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Surface tension
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phospholipids
lecithin
sphingomyelin
lung
phosphatidyl
dimethylethanolamine
phosphatidyl inositol

The Biochemical Development of Surface Activity in Mammalian Lung

I. The Surface-Active Phospholipids; the Separation and Distribution of Surface-Active Lecithin in the Lung of the Developing Rabbit Fetus

L. Gluck[31], E.K. Motoyama, Helen L. Smits and Marie V. Kulovich

Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, USA

Extract

A simple technique, precipitation with acetone, was described to separate the surface-active lecithin fraction from the nonsurface-active fraction. Surface activity in lung phospholipids was found in the acetone-precipitated fractions of lecithin, sphingomyelin, phosphatidyl dimethylethanolamine and phosphatidyl inositol. Normal surface activity of saline extract of pooled fetal rabbit lung was observed from 28 days of gestation. It was possible to isolate surface-active lecithin from lung parenchyma long before the 29th day of gestation when surface-active lecithin first is found in the alveolar wash. During the nonbreathing fetal state, even at term, only 11 % of lecithin from alveolar wash is surface-active increasing after one hour's breathing to approximately 50 % of the total lecithin. The rabbits delivered prematurely after 28 full days of gestation clinically had respiratory distress and their percentage of surface-active lecithin in alveolar wash increased at a slow rate compared to full-term animals. Good temporal correlation was seen between intracellular storage of surface-active lecithin during the fetal state and the findings with electron microscopy of increasing numbers of osmiophilic inclusion bodies as gestation progresses.

Speculation

Surface activity is shared by several phospholipids in lung but is related principally to lecithin. During fetal development there is production of intracellular surface-active lecithin with storage possibly in osmiophilic lamellar inclusion bodies until near term when some (11 %) begins to appear in alveolar wash. After breathing, a great release of surface-active lecithin into alveolar wash occurs, with 50 % of alveolar lecithin being surface active throughout the life of the animal. Prematurely delivered rabbits take much longer to increase their surface-active alveolar lecithin.

Introduction

The alveoli of mammalian lungs are lined by a material with unique surface tension properties (or activity) which tends to prevent alveolar collapse at small lung volumes, thereby contributing to the stability of the lungs. A saline extract of minced normal adult mammalian lung forms a surface film which, in a modified Wilhelmy balance, exhibits low surface tension on compression of the surface and higher tension on expansion [10]. Bubbles expressed from the cut surface of the lung are stable and retain air for prolonged periods [23]. These properties of mammalian lung, indicative of its surface activity, are found in the phospholipid fraction of lung extract [19], principally in lecithin, the most abundant phospholipid in lung, but

have been described also in sphingomyelin [12, 13, 19, 26] and phosphatidyl dimethylethanolamine [20]. Both surface activity of extracts of lung and stability of bubbles expressed from lung have been demonstrable only during the late development of the mammalian fetus and thereafter [3, 7, 17, 22, 24].

Of particular clinical interest have been the observations that lungs from infants dying with the respiratory distress syndrome (RDS or hyaline membrane disease) exhibit neither low surface tension of saline extracts [3] nor stable bubbles from the cut surface [25]. A significant depletion of lecithin in lungs of infants dying with RDS has been reported [1, 9].

Affecting almost exclusively prematurely born infants, RDS undoubtedly is related to immaturity and incomplete biochemical development of the pulmonary alveolus. However, few studies have described the changes in concentration of lipids or the biosynthesis of phospholipids in the lungs of the mammalian fetus during development [8, 11, 16]. No study of changes in the chemical structure of lecithins nor of their appearance in alveoli during development has previously been reported although this information is fundamental for an understanding of both normal and abnormal lung function.

In this and succeeding reports are described a series of studies designed to examine in detail the normal biochemical development of surface activity[1] in mammalian fetal lung (including human) and how this is influenced by such factors as prematurity, breathing, species variation, chemical structures of lecithin and abnormal physiological states.

The present report describes a procedure for separating the surface-active lecithin component from the total lecithin extracted from lung. Surface tension measurements of purified phospholipids isolated from lung and other organs of different ages and species are presented. The presence of surface-active lecithin in lung parenchyma, its appearance in alveoli both in the nonbreathing and breathing states and some of the significant changes in alveolar cells shown by electron microscopy are correlated with development of the rabbit fetus.

