AMNIOTIC FLUID LAMELLAR BODY PHOSPHOLIPID CONTENT AND FETAL PULMONARY MATURITY

by

Wm. Michael Ivie

A THESIS

Presented to the Department of Clinical Pathology
and the Graduate Division of the Oregon Health Sciences University
in partial fulfillment of
the requirements for the degree of

Master of Science
August 1981

APPROVE [redacted]

..... (Professor in Charge of Thesis)

[redacted]

..... (Chairman, Graduate Council)

ACKNOWLEDGEMENTS

I would foremost like to thank my major advisor, Dr. J. Robert Swanson, for the assistance, guidance, and advice he has willingly provided during my graduate study. The support and interest of the faculty and staff of the Department of Clinical Pathology is also appreciated

I am grateful to Drs. M.J. Novy, J.P. O'Grady, and J.W. Reynolds for their assistance of this project. The services of Dr. M. McClellan providing the electron microscopy examination are appreciated.

This thesis is dedicated to my wife, Patricia J., for her patience and understanding.

Financial support of this project provided by the Medical Research Foundation of Oregon Grant No. 8010 and funds for equipment purchases provided by a Biomedical Research Support Grant is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

The respiratory distress syndrome (RDS)caused by hyaline membrane disease (HMD) is a leading cause of mortality in the prematurely born infant (1,2). RDS occurs when the infant is delivered prior to physiological and functional maturation of the lungs which is reflected in the production of pulmonary surfactant. Without adequate pulmonary surfactant to maintain the integrity of the alveoli atelectasis occurs preventing adequate oxygenation of the blood resulting in cyanosis and hypoxia. Pregnancies complicated by such factors as diabetes mellitus, hypertension, hemolytic disease of the fetus, and premature rupture of the membranes are at risk of premature delivery. Management of such pregnancies as well as elective repeat cesarean section relies upon the accurate assessment of fetal pulmonary maturity. The incidence of HMD is related to prematurity as gauged by either birth weight or gestational age. In a survey of the literature the average incidence of HMD for infants weighing less than 2500 g at birth is 14%(2). Of the approximately 3,000 deliveries per year at the Oregon Health Sciences University by the obstetrical service 15-20% of the patients are at risk for development of HMD[1] and are thus benefited by the diagnosis of fetal pulmonary maturity from biochemical analysis of amniotic fluid.

It is appropriate to briefly review the significance of pulmonary surfactant, its relation to hyaline membrane disease, and the development of biochemical assays used for its assessment prior to the statement of the problem which this research project addresses.

---

[1]Personal Communication. Department of Obstetrics and Gynecology. Oregon Health Sciences University.

A. SURFACTANT

Pulmonary surfactant refers to a substance which lowers the surface tension generated at the air-tissue interface of the lung. Surface tension is the force acting to reduce the surface area of the alveoli and is the result of intermolecular attractions present in the two phases. Thus, surface tension acts to inhibit distension of the alveoli and to promote collapse or atelectasis of the alveoli. The behavior of alveoli when considered as small bubbles can be described in terms of the theorem of Laplace.[2] Therefore, when the diameter of the alveoli decreases upon expiration the surface tension increases resulting in a higher pressure promoting collapse. However, the air-tissue interface of the lung is coated with a surfactant material that reduces the surface tension. This serves to decrease the pressure needed to distend the lungs during inspiration and to enhance stability of the alveoli at low lung volumes. The surfactant also accounts for the presence of a residual volume at zero pressure (2,3,4).

Chemical analysis of surfactant by density gradient centrifugation of different mammalian species (dog, rat, rabbit) has demonstrated that dipalmitoyl phosphatidyl choline (DPPC) is the only surface-active phospholipid present in sufficient quantity (41-58% w/w) possessing the physical properties of surfactant to form a monolayer to cover the whole alveolar area (5,6). Lipids comprise approximately 85% of the surfactant material of which 5-13% are neutral lipids with cholesterol the largest fraction. Of the remaining surface active phospholipids

---

[2] $P = 2T/r$, where P is equal to the pressure exerted on the sphere, T is the surface tension, and r is the radius of the sphere.

unsaturated phosphatidyl cholines comprise 11-29%, phosphatidyl ethanolamine 5-10%, and acidic phospholipid 4-18% of which phosphatidyl glycerol may constitute up to 10%. Trace amounts of carbohydrate are also present (5,7,8). A small percentage (10-15%) of surfactant consists of nonserum proteins (5,9). This is primarily a 35,000 molecular weight apoprotein which appears in alveolar surfactant along with DPPC (10).

