of 0.5 ml, and with continuous shaking at 37°, 0.4 ml of fraction S were incubated in 20 mM Tris succinate (pH 7.5), 10 mM MgCl₂, 5 mM CTP sodium salt (Sigma), and 1 mM phosphocholine chloride calcium salt (Sigma), containing 50 nCi phospho[methyl-¹⁴C]choline (55 Ci/mol; NEN Chemicals GmbH, Dreieich, W. Germany). Samples of 0.1 ml were taken at 0, 10, 20, and 40 min, and heated at 90° for 5 min to precipitate protein. After cooling, the addition of carrier phosphocholine and CDPcholine, and centrifugation, the supernatant was applied to silica gel thin layer chromatographic plates (Type LK5D from Whatman Laboratory Products Ltd., Maidstone, Kent, United Kingdom), which were developed in ethanol:water:ammonia solution (specific gravity, 0.88) (100:97.5:2.5, by vol) [modified from Sundler (53)]. The substrate and product spots were located by exposing the plate to iodine vapour, and scraped off for scintillation counting. The extent of the reaction was proportional to time for at least 40 min, and to protein concentration over the range used. No activity was seen in the absence of added protein. In order to reduce the tailing caused by sucrose on thin layer chromatography, it was necessary to dialyse fraction S overnight against 100 mM ammonium acetate. This had no effect on the enzyme activity.

Cholinephosphotransferase assay. The assay conditions were modified from Oldenborg and van Golde (41). In a final volume of 0.5 ml, and with continuous shaking at 37°, 0.3 ml of fraction M were incubated in 50 mM Tris·HCl (pH 8.0 at 37°), 20 mM MgCl₂, 5 mM EGTA [ethyleneglycol-bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid], 4 mM dithiothreitol, 0.8 mM cytidine-5'-diphosphocholine sodium salt (Sigma) containing 250 nCi cytidine-5'-diphospho[methyl-¹⁴C]choline ammonium salt (60.9 Ci/mol; Amersham International Ltd., Amersham, Bucks., United Kingdom), and 12 mM diacylglycerol (prepared from egg phosphatidylcholine and supplied by Koch-Light Ltd., Haverhill, Suffolk, United Kingdom) dispersed in ethanol/ Triton X-100/ water according to Rooney and Wai-Lee (44). The addition of EGTA and the increased Mg²⁺ concentration were to reduce the inhibition of cholinephosphotransferase by Ca²⁺ (29) and to inhibit the activity of microsomal phospholipases (44). Samples of 0.1 ml were taken at 0, 5, 10, and 20 min, and mixed with 0.35 ml chloroform: methanol (1:2, v/v) for extraction of lipids (7). Phosphatidylcholine (25 μg) was added as carrier. The extracted phospholipids were separated by thin layer chromatography on Whatman LK5D plates with chloroform: methanol:water (65:25:4, by vol.). Phosphatidylcholine was located by exposing the plate to iodine vapour, and the spot was scraped off for scintillation counting. The extent of the reaction was proportional to protein concentration over the range used, but not proportional to time, so the rate of reaction over the first 5 min was used to determine enzyme activity. No activity was seen in the absence of added protein.

Lysosomal phospholipase A₁ and A₂ assay. Assays were performed in a similar manner to that previously described (24, 25). The substrate was 1-palmitoyl-2-[2-¹⁴C]oleoyl phosphatidylcholine (55 Ci/mol; Applied Science Laboratories Inc., State College, PA) diluted to 1 Ci/mol with egg phosphatidylcholine (Sigma) and prepared as liposomes by ultrasonication for 3 min. The reaction mixture (0.2 ml) contained 0.1 ml of fraction LS, 0.05 mM substrate, 40 mM sodium acetate (pH 4.0), and 5 mM Na₂EDTA. After 1 h at 37°, with continuous shaking, the reaction was terminated by the addition of 0.3 ml chloroform:methanol (2:1, v/v) containing 25 μg each of oleic acid and lysophosphatidylcholine. The chloroform layer was separated by centrifugation and analysed by thin layer chromatography on Whatman LK5D plates developed in hexane:chloroform:methanol:acetic acid:water (100:75:25:2:1, by vol.), and the spots corresponding to free fatty acids, phosphatidylcholine and lysophosphatidylcholine were located by exposing the plate to iodine vapour. The spots were scraped off for scintillation counting. Phospholipase A₁ activity was calculated from the yield of labeled lysophosphatidylcholine, and phospholipase A₂ from the yield of labeled free fatty acid.