[1] This term 'surface activity' is used throughout the manuscript to describe those unique properties both of saline extract of minced normal lung and of certain purified phospholipids exhibiting low surface tension on compression of the surface of the modified Wilhelmy balance and higher tension on expansion of the surface while describing a hysteresis during cycling of the balance. The low surface tensions on compression for either lung extract or purified phospholipids which would be considered to show 'normal surface activity' are described in the following section on materials and methods.

Materials and Methods

A pure-bred strain of albino New Zealand white rabbits with an average of nine fetuses per litter was used. Times of matings were known within 8–12 hours. Lungs from fetal and newborn animals with gestational ages ranging from 21 days to term (30–31 days) were studied. The program pregnant rabbits were given sodium pentobarbital intravenously and were sacrificed by electrocution. The total time from pentobarbital injection to hysterectomy was about 1 ½ to 2 minutes.

In the studies of fetal lungs the fetuses quickly were shelled out of their amniotic sacs, their tracheas clamped and they were immediately decapitated. The lungs, removed before the onset of respiratory movements, were pooled for the biochemical and surface-activity studies. For each gestational age studied, unless otherwise listed, the data reported are based on analyses of five to eight separate litters.

In the studies of lungs after the onset of respiration three to four litters of fetuses for each day of gestation from 28 to 31 days were delivered by cesarean section and approximately half placed immediately in a highly humidified incubator at 37.5°. The residual fetuses were immediately sacrificed as described above and served as additional fetal controls. The rabbits were allowed to breathe for intervals varying from 30 minutes to 6 hours. The lungs from each of these were lavaged and analyses performed on the residual lung as well as the washings.

The lavage was performed as follows: Each pair of lungs was gently expanded with air from a syringe connected to the trachea with appropriately sized polyethylene tubing. This was followed by five gentle lavagings, each with 2 ml of 0.9 % saline (20°). The washings, which totalled 10 ml for each pair of lungs, were pooled by litter groups and the cells and debris in the lavage fluid removed by centrifugation (1000 g at 2°). Lung extracts and lung washings from each pregnant adult rabbit sacrificed plus 6 male adult rabbits were also studied for purposes of comparison. Five lavagings of 10 ml of 0.9 % saline (20°) were done for each adult lung.

Surface activity of saline extracts from 3 g of pooled minced lung tissue was measured with a modified Wilhelmy balance as described by CLEMENTS *et al.* and modified by others [6, 10, 27]. The minimum surface tension during compression of the surface was recorded after 2 ½ hours of cycling (10 cycles) or when three consecutive cycles had shown the same minimum surface tension. If the minimum surface tension thus obtained was below 15 dyn/cm, the lung extract or wash-

ing was considered to have 'normal' surface activity [27]; if not, cycling was continued for as long as 24 hours. If during this time the minimum surface tension decreased to below 15 dyn/cm, the extract was considered to have 'borderline' surface activity.

Surface activity of individual purified phospholipid fractions from lung and other organs also were studied. In addition, lecithin fractions (total, acetone-soluble and acetone-precipitable fractions) from lung homogenates, alveolar lavage and from the residual lungs after alveolar lavage were examined. Each purified phospholipid was made up in 100 mg % solution in chloroform and an aliquot containing 25 μg was gently layered on the surface of 0.9 % saline in the trough of a modified Wilhelmy balance. After allowing the chloroform to evaporate from the surface, the surface area of the trough was alternately compressed and expanded and the minimum surface tension on compression recorded for 3 to 5 consecutive loops. A purified phospholipid was considered surface active if a minimum surface tension 0–5 dyn/cm were thus recorded and if good hysteresis was obtained. An occasional purified compound (table II) described good hysteresis but the lowest surface tension did not achieve 5 dyn/cm, but did measure less than 10 dyn/cm. This was considered surface active because of the hysteresis (fig. 1). When the minimum surface tension did not fall below 15 dyn/cm, 'normal' for saline extracts of minced lung [27], the experiment was repeated with increased amounts (100–150 μg) of the phospholipid to assure that adequate phospholipid was present to form a surface film. In no instance was low surface tension observed with the increased amounts when it was not present with 25 μg.

Extraction and separation of lipids and isolation of individual phospholipids from lung homogenates, alveolar wash and from other tissues were done by the procedure described by GLUCK *et al.* [15]. With this method, the lipids were extracted from tissues by homogenizing at high speed (30,000 rpm in a Virtis '45' homogenizer) in chloroform and methanol. The saline alveolar wash was stirred with an equal volume of methanol and the lipids extracted with two volumes of chloroform. Lipid extracts were separated on N, N-diethylaminoethyl (DEAE) cellulose acetate microcolumns into neutral lipids, nonacidic phospholipids (sphingomyelin, lecithin, phosphatidyl ethanolamine and, when present, phosphatidyl mono- and dimethylethanolamine) and acidic phospholipids (principally phosphatidyl serine and phosphatidyl inositol). Purification of individual phospholipids was done with preparative thin layer chromatography on Silica Gel H (E. Merck, Darmstadt, Germany) in a nitrogen atmosphere.