The type II alveolar epithelial cell is known to be the site of synthesis for DPPC (11,12). All of the enzymes necessary for DPPC synthesis have been demonstrated to be present in lung tissue (2). The primary pathway of DPPC synthesis in the lung is by the so-called Kennedy and Weiss pathway (13) in which choline first reacts with adenosine triphosphate to form phosphocholine which then reacts with cytidine triphosphate to form cytidine diphosphate choline. The phosphocholine portion of cytidine diphosphate choline is then transferred to a 1,2-diglyceride molecule to form DPPC (1,2,6,14).

From studies using radiolabeled precursors and electron microscopy it was demonstrated that multilamellar bodies within the type II cells serve as storehouses of surfactant. DPPC is synthesized in the rough endoplasmic reticulum, progresses through the golgi apparatus to lamellar bodies where it is stored prior to release (14,15). Synthesis of phosphatidyl glycerol (PG) has also been demonstrated in the microsome fraction of type II epithelial cells. The mechanism involves cytidine diphosphate diglyceride and sn-glycerol phosphate as precursors to phosphatidyl glycerol phosphate which is subsequently hydrolyzed to phosphatidyl glycerol (16).

The lamellar bodies (LB) are secreted by exocytosis by fusion of the limiting membrane of the lamellar body with the plasma membrane of the type II epithelial cell (17). Once the LB have been secreted into the subphase of the alveolar lining layer tubular myelin is formed which is thought to be a liquid crystal state of surfactant (18-21). The phospholipids form a monolayer at the surface of the alveolar lining layer with their hydrocarbon chains towards the air and the polar heads in the aqueous phase (6,15). The presence of the unsaturated phosphatidyl cholines, phosphatidyl glycerol, neutral lipids, and apoprotein are thought to enhance the adsorption and stability of DPPC at the surface of the alveolar lining layer (5,22,23).

B. BIOCHEMICAL ASSAYS

Avery and Mead in 1959 (4) were the first to suggest a correlation between surfactant properties and hyaline membrane disease. It was not until 1967 that the association of HMD and low content of lung DPPC was demonstrated (24). Biezenski, Pomerance, and Goodman (25) observed that phospholipids in the amniotic fluid tended to increase in concentration with gestational age. The relationship between low amniotic fluid phospholipid content and the developement of respiratory distress syndrome was first examined by Nelson (26). On the assumption that an increasing concentration of lecithin in the amniotic fluid is a reflection of pulmonary maturity Gluck and associates (27) recommended a thin layer chromatography method for determining the ratio of lecithin to sphingomyelin (L/S ratio). The concentration of lecithin increases near term while sphingomyelin remains relatively constant throughout gestation. Subsequently it was demonstrated that

surfactant material secreted into the fetal lung contributes to the amniotic fluid composition verifying that the phospholipids pass from the lung via the trachea into the amniotic cavity (28-30).

The L/S ratio method has been widely incorporated into the clinical laboratory and its usefulness verified by a number of authors (1,2). An increase in the L/S ratio occurs late in gestation (about 35 weeks) due to an increase in the synthesis and release of lecithin. A L/S ratio approximating 2.0 has been demonstrated to indicate maturity (1,2,27,31). The method relies upon the separation of fully saturated surface active phosphatidyl choline (primarily DPPC) and sphingomyelin from other phospholipids present in amniotic fluid by use of cold acetone precipitation (32). However, the use of this extraction step has been questioned by others (1) and has been shown to be a semi-quantitative technique for concentrating the surface active lecithin which may account for immature L/S ratio determinations for fetuses with mature lungs (33). Another methodological problem is the preparative centrifugation of amniotic fluid which if centrifuged at high relative centrifugal forces will increase the number of false immature L/S ratios while centrifugation at low relative centrifugal forces will increase the number of false mature L/S ratios (28,34,35). Problems in quantitation of the L/S ratio on the thin layer chromatography plate exist due to variations in charring properties of phospholipids, spot size and migration variables of phospholipids, and the variables of densitometry which include light scattering and nonlinearity (1,36). Contamination of

