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Developmental Study of a Lamellar Body Fraction Isolated From Human Amniotic Fluid

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Summary

Several properties of a pellet fraction obtained on centrifuging amniotic fluid at 10,000 × *g* for 20 min were investigated. From these analyses, we defined a developmental profile which appears to describe the maturational process of the fetal lung surfactant system. At 14 to 18 wk gestation, the pellet fraction consisted of membrane-bound vesicles without internal lamellae. The phospholipid composition did not resemble that of surfactant, the major phospholipid being sphingomyelin. This stage, designated as presurfactant, persisted until 30 to 32 wk gestation. After this time, the phospholipid concentration of the pellet fraction increased continuously throughout development, and gradual but continuous changes in phospholipid composition were observed. Lecithin and phosphatidylinositol increased between 30 and 35 wk gestation. Interruption of pregnancies at this stage, termed onset of surfactant synthesis, resulted in 100% incidence of respiratory distress syndrome. From 36 wk gestation to postterm, the pellet fraction contained structures with the characteristic morphology of the lamellar inclusion bodies. The presence of single membrane components in these preparations did not contribute to the phospholipid composition. Early formed lamellar bodies lacked phosphatidylglycerol and had a high content of phosphatidylinositol. Once phosphatidylglycerol appeared in the lamellar body fraction, it continued to increase, accompanied by a decrease in phosphatidylinositol with little change in lecithin. A phosphatidylglycerol value of greater than 1% of the total phospholipids appeared to represent the stage of maturity at which there was no risk of respiratory distress syndrome.

Speculation

The characterization of a pellet fraction obtained from human amniotic fluid led to the description of a developmental profile which appears to define the individual stages involved in the biochemical maturation of the fetal lung surfactant system. The development of respiratory distress syndrome appears to be very much dependent on the stage at which this process is interrupted. It is expected that such analyses can be adapted to provide a simple, yet specific index of fetal pulmonary maturity.

Several methods are currently used for the measurement of surfactant in amniotic fluid as an index of fetal pulmonary maturity. The usefulness of this measurement in the prevention of the respiratory distress syndrome (RDS) is unquestionable; however, there appears to be little agreement as to which type of measurement should be made. The lecithin:sphingomyelin (L:S) ratio method (10) gained the most widespread clinical acceptance but has been so variously modified by individual investigators that interpretation is confusing and comparisons difficult (3). Other measurements, based on chemical (19, 25, 26) or physical (4, 5) properties of surfactant, have not yet proven to be superior to the L:S ratio measurement (14). Interest recently focused on the measurement of amniotic fluid phosphatidylglycerol, an acidic phospholipid present in lung surfactant (23). The pattern of accumulation of this phospholipid in amniotic fluid, however, needs elucidation (6, 15).

If the properties of surfactant as it exists in amniotic fluid were better understood, then the confusion surrounding its measurement would be cleared. Studies suggest that surfactant material is secreted into amniotic fluid as intact lamellar bodies (17, 20). Such structures, identified in amniotic fluid from women near term, were found to represent an insoluble or particulate fraction of amniotic fluid (16). Recent observations in our laboratory support this concept. We showed that on differential centrifugation of amniotic fluid, surfactant material was recovered in the pellet fraction, whereas nonsurfactant components, including phospholipids, remained in the soluble supernatant. We suggested that some measurement of this surfactant-containing pellet fraction should provide a specific index of fetal pulmonary maturity (22). To investigate this possibility more fully, we undertook an extensive characterization of the properties of this fraction obtained from human amniotic fluid specimens at various gestational ages. This report describes the details of these investigations.

MATERIALS AND METHODS

MATERIALS

Amniotic fluid specimens taken at 14 to 18 wk (at therapeutic abortion) and 28 wk to postterm via indicated amniocenteses (with informed consent) were used in this study.

Authentic lipid standards were purchased from Serdary Research Laboratories, Inc., London, Ontario, Canada or from Applied Science Laboratories, Inc., State College, PA. Silica Gel G plates (Analtech, Inc.) were obtained from Fisher Scientific Co., Montreal, Quebec, Canada. Taab's resin was purchased from Marivac Ltd., Halifax, Nova Scotia, Canada. All other chemicals and solvents were reagent grade and were purchased from Canadian Laboratory Supplies, Ltd., Halifax, Nova Scotia, Canada.