(There are several advantages of this method over the use of silicic acid columns or of direct thin layer chromatograph. This procedure allows very rapid quantitative separation of phospholipids from a total lipid extract. By separating the nonacidic and acidic phospholipid groups there is no overlap in resolution on thin layer plates of these two groups and permits easy and uncontaminated separations of individual compounds. In addition, when only very small total lipid extracts (100 μg and less, e.g.) require separation, these are easily chromatographed on the DEAE cellulose acetate microcolumn. Perhaps most important no breakdown of phospholipids occurs with this column.)

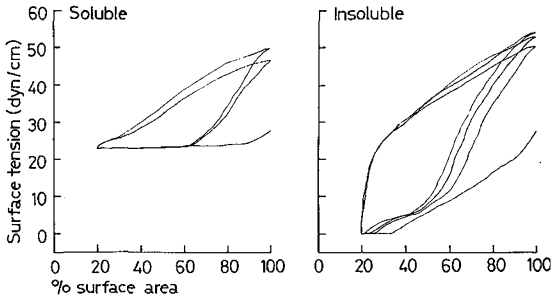

Fig. 1. Surface tension/area diagrams recorded by acetone-soluble and acetone-precipitated fractions of purified total lecithin from lung.

Separation of purified lecithin into acetone-precipitated and acetone-soluble fractions was done after thorough drying of the lecithin extract under prepurified nitrogen in a glass stoppered conical centrifuge tube. A minute amount of chloroform (10–20 μl/3–4 mg lecithin) was added to the purified total lecithin fraction and excess cold (0°) acetone added dropwise until no more milky precipitate formed. The stoppered tube was centrifuged briefly at 0–4° and the supernatant decanted into another stoppered tube. To the precipitated lecithin was added cold acetone (approximately 1 ml/mg) and both tubes placed in the freezer (0 to –5°) overnight. The precipitates were collected the following day. If an unusually large precipitate formed, as for example, with adult lung tissue, then one or two more similar washings with small amounts of cold acetone were done to assure removal of all acetone-soluble lecithins. Similar acetone precipitation of the other purified phospholipids [2] to be checked for surface activity also was done.

Phosphorus determinations were done by the method of BARTLETT [4].

Results

Alveolar Wash

The completeness and reproducibility of the procedure of alveolar lavage with 0.9 % saline at 20° in removing phospholipids were studied. Since no published studies were known which quantify the phospholipids recovered from alveolar wash, a study was set up in which the lungs from each of a litter of 8 full-term fetuses were lavaged as described above in Methods, using 15–2 ml portions of saline per lung. The washings for each pair of lungs were centrifuged as described, combined, the lipids extracted and lipid phosphorus determinations done. The values (×25) for the pairs of lungs were averaged as the theoretical maximum amount of phospholipid recoverable per lung (100 % in table I). Correspondence between total recoveries for the 8 pairs of lungs was within 10 % of the average.

Intact lungs were then removed from another litter of 9 full-term fetuses and similarly lavaged with 15–2 ml portions of saline at 20°. Each 2 cc portion was separately analyzed for lipid phosphorus. As shown in table I, significant variability of recovery of lipid phosphorus was found only in the first 2 washings. With 3 washings, 95 % of the lipid phosphorus recoverable after 15 washings was removed, with little variability; after 5–2 cc washings, 98 % was recoverable.

Table I. Percent recovery of lipid phosphorus from alveolar lavage of rabbit fetal lung with consecutive 2 ml portions of 0.9 % saline at 20°

Wash number	Percent of total[1] lipid phosphorus recovered in 2 cc saline wash	Mean cumulative % of total recovered
	Mean[2] ± S.D.	
1	77. ±12	77
2	14. ± 3	91
3	4. ± 0.5	95
4	2. ± 0.5	97
5	1. ± 0.5	98
6	0.4	
7	0.3	
8	0.2	
9	0.2	
10	0.1 (approximately)	
11	0.1 (approximately)	
12	0.1 (approximately)	
13	0.1 (approximately)	
14	0.1 (approximately)	
15	0.1 (approximately)	

[1] 'Total' is based on the average total lipid phosphorus obtained from 15–2 ml lavagings of 8 pairs of intact lung from a litter of full-term rabbit fetuses. Phosphorus determinations [4] were done on aliquots from the 30 cc total wash from each lung. The average of the 8 determinations agreed to within 10 % of each of the individual totals.