amniotic fluid with blood and meconium is known to affect the L/S value especially when the L/S ratio is near the critical maturity level of 2.0 (32,37,38).

A review of the literature indicates that the L/S ratio is a good indicator of maturity when the L/S ratio is mature (>2.0) having an occurence rate of RDS of only 1-2% (1,2,39). The occurence of RDS when the L/S ratio is less than 2.0 is much less predictable with reported incidence rates varying greatly (1,2). The usefulness of the L/S ratio in pregnancies complicated by diabetes mellitus has also been questioned with a number of cases of RDS developing in pregnancies with mature L/S ratios (1).

Due to the uncertainty of the L/S ratio in the situations cited above, other investigators have proposed alternative methods for evaluating fetal pulmonary maturity. The rapid surfactant test (RST) (40) is one method commonly employed. This simple procedure depends on the ability of surface active phospholipids to form stable surface films that can support the structure of a foam in the presence of an ethanol-water mixure. Unsaturated phospholipids form unstable foam and other surface tension lowering substances are excluded from the film by the ethanol. Evaluation of the test has revealed somewhat contradictory results but generally the effectiveness of the test is similar to that of the L/S ratio. A low incidence of RDS is associated with a mature result while an immature and intermediate result is not nearly as reliable in assessing lung maturity (1). Also, false mature results occur in the presence of contamination by blood or meconium (37,40,41).

The presence of phosphatidyl glycerol (PG) which occurs late in gestation at approximately 35 to 36 weeks has of late been recommended as an additional indicator for fetal pulmonary maturity (42-44). A few authors utilizing thin layer chromatographic separation of phospholipids have indicated the absence of RDS when PG is present but have noted that the converse is not always the case(42-44,39,45). This method appears to have the advantage of not being affected by contamination from blood due to its relatively high concentration in the lung surfactant and its low concentration or absence in other tissue (42,46).

Numerous other methods for measurement of amniotic fluid phospholipids have been reported and related to the development of hyaline membrane disease. These include absolute and total lecithin concentration (47,48), lecithin as a percentage of total phospholipid (49), palmitic acid content (50,51), assessment of surfactant apoprotein(52), enzyme activity for lecithin biosynthesis (53,54), fluorescence depolarization (55,56), optical density measurement (57), and the determination of disaturated phosphatidyl choline utilizing osmium tetroxide oxidation of unsaturated phospholipids (58).

The determination of lamellar body phospholipid content of amniotic fluid serves as another possibility for assessment of fetal lung maturity (35,59,60). Lamellar bodies as well as tubular myelin figures have been demonstrated in the amniotic fluid and have been isolated by differential and/or density gradient centrifugation (28, 61-63).

Differentiated type II epithelial cells do not usually contain lamellar bodies until after the 24th week of gestation (64) and the presence of lamellar bodies in the amniotic fluid does not occur prior to 26 weeks of gestational age (63).

As a function of gestational age 50% of the specimens at 28-32 weeks gestation contained lamellar bodies and 85% of specimens at term contained lamellar bodies as demonstrated in a qualitative examination of amniotic fluid by electron microscopy (63).