PREPARATION AND PURIFICATION OF THE SURFACTANT FRACTION

Each specimen was centrifuged at 140 × *g* for 5 to 10 min in an IEC-model PR6 centrifuge using rotor IEC-269 to remove cellular material. The resultant supernatant was then centrifuged at 10,000 × *g* for 20 min in an IEC B20A high-speed centrifuge using rotor IEC-870. The supernatant was discarded. The pellet, containing the surfactant material (22), was retained for chemical analysis. In several experiments, this crudely prepared surfactant fraction was further purified by density gradient centrifugation according to Frosolono *et al.* (9). For these experiments, only freshly obtained, unfrozen specimens were used. Chemical analyses and electron microscopy were performed on both the crudely prepared and the purified surfactant fractions.

CHEMICAL ANALYSES

Lipid extraction, phospholipid concentration, and lecithin:fatty acids analyses were performed as previously described (22). Individual phospholipids were separated by two-dimensional thin-layer chromatography using chloroform:methanol:water (65:25:4, v/v/v) in the first dimension and butanol:acetic acid:water (90:30:30, v/v/v) in the second. Phosphorus analysis of the individually scraped phospholipids was performed according to Bartlett (1).

Individual neutral lipids were separated by thin-layer chromatography using petroleum ether:diethylether:acetic acid (80:20:1, v/v/v) as solvent. The spots were visualized by spraying with 5% phosphomolybolic acid in 3.5% $HClO_4$ and heating for 8 min at 85°C. Individual spots were measured by reflectance densitometry. Individual standard curves were prepared for each neutral lipid component. Protein was measured according to Lowry *et al.* (18).

ELECTRON MICROSCOPY

Portions of the 10,000 × *g* pellet and the postdensity gradient material were fixed for 1 to 2 hr in 2.5% glutaraldehyde at 4°C and postfixed in 1% osmium tetroxide for 2 hr at 4°C. The fixatives were buffered in 0.1 M cacodylate (pH 7.3). Specimens were stained overnight in 5% aqueous uranyl acetate prior to routine dehydration with ethanol and embedding with Taab's resin. Thin sections were cut with glass knives on an LKB-Huxley Ultratome and double stained with 2% aqueous filtered uranyl acetate for 5 min and lead citrate (27) for 2 min. Observations were made using a Phillips E.M. 200.

DIAGNOSIS OF RDS AND GESTATIONAL AGE

The diagnosis of the idiopathic RDS was made without knowledge of the results of the amniotic fluid studies. RDS was diagnosed when an infant had expiratory grunting, subcostal retractions, and decreased breath sounds on auscultation, beginning at birth and persisting beyond the first 6 hr of life. Transient respiratory distress was diagnosed when an infant had unexplained respiratory distress lasting more than 1 hr but less than 6 hr. Infants who had positive blood or positive cerebrospinal fluid cultures during the acute phase of the disease and infants who had other causes of respiratory distress identified by X-ray were excluded.

Gestational age was calculated from the onset of the last normal menstrual period and checked by neonatal assessment. If clinical assessment of gestation was within two wk of assessment by dates, calculation by dates was accepted as accurate. Otherwise, gestation was assigned from neonatal assessments.

RESULTS

Light microscopy of the 10,000 × *g* pellet revealed the presence of intact globular structures of approximately 1 μm dimensions (Fig. 1*A*). Those present at 14 to 18 wk gestation were indistinguishable at this level of microscopic analysis from those present at later developmental stages. However, examination of these pellets by electron microscopy showed that the structures obtained at 14 to 18 wk gestation lacked an intralamellar appearance (Fig. 1*B*), whereas those obtained at later developmental stages, *i.e.*, from 36 wk gestation to postterm, had the characteristic morphology of the lamellar inclusion bodies of the alveolar type II cell (Fig. 1, *C* and *D*). Although the lamellar-like structures represented the major portion of the pellet fraction by electron microscopic evaluation, other unidentified membrane components were present in these crude preparations (Fig. 1*C*).

Density gradient centrifugation of the crude lamellar body preparation (10,000 × *g* pellet) resulted in a fraction enriched in lamellar bodies but devoid of the extraneous membrane components observed in the crude preparation (Fig. 1*D*). The purified lamellar body fraction banded at the interface between 0.68 and 0.25 M sucrose on the gradient, and 90 to 92% of the phospholipid present in the crude preparation were recovered in this fraction.