[2] Mean values were determined from washouts of lungs from a litter of 9 full-term fetuses.

Surface Activity of Purified Phospholipids

Table II shows the minimum surface tensions of purified phospholipids from rabbit and human lung and from other tissues measured on the modified Wilhelmy balance. Surface activity in lung phospholipids was found in the acetone precipitated fractions of lecithin, sphingomyelin, phosphatidyl dimethylethanolamine and phosphatidyl inositol. Phosphatidyl serine and phosphatidyl ethanolamine were not surface active. Surface activity was found also in acetone-precipitated lecithin from rabbit intestine and mesenteric fat and in lysolecithins which were prepared from alveolar wash lecithin of the 6-hour-newborn rabbit and from synthetic dipalmitoyl lecithin. The acetone-precipitated lecithin from liver, kidney, skeletal muscle, heart, plasma, red blood cells and egg were not surface active, nor was phosphatidyl methylethanolamine. All of the surface-active compounds described large hysteresis loops on compression and expansion of the surface film while those that were not surface active showed much less hysteresis. Figure 1 shows the surface tension-area diagrams of the acetone-precipitated and soluble fractions of total lecithin.

Surface Activity of Minced Fetal Lung Saline Extracts vs. Gestational Age

The minimum surface tensions of pooled fetal rabbit lung extracts are shown in table III. Surface tension was above 15 dyn/cm even after prolonged aging of the surface in the lungs from fetuses of less than 25 days gestation. Borderline surface activity was observed at 26 days of gestation and normal surface activity from 28 days of gestation. At term, 30–31 days of gestation, the minimum surface tension was 4 dyn/cm. Minimum surface tensions of lung extracts from adult rabbits were between 6 and 12 dyn/cm.

Surface Activity of Lecithins from Homogenized Lung vs. Gestational Age

The lowest surface tensions of total lecithin and its acetone-soluble and acetone-precipitable fractions isolated from homogenized whole lung are plotted in figure 2 against gestational age. Neither purified total lecithin nor the acetone-soluble fraction showed normal surface activity at any time during gestation or even in the adult lung. However, the acetone-precipit-

Table II. Surface activity of purified phospholipids

Phospholipid and source	Minimum surface tension on modified Wilhelmy balance (dyn/cm)	
	Acetone precipitated	Acetone soluble
1. Total nonacidic phospholipid fraction		
a) Adult rabbit lung	0–2	
2. Lecithin		
a) Newborn and adult rabbit lung, alveolar wash or lung homogenate[1]	0–4	23–25
b) Human infant tracheal aspirates, both full term and premature[1]	0–0.5	21
c) Adult rabbit kidney	16	25
d) Adult rabbit liver	26	25
e) Adult rabbit mesenteric fat[1]	2	
f) Adult rabbit intestine[1]	2	
g) Adult rabbit heart	22	
h) Adult rabbit skeletal muscle	20	
i) Egg yolk	26	
j) Repurified commercial synthetic dipalmitoyl lecithin [1]	0–2	
k) Adult rabbit red blood cells	18	25
l) Adult rabbit blood plasma	17	27
3. Sphingomyelin		
a) Newborn and adult rabbit lung homogenate[1]	4–5	
b) Adult rabbit kidney	46	
c) Adult rabbit liver	36	
4. Phosphatidyl ethanolamine		
a) Newborn rabbit alveolar wash	23	31
b) Newborn rabbit lung homogenate	22	27
c) Adult rabbit alveolar wash	23	28
5. Phosphatidyl serine		
a) Adult rabbit alveolar wash	24	35
b) Adult rabbit lung homogenate	22	36
6. Phosphatidyl inositol		
a) Adult rabbit alveolar wash[1]	5	24
b) Adult rabbit lung homogenate[1]	6	23
7. Phosphatidyl methylethanolamine		
a) Adult rabbit alveolar wash	22	27
8. Phosphatidyl dimethylethanolamine		
a) Adult rabbit alveolar wash[1]	3	27
b) Human infant tracheal aspirates, both full term and premature[1]	0–0.5	24
9. Lysolecithin		
a) Palmitic lysolecithin prepared from synthetic dipalmitoyl lecithin[1]	3	
b) Prepared from 6-hour-newborn rabbit alveolar wash lecithin[1]	9	25
c) Prepared from adult rabbit alveolar wash lecithin	28	27

[1] Surface-active compounds

Table III. Surface tensions of saline extracts of rabbit lungs

Gestational age (days)	Minimum surface tension[1] (dyn/cm) recorded with modified Wilhelmy balance	
	after 2–3 h of cycling	after 24 h of cycling
25	23	24
26	20	14
27	22	15
28	13.5	—
29	10	—
30–31	4	—
Adult	6–12	—

[1] A surface tension of 15 dyn/cm or less is considered 'normal' for saline extract of minced lung.