Biochemical studies of lamellar bodies reveal that 65% of the phospholipid of amniotic fluid is recovered in the LB fraction (62). The LB fractions have been demonstrated to consist of 72-76% phosphatidyl choline, 12-13% phosphatidyl ethanolamine and/or phosphatidyl glycerol, 2.4-3.8% lysolecithin, 1.1-2.3% sphingomyelin, 7.3-9.7% phosphatidyl serine and/or phosphatidyl inositol (61). Duck-Chong (62) demonstrated that the LB fraction contains 10-14 times as much phospholipid as protein on a weight/weight basis and that 75-85% of the phospholipid was phosphatidyl choline. Also, LB isolated from the rat lung have been shown to reproduce the surface tension characteristics of pulmonary surfactant (65).

Oulton (35) employing differential centrifugation to obtain a lamellar body enriched fraction determined the phospholipid phosphorus content to quantitate the phospholipid and compared this method with the L/S ratio for assessing lung maturity. Only one infant out of eight who developed RDS had a LB phospholipid value greater than 4mg/dl.

A LB phospholipid content greater than 4mg/dl proved to be a better indicator of maturity than intermediate and immature L/S ratios. In 24 cases with an intermediate L/S ratio (1.5-1.9) that did not develop respiratory problems the LB phospholipid content was greater than 4mg/dl, and in 11 out of 37 cases with immature L/S ratios (<1.5) that did not develop respiratory problems the LB phospholipid content was greater than 4mg/dl. Thus, Oulton demonstrated that the lamellar body phospholipid content of amniotic fluid may be a better index to fetal pulmonary maturity than the L/S ratio when values less than 2.0 are obtained.

Duck-Chong (66) developed a micro-method for the isolation of lamellar bodies from amniotic fluid which correlated well with conventional isopycnic density gradient ultracentrifugation. This procedure was utilized in a comparison of LB phospholipid content and the L/S ratio of amniotic fluid in assessing fetal lung maturity. The two methods disagreed in their assessment of lung maturity in 25% of the pregnancies (i.e., one method predicted lung maturity while the other method predicted lung immaturity) with 80% of these being cases of immature L/S ratios (<2.0) with LB phospholipid values indicating maturity (greater than 3.5mg/dl). The LB phospholipid content was less than 3.5mg/dl in seven cases of RDS that were analyzed within three days of delivery. In 185 cases free of respiratory problems that were analyzed within two days of delivery the LB phospholipid content was greater that 3.5mg/dl in 182 (59).

In a more extensive evaluation the LB phospholipid content of amniotic fluid was correlated to respiratory status of the infant.

Eleven of 12 infants analyzed within 2 days of delivery with LB phospholipid values of less than 2.5mg/dl developed serious respiratory problems. The LB phospholipid values for 15 cases of HMD assayed within three days of delivery ranged from 1.0-4.7mg/dl(60).

These results show promise for the determination of lamellar body phospholipid content of amniotic fluid for the assessment of fetal pulmonary maturity.

STATEMENT OF THE PROBLEM

As noted above the assessment of fetal pulmonary maturity by current methods suffers from a variety of problems. Briefly, this includes the following: poor predictive value of results indicating immaturity making such values inaccurate and unreliable for the management of pregnancies which are at risk for premature delivery; difficulty in accurately analyzing amniotic fluid samples which are contaminated with blood or meconium; a lack of specificity for surface-active phospholipids; and possibly poor reliability in the assessment of pregnancies complicated by diabetes mellitus.

The primary objective of this project was to measure the clinical effectiveness of the lamellar body phospholipid assay of amniotic fluid in predicting fetal pulmonary maturity, and to compare the effectiveness of this test with the L/S ratio, phosphatidyl glycerol assay, and the rapid surfactant test. Pregnancies at risk for premature delivery constituted the population studied. Amniotic fluids collected within three days of delivery by transabdominal amniocentesis and at

the time of cesarean section were used for evaluation in the assessment of lung maturity and the development of hyaline membrane disease. The lamellar body phospholipid value was also compared to the pediatrician's estimate of gestational age to determine the correlation with gestational age.

The second objective of the project was to examine the analytical parameters of the method for the analysis of lamellar body phospholipid content. This included determination of the accuracy and precision of the phospholipid assay, and the effect of contamination by blood and meconium on the lamellar body phospholipid content of amniotic fluid.