Chemical analysis of the crude and purified lamellar body fractions (Table 1) revealed that the purification resulted in the removal of protein and neutral lipid components from the crude preparation. These components were largely recovered in the pellet fraction of the gradient, and their removal from the lamellar body preparation resulted in an enrichment of phospholipid in this fraction. No significant differences were observed in the phospholipid composition of the crude and purified lamellar body preparations. On density gradient centrifugation of 10,000 × *g* pellets obtained at 14 to 18 wk gestation, no material banded in the region occupied by lamellar bodies but instead formed a small sediment at the bottom of the tube.

Routine phospholipid analyses were performed on the 10,000 × *g* pellet fractions without further purification by density gradient purification. Representative results obtained at three individual stages at which electron microscopy was also performed are presented in Table 2. There was an increase in phospholipid concentration between 14 and 18 wk gestation (at which time the pellet fraction contained nonlamellated structures) and 36 wk gestation, at which time the pellet fraction was enriched with lamellar bodies. There was a further large increase in the lamellar body-enriched pellet between 36 and 42 wk gestation. Differences in phospholipid composition were observed at these three stages. In the nonlamellated structures, sphingomyelin was the most abundant phospholipid, followed by lecithin. Phosphatidylglycerol was absent from these structures. Lecithin was by far the most abundant phospholipid in the lamellar body-containing pellets, but in somewhat higher proportions in those at postterm than those present at 36 wk. Phosphatidylglycerol was absent in the lamellar body fraction of 36 wk gestation, and phosphatidylinositol represented the second most abundant phospholipid at this time. In postterm preparations, phosphatidylglycerol was the second most abundant phospholipid, and phosphatidylinositol represented a minor component. Furthermore, fatty acids analyses revealed that palmitic acid represented less than 50% of the total lecithin-esterified fatty acids in the nonlamellated structures but was greatly increased in the lamellar body-enriched pellets.

A more complete developmental profile of the phospholipid analyses of the pellet fractions (Fig. 2) illustrated that the concentration (expressed as μg phospholipid in the pellet fraction per ml amniotic fluid) increased continuously throughout development,

Table 1. *Composition of lamellar body fraction: pre- and postdensity gradient centrifugation*[1]

	Pregradient	Postgradient
Component	Wt %[2] (n[3] = 4)	
Protein	16.22 ± 2.32[4]	4.92 ± 1.13
Neutral lipid[5]	17.63 ± 2.26	8.19 ± 1.57
Phospholipid	66.16 ± 1.84	86.89 ± 1.01
Individual phospholipids	Total lipid phosphorus (n = 5)	
	%	
Phosphatidylserine	2.04 ± 0.82	1.07 ± 0.37
Phosphatidylinositol	7.10 ± 0.87	6.87 ± 1.07
Sphingomyelin	1.81 ± 0.39	1.14 ± 0.15
Lecithin	79.13 ± 1.12	81.59 ± 1.56
Phosphatidylglycerol	4.81 ± 1.09	4.72 ± 1.12
Phosphatidylethanolamine	3.33 ± 0.33	2.89 ± 0.33
Unidentified	1.77 ± 0.60	1.73 ± 0.52

[1] For individual specimens, analyses were performed on the 10,000 × *g* pellet (pregradient) and on the purified lamellar body fraction on density gradient centrifugation of the 10,000 × *g* pellet suspension (postgradient).

[2] % of total (lipid and protein).

[3] *n*, number of determinations.

[4] Mean ± S.D.

[5] Includes cholesterol, both free and esterified, and triglycerides.