Lecithin from whole lung homogenates

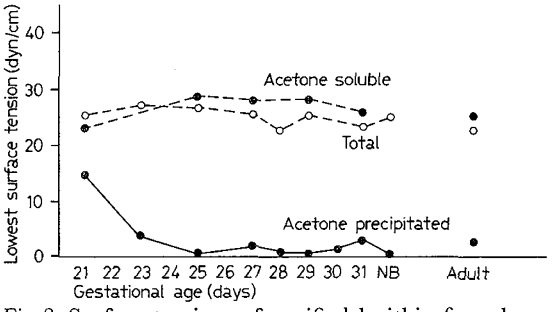

Fig. 2. Surface tensions of purified lecithin from lung homogenates during gestation. Total lecithin extracted from lung is not surface active. Acetone-precipitated surface-active lecithin was present in lungs from rabbit fetuses of all gestational ages examined.

Lecithin from alveolar wash

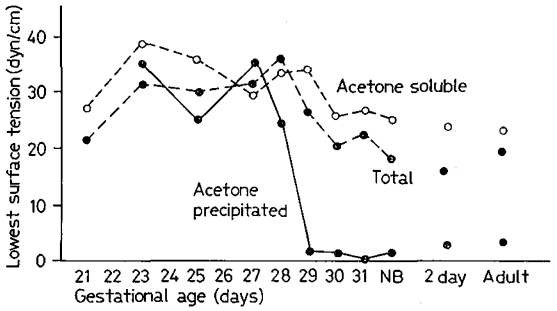

Fig. 3. Surface tensions of purified lecithin from alveolar wash during gestation. Norma surface activity of acetone-precipitated lecithin is first found on day 29 in alveolar wash.

Lecithin from homogenates of lung after alveolar wash

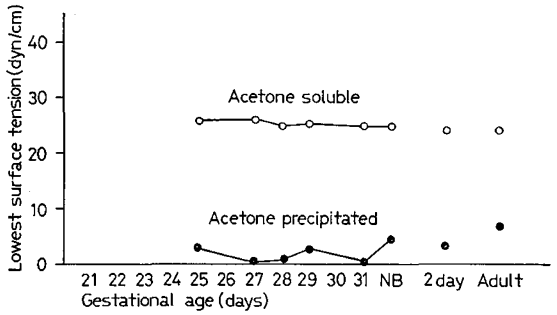

Fig. 4. Surface tensions of purified lecithin from lung homogenate after alveolar wash. Acetone-precipitated surface-active lecithin was found in the parenchyma of all gestational ages examined.

Acetone-precipitated lecithin in alveolar wash after breathing

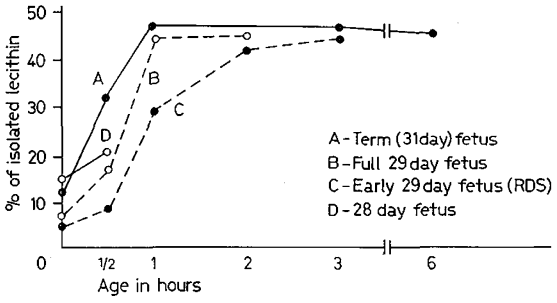

Fig. 5. Mean increase with time in percent of acetone-precipitated lecithin in alveolar wash after breathing of rabbit fetuses of different gestational ages delivered by cesarean section. Standard deviations are not plotted. The values were remarkably homogeneous about the mean, varying only by ± 0.5–2.5 %. The greatest standard deviation (± 5 %) was found in the 1-hour value for the early 29 day fetus (RDS).

able fraction of lecithin showed surface activity even at 21–23 days of gestation.

Surface Activity of Lecithins in Alveolar Washings vs. Gestational Age

The minimum surface tensions for the total, acetone-soluble and acetone-precipitable lecithin lavaged from the alveoli are plotted vs gestational age in figure 3. Again, neither the total nor the acetone-soluble lecithin is surface active. The acetone-precipitable lecithin, although present by day 23 in alveolar wash, was not surface active until the 29th day of gestation.