Microsomal phospholipase A₂ assay. Assays were performed as for lysosomal phospholipases, with the following differences: incubation of 0.1 ml of fraction M took place in 50 mM Tris·HCl (pH 8.0 at 37°), containing 12.5 mM CaCl₂, these conditions corresponding to the broad optima for pH and Ca²⁺ concentration shown by the enzyme. The reaction mixture was acidified by the addition of 25 μl 0.6 M H₂SO₄ before the extraction of lipids for analysis. The extent of reaction was proportional to time for at least 1 h, and to protein concentration over the range used. Blanks with no added protein were always included. The addition of 1 mM sodium deoxycholate to the incubation medium caused a 14-fold increase in activity, presumably by converting the liposomal substrate to a micellar form. But, because a liposomal substrate was considered to be more relevant physiologically, detergent was not added to the standard assay. Phospholipase A₁ activity was always insignificant.

β-galactosidase and β-glucuronidase assays. Assays were performed as described by Barrett and Heath (3), using 4-nitrophenyl β-D-galactopyranoside and 4-nitrophenyl β-D-glucuronide (both from Sigma), respectively, as substrates. In a final volume of 1.5 ml, and with continuous shaking at 37°, 0.1 ml of fraction LS were incubated in 100 mM sodium acetate (pH 4.5), and 5 mM substrate. After 0, 20, or 40 min the reaction was stopped by the addition of 1.5 ml 0.5 M NaHCO₃, 0.5 M Na₂CO₃. The tubes were kept cold and dark until the absorbance at 420 nm had been determined. The extent of the reaction was proportional to time for at least 40 min, and to protein concentration over the range used. No activity could be detected in the absence of added protein.

RESULTS

The mean lung wet weight per animal was determined for each litter, and these values were averaged for each time point. No attempt was made to remove blood or any other liquid from the excised lungs before weighing. Lung wet weight increased from 0.36 g/animal (*n* = 1) on d 24 to 1.43 ± 0.07 g/animal (SEM, *n* = 4) on d 30. There followed an abrupt fall of 40% in lung wet weight between d 30 fetuses and d 0 neonates, representing the loss of fluid on conversion to air breathing. The value of lung wet weight for the does studied was 15.9 ± 1.3 g (SEM, *n* = 5). The histologic study confirmed the gestational accuracy of the chronologic ages of the fetuses. The mean litter size for fetal and neonatal New Zealand White rabbits was 7.8 ± 2.4 (SD, *n* = 32). Control experiments showed that the use of barbiturates to kill some of the does did not affect the enzyme levels in fetal lungs.

Cytidyltransferase. Figure 1 shows the developmental profile of cytidyltransferase as specific activity in the cytosol fraction, S. Activity declines from d 25 to d 29 (d 28 *versus* d 29, Student's

t test: $P < 0.05$), then rises sharply in neonates (d 30 versus d 0, $P < 0.05$) to adult values. As shown in Figure 8, there is a general increase in the total activity per lung.

Cholinephosphotransferase. Figure 2 shows the developmental profile of cholinephosphotransferase as specific activity in the microsomal fraction, M. Activity changes little from d 25 to d 0, apart from a marked peak on d 29. This increase in activity is, however, not significant ($P > 0.05$) relative to either d 28 or d 30. There is a sharp rise to adult levels on d 1 (d 0 versus d 1, $P < 0.01$). Figure 8 shows a steady increase in total activity per lung, with a superimposed (but not significant) peak on d 28 and 29.

Microsomal phospholipase A_2 . Figure 3 shows the developmental profile of the alkaline-active phospholipase A_2 as specific activity in the microsomal fraction, M. Activity declines from d 25 to reach adult values in the neonate [d 25 versus (day 0 + d 1), $P = 0.05$]. Figure 8 shows that the total activity per lung remains nearly constant in the fetus, then declines in the neonate.

Lysosomal phospholipase A_2 . Figure 4 shows the developmen-

Fig. 1. Cytidyltransferase, developmental profile of specific activity in cytosol fraction, S. Activity was determined for each litter as described in "Materials and Methods." Values are plotted as mean $\pm$ SEM for each time point. The numbers of litters used are shown in parentheses; Ad, adult. The vertical line marks term (31 d).