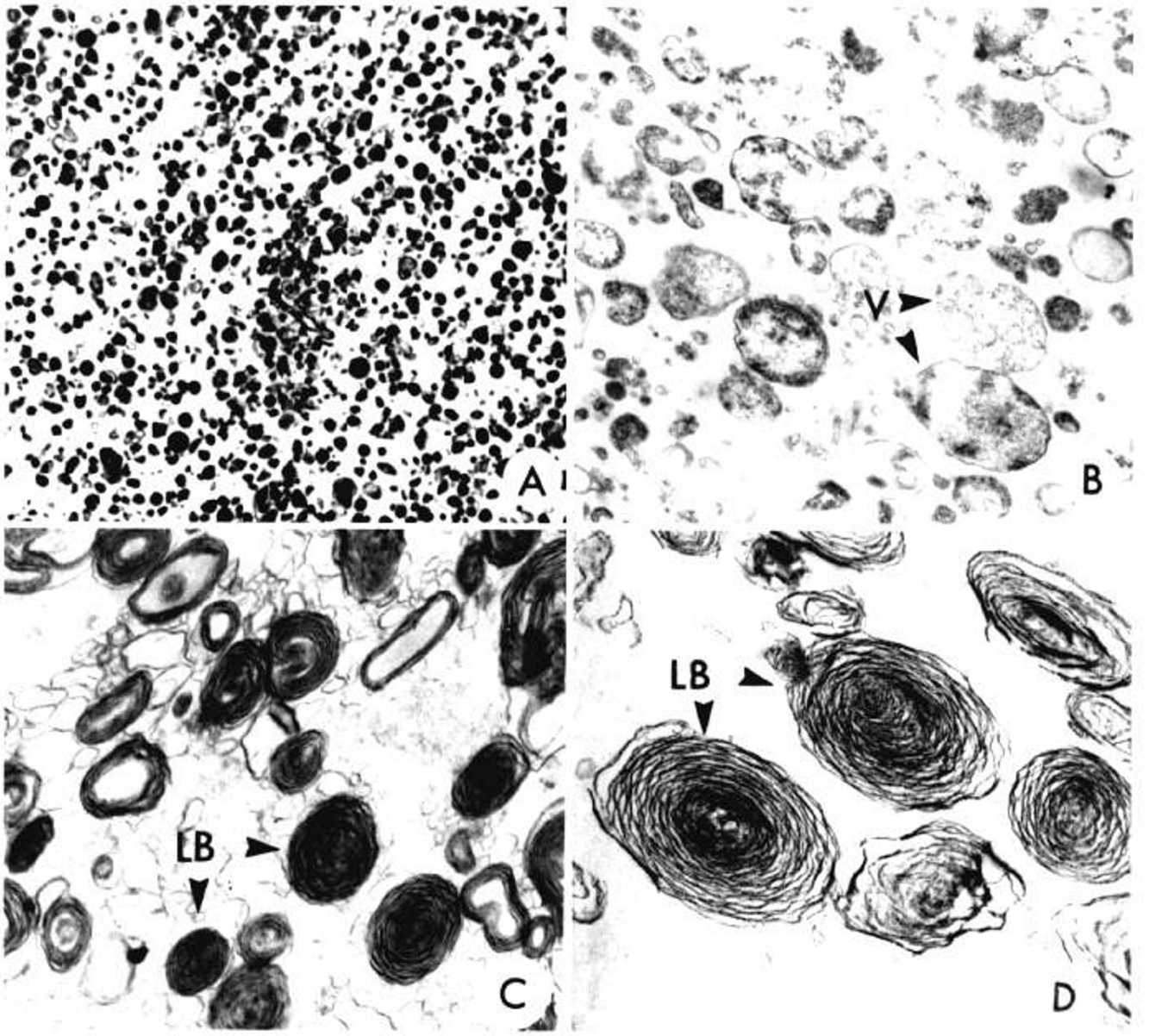

Fig. 1. Photographs illustrating the 10,000 × *g* pellet of human amniotic fluid. *A*, photograph of a pellet taken near term as seen with optical microscopy of semi-thin plastic section stained with toluidine blue. × 800; *B*, electron micrograph of early-gestation pellet (14 to 18 wk). Membrane-bound vesicles (*V*) but absence of lamellated structures. × 15,000; *C*, electron micrograph of late gestation pellet (36 wk) depicting lamellar bodies (*LB*). × 7,200; *D*, lamellar bodies as seen in pellet following density gradient centrifugation. Absence of extraneous membrane components × 13,500.

most particularly after 32 wk gestation. Compositional changes were gradual but continuous to postterm.

Early changes, *i.e.*, between 30 and 35 wk gestation, were marked by an increase in the relative percentage of both lecithin and phosphatidylinositol. Phosphatidylglycerol was undetectable throughout this period. The relative percentage of phosphatidylinositol reached a maximum value at 35 wk gestation and then continuously decreased throughout later stages of development. The decline in phosphatidylinositol was accompanied by the appearance and gradual accumulation of phosphatidylglycerol. During this latter period of development, there was little change in the relative percentage of lecithin.