Surface Activity of Lecithin in Residual Lung Parenchyma after Alveolar Wash vs. Gestational Age

The lowest surface tensions of total lecithin and its acetone-soluble and acetone-precipitable fractions isolated from homogenized residual lung after alveolar lavage are plotted in figure 4 against gestational age. Only the fraction precipitated in acetone was surface active. This was not removed by alveolar wash, but was found in the parenchyma throughout the gestational period examined.

Proportions of Lecithin Fractions in Alveolar Washings during Development

The proportions of acetone-soluble and acetone-precipitable lecithin of the total lecithin obtained from alveolar lavage are shown in table IV. There is generally a slight increase in the proportion of the acetone-precipitable fraction as gestation progresses, but even in the term fetus, this represents only about 11 % of the total lecithin.

Table IV.
Percentages of acetone partitioned lecithin in alveolar wash of rabbit

Gestational age (days)	Percent of total lecithin	
	Acetone precipitated	Acetone soluble
21	5.4	94.6
23	6.8	93.2
25	6.8	93.2
27	9.0	91.0
28	15.1	84.9
29	7.4	92.6
30	11.7	88.3
31	11.1	88.9
6 h newborn	45.2	54.8
2 day	44.9	55.1
Adult	57.3	43.7

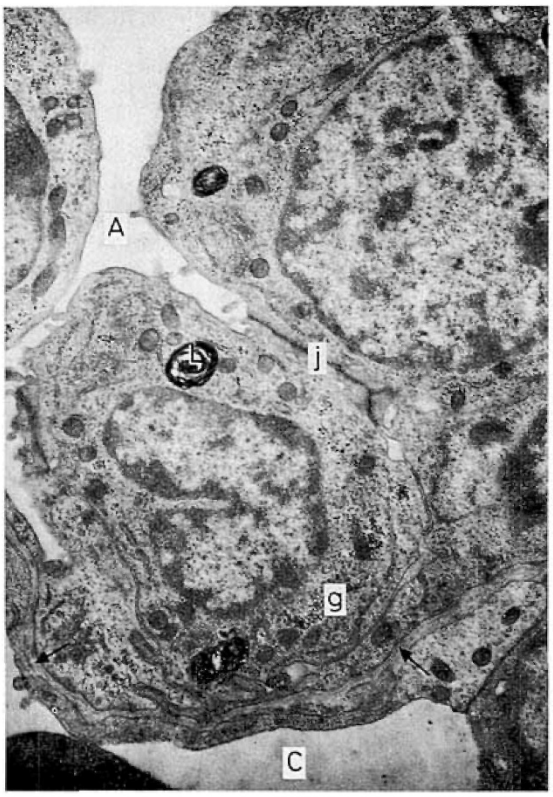

Fig. 6. Granular pneumonocyte (Karrer type II cell) from the non-breathed lung of a 28-day rabbit embryo. This cell rests on the alveolar basement membrane (arrows) and extends a few small microvilli into the alveolar space (A). Two typical lamellar bodies (L) and numerous glycogen granules (g) are found within the cytoplasm. A desmosome or tight junction (j) joins an adjacent granular pneumonocyte containing one lamellar body. A portion of an erythrocyte can be seen within a capillary (C). (21,600×, glutaraldehyde and 0_s0_4 fixation; lead citrate and uranyl acetate stain.)