Fig. 2. Cholinephosphotransferase, developmental profile of specific activity in microsomal fraction, M. For method of presentation, see Figure 1.

Fig. 3. Alkaline-active phospholipase A_2 , developmental profile of specific activity in microsomal fraction, M. For method of presentation, see Figure 1.

Fig. 4. Acid-active phospholipase A_2 , developmental profile of specific activity in lysosome soluble fraction, LS. For method of presentation, see Figure 1.

tal profile of the acid-active phospholipase A_2 as specific activity in the lysosome soluble fraction, LS. Activity rises initially (but d 25 versus d 28, not significant) then falls steeply in the neonate (d 28 versus d 0, $P < 0.05$). Adult values are generally higher. A similar profile is seen for total activity (Fig. 9).

Lysosomal phospholipase A_1 . Figure 5 shows the developmental profile of acid phospholipase A_1 as specific activity in the lysosome soluble fraction, LS. The profile generally resembles that for phospholipase A_2 (but d 28 versus d 0, not significant), as does the total activity per lung profile (Fig. 9).

β -galactosidase and β -glucuronidase. Figures 6 and 7 show the developmental profiles of these glycosidases as specific activity in the lysosome soluble fraction, LS. Activity rises initially (d 25 versus d 28, $P < 0.05$ for each enzyme) then falls in the neonates (but d 28 versus d 0, each not significant). The activities in adults are relatively less elevated than was the case with the phospholipases (Figs. 4 and 5). The similarity of profiles of total activity per lung can be seen in Figure 9. All four lysosomal enzymes studied rise to peaks on d 28, and decline thereafter.

Fig. 5. Phospholipase A₁, developmental profile of specific activity in lysosome soluble fraction, LS. For method of presentation, see Figure 1.

Fig. 6. β-galactosidase, developmental profile of specific activity in lysosome soluble fraction, LS. For method of presentation, see Figure 1.

Fig. 7. β-glucuronidase, developmental profile of specific activity in lysosome soluble fraction, LS. For method of presentation, see Figure 1.

Fig. 8. Developmental profiles of total enzyme activities in the fractions assayed, expressed on an activity per lung basis. Activities of cytidyltransferase (■), cholinephosphotransferase (□), and microsomal phospholipase A₂ (△) were determined for each litter as described in "Materials and Methods," and are plotted as mean values for each time point.

Fig. 9. Developmental profiles of total enzyme activities in the lysosome soluble fraction, LS, expressed on an activity per lung basis. Activities of lysosomal phospholipase A₁ (△) and A₂ (▲), β-galactosidase (■), and β-glucuronidase (□) were determined for each litter as described in "Materials and Methods," and are plotted as mean values for each time point.

DISCUSSION

General patterns of enzyme activities and correlation with lung development. Most of the enzymes involved in the production of pulmonary surfactant have been studied in time course in the developing rabbit lung. The developmental profiles can be roughly classified as being of five general forms, as shown in Table 1. There is a tendency for key enzymes catalysing reactions near the ends of metabolic pathways to show profiles of Forms 4 or 5 whereas enzymes involved in more fundamental processes are in the earlier groups.

The present study confirms the findings of Rooney *et al.* (45) that the activity of cytidyltransferase rises just at birth in the rabbit (Fig. 1) whereas Tsao and Zachman (56) found a declining

Table 1. *Enzymes of surfactant production in developing rabbit lung, classified according to the form of the developmental time course*