The above data were obtained by combining results from a number of individual patients at each gestational age. In several cases, serial amniocenteses were performed on a single patient, and in these events, the profile obtained was similar to that shown in Figure 2, although the entire profile was sometimes shifted towards earlier or later stages in gestation. Furthermore, this same profile was obtained in cases where the 10,000 × *g* pellet was purified by density gradient centrifugation. In every instance, it was found that the first changes in the composition of the 10,000 × *g* pellet were an increase in lecithin and phosphatidylinositol (*i.e.*, each expressed as a percentage of total phospholipids). Phosphatidylglycerol was never detected during this early accumulation of lecithin and phosphatidylinositol and was not detectable until the relative percentage of these two phospholipids reached their maximum value. Once phosphatidylglycerol appeared, it always continued to increase, and this increase was accompanied by a proportionate decrease in phosphatidylinositol with little change in lecithin.

The developmental profile (Fig. 2) was divided into individual stages, each stage representative of a particular phospholipid composition of the 10,000 × *g* pellet. These are shown in Table 3 together with the respiratory status of infants born at each partic-

Table 2. *Concentration and composition of structures present in 10,000 × g pellet at various developmental stages*[1]

Gestation (wk)	Structures observed	Concentration (μg phospholipid/ml amniotic fluid)	Phospholipid composition (wt % of total phospholipid)							
			Phosphatidylserine	Phosphatidylinositol	Sphingomyelin	Lecithin	Phosphatidylglycerol	Phosphatidylethanolamine	Unidentified	Palmitic acid (% of lecithins fatty acids)
14–18	Nonlamellated structures	4.0	13.5	2.3	38.9	28.1	0.0	17.3	0.0	43.6
36	Lamellar bodies	52.3	3.2	13.7	4.2	72.8	0.0	5.3	0.9	80.0
42	Lamellar bodies	288.5	1.4	2.8	1.1	82.9	7.4	3.1	1.4	82.1

[1] Electron microscopy was performed on five specimens at 14 to 18 wk gestation and 10 specimens from 36 wk to postterm. Chemical analyses represent results obtained from one specimen at each stage indicated. Those of 36 and 42 wk gestation were from the same patient.

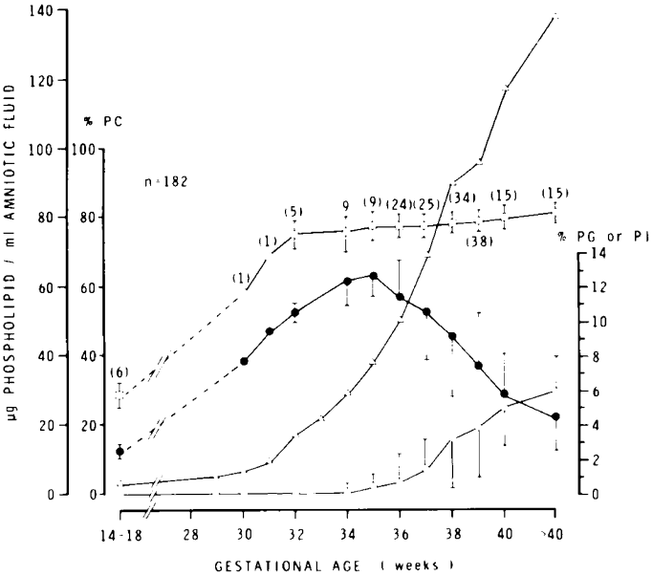

Fig. 2. Phospholipid profile of amniotic fluid 10,000 × *g* pellet during development. Concentration (△) is expressed as μg phospholipid in the pellet fraction per ml amniotic fluid. Individual phospholipids [lecithin (□), phosphatidylinositol (●), and phosphatidylglycerol (○)] are expressed as a percentage of total phospholipids in the pellet fraction. Each value represents the mean ± S.D. for the number of determinations shown in *parentheses*.