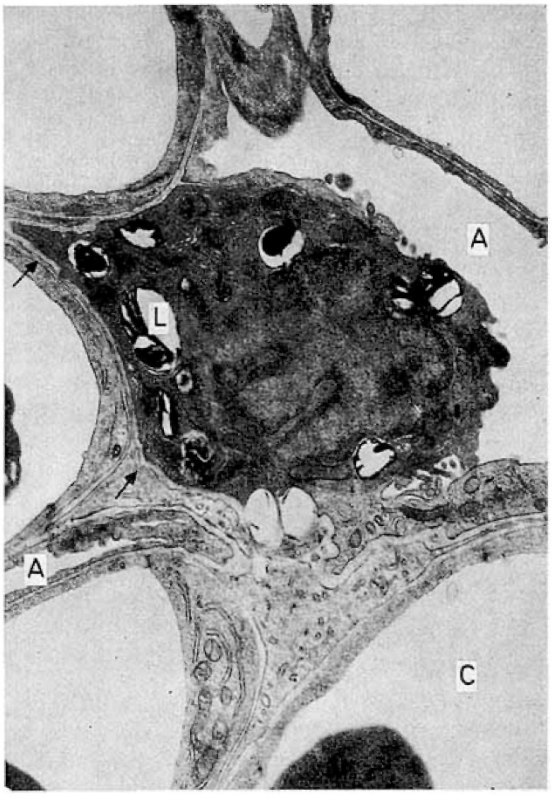

Fig. 7A

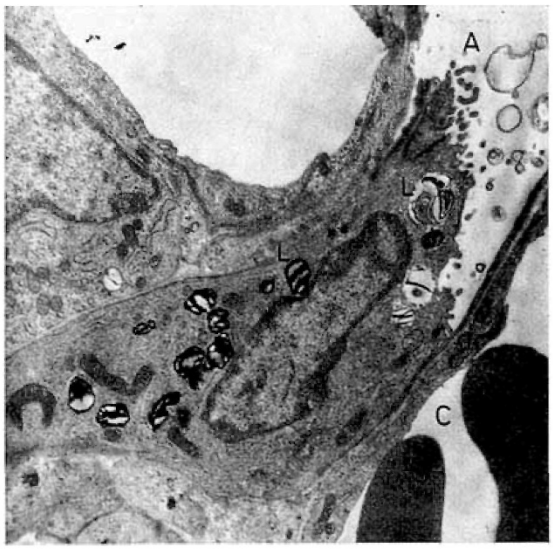

Fig. 7B

Fig. 7A. Granular pneumonocyte from the lung of a newborn rabbit approximately 8 hours after delivery. This cell is similar in position to that in fig. 6. The cytoplasm contains numerous lamellar bodies (L); this is typical of the fully developed animal. No glycogen granules are seen. Several capillaries (C) as well as a portion of two alveoli (A) are visible (21,600×, glutaraldehyde and 0_s0_4 fixation; lead citrate and uranyl acetate stain.)

B. Another type II alveolar cell of 8-hour breathing full-term rabbit showing presumed extrusion of osmophilic lamellar body (L) contents into alveolar space (A). Portions of erythrocytes are seen within a capillary (C).

Amounts and Proportions of Lecithin Fractions in the Alveolar Lavage after Breathing

The proportion of acetone-soluble and acetone-precipitable fractions of the total lecithin in the alveolar washings after breathing is shown in figure 5. In the full-term (31-day gestation) fetuses the percentage of surface-active acetone-precipitable lecithin from the alveolar wash increased to approximately 50 % of the total lecithin (the same proportion found in adult rabbit lung) after 1 hour of breathing. No further significant increase in this proportion was seen even after 6 hours of breathing. Previable fetuses, barely 28 days of gestation lived about 20 minutes with almost no detectable appearance of surface-active lecithin in the alveolar wash. These animals showed obvious signs of respiratory distress. The prematurely delivered rabbits (early 29th day of gestation) developed respiratory distress and increased their proportion of acetone-precipitable lecithin at a much slower rate than the full-term. Fetuses delivered late on the 29th day of gestation differed very little in the rate of increase of acetone-precipitable lecithin from the full-term animals.

Findings with Electron Microscopy

Increasing numbers of osmiophilic lamellar bodies in granular pneumonocytes (type II alveolar cells) with advancing gestation were found in the rabbit as they have been in other species previously [18, 28]. Figures 6 and 7 are representative sections by electron microscopy showing few lamellar bodies in alveolar cells of the nonbreathing lung of the 28-day fetus (fig. 6) and larger numbers of inclusion bodies in the alveolar cells of the lung at term (fig. 7 A and B, shown here after 8 hours of breathing). In the fetal state, as has been described by others [18, 28], these inclusion bodies appear to be smaller and deep within the cytoplasm of the granular pneumonocytes but in the term, breathing animal some inclusion bodies are superficial and distended. The appearance of some of these are similar to descriptions for other species [18] where it has been speculated that contents are being extruded into alveoli.

Discussion

The studies presented show that the unique surface activity described is a property shared by several phospholipids and is found also in lecithin from tissues other than lung. Surface activity of lecithin, sphingomyelin, lysolecithin and phosphatidyl dimethylethanolamine have been reported by others [12, 13, 19, 20, 26]. The present studies describe, in addition, surface activity in phosphatidyl inositol and in lecithin of mesenteric fat and intestine.