Enzyme	References	Suggested role
1) No significant change		
Acetyl-CoA carboxylase	19	Fatty acid synthesis
Phosphatidate phosphatase (cytosol forms)	12, 47	Phosphatidylcholine synthesis
2) Declining from higher values in early gestation to lower values at and beyond term		
Glycogen synthase	2	Glycogen accumulation
Glycerol-3-phosphate phosphatidyltransferase (mitochondrial form)	20, 45	Phosphatidylglycerol synthesis
Choline kinase	45, 56	Phosphatidylcholine synthesis
Phospholipase A ₂ (microsomal form)	*	Phosphatidylcholine remodelling
3) Rising in late gestation to fall again at or before term		
Phosphorylase	2	Glycogen mobilisation
Fatty acid synthetase	13, 19	Fatty acid synthesis
Phospholipase A ₂ (lysosomal form)	*	Phosphatidylcholine remodelling
4) Rising in late gestation and retaining or resuming elevated values beyond term		
Fatty acid elongation enzymes	19	Fatty acid synthesis
Lysophosphatidate acyltransferase	45	Phospholipid synthesis
Phosphatidate cytidyltransferase	20, 32	Acidic phospholipid synthesis
Glycerol-3-phosphate phosphatidyltransferase (microsomal form)	20	Phosphatidylglycerol synthesis
<i>myo</i> -inositol phosphatidyltransferase	6, 20	Phosphatidylinositol synthesis
Phosphatidate phosphatase (microsomal forms)	12, 20, 47	Phosphatidylcholine synthesis
Lysophosphatidylcholine acyltransferase	45, 56	Phosphatidylcholine remodelling
Lysophosphatidylcholine:lysophosphatidylcholine acyltransferase	56	Phosphatidylcholine remodelling
11 β -hydroxysteroid dehydrogenase	37	Control of surfactant secretion
Adenylate cyclase (with activators)	4, 42, 62	Control of surfactant secretion
5) Rising only at or just after birth		
Phosphocholine cytidyltransferase	45, *	Phosphatidylcholine synthesis
Cholinephosphotransferase	18, 22, 45 *	Phosphatidylcholine synthesis

* Data from this investigation.

profile (Form 2). The profile determined here for cholinephosphotransferase (Fig. 2) has a significant rise after birth, as seen previously in some rabbit studies (18, 22, 45), though not in others (47, 56). There is also an increase in late gestation (d 29), but this is limited to that single day (Fig. 2) so the variation of gestational stage for a given chronologic age will make it difficult to obtain a statistically significant result. This problem may explain the absence of a peak in the results of some rabbit investigations. In others, d 29 was not sampled (47, 56). These enzyme profiles can be set against other changes in developing rabbit lung. Kikkawa *et al.* (30) gave a detailed account of cytologic changes. Lamellar bodies first appear on d 25 and increased to fill all Type II cells by d 27. On d 28, osmiophilic material appears in the alveolar lumina, and the amount rapidly increases until by d 31 all the alveolar spaces contain some. Also from d 28, the fatty acid composition of lung phosphatidylcholine becomes mature (17, 38, 49). The CDPcholine pool size reaches a peak on d 26 and 27 and falls thereafter (43, 54), an effect which could result from an increase in cholinephosphotransferase activity (Fig. 2).

Patterns of activity of phospholipases and other lysosomal enzymes. The present investigation is the first to examine phospholipase activities in developing rabbit lung. The declining profile of the alkaline-active phospholipase A₂ is shown in Figure 3. Melin *et al.* (34) studied a similar activity in the rat and found an increase through late gestation with elevated levels persisting in the neonate. The activity was measured on a postmitochondrial supernatant, with phosphatidylethanolamine as substrate. The enzyme activity was increased by the addition of lysed platelets (34). Rat platelets contain a phospholipase A₂ which is only active in the presence of an endogenous activating factor (16).

The acid-active phospholipase A₂ from rabbit lung lysosomes shows an interesting profile (Fig. 4), with activity elevated from

d 26–d 28. A rather similar pattern is seen for acid phospholipase A₁ (Fig. 5), an enzyme not obviously involved in remodelling processes. As two lysosomal enzymes showed profiles of this form, two further enzymes were examined: glycosidases with no known role in surfactant production. The profiles for β -galactosidase (Fig. 6) and β -glucuronidase (Fig. 7) were also of Form 3. The total activity patterns for these four enzymes can also be compared in Figure 9. One other possible lysosomal activity was investigated in developing rabbit lung. Bandstrup and Kretschmer (2) assayed a glucose-6-phosphatase at pH 6.6, and reported that there was also activity against glycerol-3-phosphate. This acid phosphatase had a Form 3 profile with peak activity on d 26 and 27. In human lung, β -galactosidase activity increased from wk 10–wk 25, as did acid phosphatase (48), but this period is rather early in gestation (equivalent to before d 19 in the rabbit). There was no change in the activities of α -galactosidase or α -glucosidase (48). In the rat, α -glucosidase and β -*N*-acetylglucosaminidase showed little change in the lung during late gestation (8).