ular stage. Stage I represents the composition observed for nonlamellated structures. This composition was often found as late as 30 to 32 wk gestation. However, in this study, no deliveries occurred at any time this composition was found. In the earliest stage of development in which delivery occurred, stage II, both the lecithin and phosphatidylinositol values were greater than those of the nonlamellated structures, but neither had reached its maximum value; phosphatidylglycerol was absent. Six deliveries occurred at this stage; the six neonates developed RDS. Analysis of 10,000 × *g* pellets of pharyngeal aspirates obtained from four other infants with RDS (but for whom we have no amniotic fluid data) revealed a phospholipid composition characteristic of this stage. In stage III, both lecithin and phosphatidylinositol reached their maximum value but phosphatidylglycerol did not appear. Only one of twelve infants delivered at this stage was free of respiratory problems. Of the 11 infants who developed resipiratory problems, in two cases the condition was transient, but for the remaining nine, RDS ranged from mild to severe. Stage IV is represented by the appearance of phosphatidylglycerol but at levels representing less than 1.0% of the total phospholipids. Two of nine infants delivered at this stage developed RDS. In both cases, RDS was mild, and both were delivered at 36 wk gestation of diabetic mothers, one being White's class C and the other White's class R (28). The final stage represents the accumulation of phosphatidylglycerol and includes all cases in which this phospholipid comprised more than 1.0% of the total phospholipids. Of 117 infants delivered in this group, two developed respiratory problems which were classified as transient respiratory distress; the remaining 115 were free of respiratory problems. Several 10,000 × *g* pellets obtained from pharyngeal aspirates of neonates free from RDS also had a composition characteristic of this stage.

DISCUSSION

In this study, we investigated several properties of a pellet fraction obtained on centrifuging amniotic fluid at 10,000 × *g* for 20 min. In a previous communication (22), we suggested that this pellet fraction represented fetal pulmonary surfactant, the soluble

supernatant containing phospholipids (and other components) of nonpulmonary origin. The demonstration of lamellar-like structures morphologically similar to the lamellar inclusion bodies of the pulmonary alveolar type II cell in the pellet fraction supports this suggestion. Other investigators identified lamellar bodies in amniotic fluid pellets (16, 20). Our observations confirm these findings and give further credence to the concept that surfactant material is extruded in this form from the fetal lung into the amniotic compartment.

Single-membrane components were observed in the lamellar body-containing pellet fractions. The source of these membranes is not known; however, they represented a very small portion of the pellet fraction and could be removed on density gradient centrifugation, and chemical analysis revealed that their presence did not significantly contribute to the phospholipid composition of the pellet fraction. Thus, the phospholipid composition of the lamellar bodies present in the 10,000 × *g* pellet could be determined directly on this crude preparation without the necessity of further purification. Pellet fractions obtained from amniotic fluid specimens at 14 to 18 wk gestation, at which time the fetal lung has not yet begun to synthesize surfactant (2), were devoid of lamellar bodies but consisted of membrane-bound vesicles without internal lamellae. The phospholipid composition of these pellets is somewhat similar to that reported for lung lavage material obtained from rabbit fetuses prior to the onset of surfactant synthesis (24), suggesting the possibility of a pulmonary origin for this material.

From an analysis of the phospholipid concentration and composition of the pellet fraction obtained from amniotic fluid at various gestational ages, we defined a developmental profile which appears to describe the maturational process of the fetal lung surfactant system. Correlation of electron microscopic analysis of the pellet fraction, studies on neonatal pharyngeal aspirates, and the respiratory status of infants born at various stages of the developmental process support this proposal. Data obtained at each individual stage are summarized in Table 4 together with the developmental stage in the maturational process of the fetal lung surfactant system which each appears to represent.

In stage I, the phospholipid concentration of the pellet fraction is very low (usually less than 5 μg/ml amniotic fluid), electron microscopy revealed the absence of lamellar bodies, and although the phospholipid composition suggested the possibility of a pulmonary origin, it in no way resembled the phospholipid composition of surfactant. This stage has thus been designated presurfactant. Electron microscopy at this stage was performed on pellets obtained at 14 to 18 wk gestation only; however, analysis of the phospholipid concentration and composition indicated the persistance of this stage until 30 to 32 wk gestation. In the present study, no infants were delivered at a time when analysis suggested this stage of lung development. However, it would be expected that delivery under these circumstances would result in serious respiratory problems.