Although other phospholipids also exhibit surface activity, lecithin is by far the most abundant of the pulmonary phospholipids and alone of all of these shows changes that correlate closely with surface activity of the lung. Other phospholipids probably also contribute to surface activity of the alveolar layer since the total nonacidic phospholipid fraction extracted from lung is surface active, but the concentration of lecithin in lung is greater than the total of the other surface-active phospholipids combined (to be reported) and there can be no doubt of the central role of lecithin in surface activity of lung.

Not all lecithin is surface active and a simple effective technique precipitation with acetone was described to separate the surface-active lecithin fraction, when present, from the nonsurface-active total lecithin extracted from lung. Separation of lecithin into fractions according to solubility in acetone, while metabolically artificial, has great significance functionally. With this procedure, very minute amounts of surface-active lecithin present in a total lecithin fraction are detectable.

In their studies, FUJIWARA, ADAMS and SCUDDER [14] 'titrated' synthetic dipalmitoyl lecithin on the modified Wilhelmy balance with a surface area of 60 cm^2 and found that as little as 20 μg was sufficient to give good surface activity. This agreed with calculations by MOTOYAMA [21] based on the surface area of one molecule of dipalmitoyl lecithin. The presently reported measurements of surface activity were done with a standard amount of 25 μg of purified lecithin (or other phospholipid) to approximate the findings with the synthetic surface-active lecithin. FUJIWARA and ADAMS [13] reported that total lecithin extracted from homogenized lung was surface active but used from 1000–2000 μg of lecithin for measurement. MORGAN, FINLEY and FIALKOW [20] similarly applied approximately 700 μg of lung lecithin on saline in the modified Wilhelmy balance to measure surface activity. These large excesses of lecithin used in other studies to demonstrate surface activity suggest a lack of homogeneity of the lecithin fraction with but a relatively small component of actual surface-active lecithin. In the present report, purified total lecithin was not surface active.

With this procedure to separate the surface-active fraction it was possible to isolate surface-active lecithin from lung parenchyma long before the 29th day of gestation in the rabbit, when surface-active lecithin first is found in the alveolar wash. The residual lung parenchyma after alveolar wash contains surface-active lecithin, suggesting intracellular storage of surface-active lecithin in the fetal lung cell considerably prior in gestation to its appearance in alveolar spaces.

During the nonbreathing fetal state, even at term, only 11 % of the lecithin from alveolar wash is acetone-precipitated surface-active lecithin. After breathing,

the proportion of the surface-active lecithin increases rapidly, the rate depending upon the gestational age. In the full-term fetus, after one hour, the acetone-precipitated fraction forms approximately 50 % of the total lecithin, which is also about the proportion found in adult rabbit alveolar wash. The late 29-day fetus (i.e. almost 29 full days gestation) differed little from the full-term fetus. The rabbits delivered prematurely after 28 full days of gestation (early 29th day) clinically had respiratory distress from birth and their percentage of acetone-precipitable lecithin in the alveolar wash increased only at a very slow rate compared to full-term animals.

Good temporal correlation was seen between the probable intracellular storage during the fetal state of surface-active lecithin (which is released rapidly after breathing) and the findings with electron microscopy of increasing numbers of osmiophilic lamellar inclusion bodies as gestation progresses. Exocrine function of these lamellar bodies have been suggested [5, 18] as the primary source of pulmonary surfactant but there is as yet no definitive evidence for this.

Summary

Surface activity among the purified phospholipids isolated from lung occurred in lecithin, sphingomyelin and dimethylphosphatidyl ethanolamine, as previously described, and in phosphatidyl inositol, hitherto unknown to be surface active. Surface-active lecithin, from the total fraction of lecithin, was separated by precipitation with acetone. Surface-active lecithin was also found in intestine and mesenteric fat of the rabbit.

During the development of the rabbit fetus, surface-active lecithin appeared to be stored in the parenchyma of the lung long before it was detected in the alveolar washings of fetal lungs. After its appearance in alveoli, surface-active lecithin was a small portion of the total lecithin in the alveolar wash, constituting only about 11 % of total alveolar lecithins in the term fetus. However, with breathing, this increased within one hour to adult levels of approximately 50 % in the term newborn rabbit. The prematurely delivered fetus (early 29-day gestation) developed RDS and increased his percentage of surface-active lecithin in alveolar wash much more slowly. Good temporal correlation of storage and increase of lecithin in alveolar wash was found with the appearance of the osmiophilic lamellar inclusion bodies in the alveolar type II cells.

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31. GLUCK, LOUIS, M.D., Associate Professor of Pediatrics, Department of Pediatrics, Yale University School of Medicine, New Haven, Conn. 06520 (USA).