The general increase in lysosomal enzymes in developing rabbit lung would seem to indicate an increase in the number of lysosomes. One possibility is that this represents a change in the proportions of the cell types in the lung. The most effective change of this kind would be an increase in the number of macrophages; however, Zeligs *et al.* (64) found that the number of alveolar macrophages in fetal rabbit lung lavage was low until a few hours before birth, from which time there was a very marked and continued increase up to the 4th postnatal wk. Another possibility is that the increase in lysosomes is a consequence of their involvement in lamellar body formation and secretion. The idea of this involvement was first proposed by Sorokin (50) and by Kikkawa *et al.* (30) on the basis of cytologic studies. Cytochemical investigations and biochemical analyses have also shown the presence of lysosomal enzymes in lamellar bodies. The early work has been reviewed (23), and later studies

have confirmed the findings for several species, and added more enzymes to the list (24, 26, 27, 51, 52, 63). It has also been demonstrated that the lysosomal enzyme activity in lamellar body preparations is not due to contamination with lysosomes (28, 39). The activity associated with lamellar bodies in immature human fetal lung explants increases as the tissue matures in culture (40). The possible roles of lysosomes in lamellar body development and surfactant secretion have been discussed (23, 24, 25). The timing of increased lysosomal enzyme activity in the present study coincides with the commencement of surfactant secretion in the developing lung (30). An involvement of lysosomes in secretory processes has been suggested for several other tissues (35, 55).

The remodelling problem. This investigation of the development of phospholipase A₂ in the lung was undertaken in order to determine the importance of such enzymes in the remodelling of phosphatidylcholine. The first suggestions that remodelling processes might be involved in the synthesis of dipalmitoyl phosphatidylcholine in lung arose from observations of the incorporation of labeled precursors (14, 21, 60). Incorporation studies have also shown that remodelling occurs in developing rabbit lung, particularly from d 28 (1, 22). Some studies of the substrate specificity of cholinephosphotransferase appeared to show that the enzyme was incapable of utilizing disaturated diacylglycerols, so that nearly all dipalmitoyl phosphatidylcholine would have to be produced by remodelling (44, 46). More recently, it has been found that cholinephosphotransferase can use dipalmitoylglycerol under appropriate conditions, and that the *de novo* formation of dipalmitoyl phosphatidylcholine is limited by the supply of dipalmitoylglycerol (36, 58). Remodelling is needed to increase the proportion of dipalmitoyl phosphatidylcholine to the level required for a functional surfactant.

Two routes of remodelling have been described. In the first, the unsaturated fatty acid at the 2-position of the phosphatidylcholine is removed by phospholipase A₂, and replaced with a palmitoyl residue transferred from palmitoyl-CoA by acyl-CoA:lysophosphatidylcholine acyltransferase (EC 2.3.1.23) (31). The second route again involves phospholipase A₂ action, but in this case the reacylation step is performed by lysophosphatidylcholine:lysophosphatidylcholine acyltransferase (15). This enzyme transfers a palmitoyl group from the 1-position of one lysophosphatidylcholine molecule to the vacant 2-position of another. Several studies have indicated that the first route is predominant in adult lung (e.g., 5, 59). The transferase activity in the second route appears to be a side reaction of a lysophospholipase (EC 3.1.1.5) (9, 11).

Remodelling probably occurs at the level of the endoplasmic reticulum (61), but may occur later, perhaps even in the lamellar body (14). We have investigated both the microsomal phospholipase A₂, presumably deriving from the endoplasmic reticulum, and also the lysosomal enzyme, part of the activity of which is located in the lamellar bodies (24, 28). As described above, the alkaline-active enzyme in the microsomes showed a steady decrease in activity from d 25 to newborn d 1, with no indication of a perinatal surge whereas the lysosomal hydrolase showed elevated activity from d 26–d 28, in parallel with the other lysosomal enzymes studied. It was suggested that this general rise in lysosomal enzyme activity indicates the presence of more lysosomes, coinciding with the beginning of surfactant secretion. The increased lysosomal involvement in lamellar body processing could cause an increased production of lysophosphatidylcholine from the action of phospholipase A₂ on unsaturated phosphatidylcholine (25, 26). This would provide substrates for remodelling. Incorporation studies show remodelling to be an important process in developing rabbit lung from about d 28 (1, 22), and it is at this time that the specific activities of lysophosphatidylcholine:lysophosphatidylcholine acyltransferase (56) and acyl-CoA:lysophosphatidylcholine acyltransferase (45, 56) rise, reaching maximum values after birth.

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