In stage II, the phospholipid concentration of the pellet fraction increased but not to a value of 25 μg/ml amniotic fluid. Because

Table 3. *Correlation of amniotic fluid 10,000 × g pellet phospholipid composition with respiratory status of the newborn*[1]

Pellet composition				No. of infants delivered	Respiratory status		
%	PC[2]	PI	PG		No RDS	TRD	RDS
I	<SPH	<10	0	0			
II	<75	<10	0	6	0	0	6 (33.6)[3]
III	>75	>10	0	12	1 (31.0)	2 (33.5)	9 (34.3)
IV	>75	>10	>0<1	9	7 (36.0)	0	2 (36.0)
V	>75	<10	>1	117	115 (39.1)	2 (39.3)	0

[1] Respiratory status is as defined in "Materials and Methods." Specimens were grouped according to their composition and are arranged in order of increasingly mature composition as ascertained in Figure 2. For groups II to IV, delivery was within 48 hr of amniocentesis. For group V, the mean interval between amniocentesis and delivery was 4.1 days.

[2] PC, lecithin, PI, phosphatidylinositol, PG, phosphatidylglycerol, TRD, transient respiratory distress; SPH, sphingomyelin.

[3] Numbers in parentheses, mean gestational age.

Table 4. *Correlation of amniotic fluid analysis and respiratory status of the newborn with individual stages of development of the fetal lung surfactant system*

10,000 × g pellet analysis		Composition					
Electron microscopy	Phospholipid concentration (μg/ml)	% PC[2]	% PI	% PG	Gestation (wk)	Respiratory status	Developmental stage
I Nonlamellated vesicles	<5	<SPH	<10	0	14–18	No infants delivered	Presurfactant
II No electron microscopy performed	<25	>SPH	<10	0	30	100% RDS	Onset of surfactant synthesis
III Lamellar bodies	>25	>75	>10	0	34–36	75% RDS 84.6% RDS and TRD	Early lamellar body
IV Lamellar bodies	>50	>75	>10	>0<1		0% RDS in NDM	Biochemical maturation of lamellar bodies
					36–37	50% RDS in ODM	
V Lamellar bodies	≫50	>75	<10	>1	36–37	0% RDS 1.7% TRD	

[1] Values shown for the phospholipid concentration and composition represent a summarization of data obtained from 6 to 117 specimens at each stage. Electron microscopy was performed on 1 to 5 individual specimens for each stage indicated. The gestations represent the approximate time at which each stage was first observed. The stage of development of the fetal lung surfactant system was assigned on the basis of these analyses.

[2] PC, lecithin; PI, phosphatidylinositol; PG, phosphatidylglycerol; SPH, sphingomyelin; TRD, transient respiratory distress; NDM, nondiabetics; ODM, overt diabetics.

of the difficulty in obtaining sufficient material for a detailed analysis, this stage is the least well characterized. Although no electron microscopy was performed, the onset of accumulation of lecithin and phosphatidylinositol observed at this stage suggests that it represents the onset of surfactant synthesis by the fetal lung. This conclusion was supported by the finding that the proportion of lecithin-bound palmitic acid at this stage reached a value of 70 to 80% (results not shown). Although this stage was generally reached by 30 wk gestation, it was observed in some instances as late as 36 to 37 wk gestation. Pregnancies interrupted at this stage of development resulted in 100% incidence of RDS.

Stage III represents our earliest observance of lamellar bodies in the pellet fraction and is designated as such. Although lamellar bodies may indeed have been present at stage II, the phospholipid composition at this stage was characteristically different from that at stage III, and therefore these stages are described separately. Early-formed lamellar bodies were characterized by their virtual absence of phosphatidylglycerol and high content of phosphatidylinositol. Pellets of this composition were generally observed at 34 to 36 wk gestation but in some instances were identified as early as 29 wk and even as late as 39 wk gestation. The phospholipid concentration of the pellet fraction of this composition was usually in the range of 25 to 50 μg/ml amniotic fluid. Interruption of pregnancies at this stage of development still resulted in a high incidence of RDS.

Stages IV and V are together designated as the biochemical maturation of lamellar bodies, characterized by the appearance and increased accumulation of phosphatidylglycerol. Stage IV represents the early phase of phosphatidylglycerol accumulation (*i.e.*, representing less than 1% of the total phospholipids) whereas V represents later stages of phosphatidylglycerol accumulation. This phospholipid was present for the most part by 36 to 37 wk gestation, but in three cases, it appeared even before 34 wk, and in six cases, it was absent at 39 wk gestation. Nevertheless, once phosphatidylglycerol appeared, it accumulated rapidly so that it comprised more than 1% of the total phospholipids just 2 to 3 days after its appearance. Interruption of pregnancies at stage IV resulted in RDS only in those cases in which the pregnancy was complicated by maternal diabetes. Although we report a 50% incidence in this circumstance, it should be pointed out that there were only four patients in this group. Deliveries in all cases were by Cesearean section at 36 wk gestation. A phosphatidylglycerol value of greater than 1% of the total phospholipids appeared to represent the stage of maturity at which there was no risk of RDS.

From the foregoing, it is evident that analysis of the phospholipid concentration and composition of a 10,000 × *g* pellet fraction of amniotic fluid offers good insight into the state of maturity of the fetal lung. A characteristic concentration and composition is observed when no surfactant material is present in amniotic fluid. Once surfactant is synthesized and secreted by the fetal lung, it can be recovered in reasonably pure form in the pellet fraction, and again, a characteristic composition indicates the state of maturity of the fetal lung surfactant system.

The compositional changes in the lamellar body fraction during later stages of development are similar to those described for lamellar bodies isolated from rabbit lungs (13) and suggested by studies on human neonatal pharyngeal aspirates (12, 21). The significance of the compositional changes associated with maturation is not clear. Although the role of phosphatidylinositol is obscure, possible roles for phosphatidylglycerol have been suggested. Feldman *et al.* (8) demonstrated its activation of CTP: cholinephosphate cytidyltransferase (EC 2.7.7.15), an enzyme involved in lecithin biosynthesis, whereas Hallman and Gluck (13) suggested that it is a possible modifier of surfactant activity. Our studies indicate that RDS does not develop when phosphatidylglycerol is present in the surfactant fraction.

With the characterization of the lung surfactant fraction throughout fetal development, it now becomes a relatively simple task to develop procedures feasible for routine use for assessing the precise stage in the maturational process. The presence of phosphatidylglycerol in amniotic fluid clearly indicates that the fetal lung surfactant system is in its final stages of maturation. This suggestion was first put forth by Hallman *et al.* (15) who proposed measurement of this phospholipid as an adjunct to the L:S ratio for the assessment of fetal lung maturity. However, their measurements are complicated by the fact that they have used a much lower *g* force in preparing amniotic fluid for the comparison of their phosphatidylglycerol measurement with the L:S ratio (15) than for their routine L:S ratio measurement (10). For phospholipid analysis, they did not separate the surfactant from the nonsurfactant components of amniotic fluid. Furthermore, they suggest that phosphatidylglycerol does not appear until the L:S ratio is at least 2.0, which in their experience is already indicative of a functionally mature lung. Gotelli *et al.* (11) have similar findings. We found in many instances that phosphatidylglycerol was present in the isolated lamellar body fraction when the L:S ratio was less than 2.0 (in some cases as low as 1.2). Because delivery under these circumstances resulted in the absence of respiratory problems, it was evident that the phosphatidylglycerol measurement provided a more accurate assessment of fetal pulmonary maturity than the L:S ratio (to be described in detail in a separate communication). In most cases, when the L:S was greater than 2.0, phosphatidylglycerol was present in the isolated lamellar body fraction. In one case where phosphatidylglycerol was absent, its absence indicated pulmonary immaturity as evidenced from the development of RDS. We noted in six cases of diabetes-complicated pregnancies a rather extended stage III. Lamellar bodies continued to accumulate during this stage, but the appearance of phosphatidylglycerol was somewhat delayed. In these cases, the L:S ratio was well above 2.0 before phosphatidylglycerol appeared. Although the significance of this is not clear, it helps to explain the high incidence of RDS often observed (6, 7, 29) in diabetic pregnancies in the event of a mature L:S ratio. Cunningham *et al.* (6) suggested the measurement of phosphatidylglycerol as an adjunctive index of fetal pulmonary maturity, particularly in diabetic pregnancies. However, these authors describe an unusual pattern of accumulation of this phospholipid. They report decreasing levels in the nondiabetic from 33 wk gestation to near term, and for diabetics, phosphatidylglycerol decreased at 34 to 37 wk and increased again at 38 wk. In view of the foregoing discussion, their data are difficult to interpret.

We suggest that for the utmost accuracy in interpretation, the surfactant fraction of amniotic fluid be separated from the nonsurfactant components which interfere with the results. From a detailed phospholipid analysis as described in this report, we have been able to assess the precise stage of the fetal lung maturation process. We are currently investigating possible means of simplifying these analyses while maintaining the same degree of accuracy in interpretation.

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