Hopefully this project will provide information and aid the development of methods that will be of value in assessing the fetal lung maturity of high risk obstetrical patients. The findings of this research project are described below.

MATERIALS AND METHODS

A. EQUIPMENT

Beckman Airfuge Ultracentrifuge with 18° A-100 Rotor. Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

5 x 20 mm Cellulose Nitrate Centrifuge Tubes. Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

Gilford Stasar III Spectrophotometer. Gilford Instrument Laboratories Inc., Walnut Creek, California.

Beckman CD-200 Computing Densitometer. Beckman Instruments Inc., Clinical Instruments Division, Fullerton, California.

Mettler H 35 AR Analytical Balance. Mettler Instrument Corp., Princeton, New Jersey.

International Centrifuge Model V. International Equipment Co., Boston Mass.

25 ul SMI Pipet. Scientific Manufacturing Industries, Emeryville, California.

10 ul Hamilton Syringe Model 701N with repeating dispenser (PB 600-1). Hamilton Co., Reno, Nevada.

Instant Thin Layer Chromatography Silica Gel (ITLC-SG) impregnated glass fiber sheets (product 61886). Gelman Instrument Co., Ann Arbor Michigan.

Gelman Developing Tank Model 51325-1. Gelman Instrument Co., Ann Arbor, Michigan.

S/P Vortex-Genie Mixer Model K-550-G. Scientific Industries Inc., Bohemia, New York.

Temp-Blok Module Heater No. 2090. Lab-Line Instruments, Inc., Melrose Park, Illinois.

Multi-Temp Blok No. 2093. Lab-Line Instruments, Inc., Melrose Park, Illinois.

-10 to 360°C Thermometer (VWR 61010-121). VWR Scientific Inc., San Francisco, California.

B. CHEMICALS

Ficoll Type 70. Sigma F-2878. Sigma Chemical Co.

Ammonium Molybdate, 4-hydrate (1-0716). J.T. Baker Chemical Co.

L-Ascorbic Acid (A-7506). Sigma Chemical Co.

Potassium Phosphate, Monobasic (4-4921). J.T. Baker Chemical Co.

Phosphatidyl Choline, Dipalmitoyl (P-6267). Sigma Chemical Co.

Lecithin/Sphingomyelin Standard Solution (LS-30). Sigma Chemical Co.

L-α-Phosphatidyl-DL-Glycerol (P-0514). Sigma Chemical Co.

U.S.I. Absolute Pure Ethyl Alcohol, Reagent Quality. U.S. Industries Chemicals Co.

Chloroform, UV Spectrophotometry grade. J.T. Baker Chemical Co.

Methyl Alcohol, Reagent Grade.

70% Perchloric Acid, Reagent Grade.

Dichloromethane, Reagent Grade.

Ziminski Reagent: dissolve 60g $(NH_4)_2SO_4$ in 50 ml H_2O, add 24 ml concentrated H_2SO_4 and dilute to 200 ml with H_2O.

0.9% Saline: dissolve 9.0g NaCl in deionized water and dilute to one liter.

C. SPECIMENS AND CLINICAL ASSESSMENT

From July, 1980, through May, 1981, a total of 229 samples of amniotic fluid from 166 pregnancies were assayed for fetal lung maturity by the lamellar body phospholipid assay, lecithin/sphingomyelin ratio, phosphatidyl glycerol assay, and the rapid surfactant test. One hundred eighty samples were collected by transabdominal amniocentesis and four vaginal drainage samples for determination of fetal maturity as part of the routine fetal maturity profile for management of complicated pregnancies performed at the Oregon Health Sciences University. An additional 45 samples of amniotic fluid were collected at the time of cesarean section. Samples contaminated with blood and meconium were analyzed as were samples from one case of twin pregnancy (separate amniotic cavities).

Assessment of hyaline membrane disease was determined by a neonatologist not aware of the laboratory results. Infants were diagnosed and classified as hyaline membrane disease (HMD), mild hyaline membrane disease (mild HMD), transient tachypnea of the newborn (TTN), or no respiratory disease based on the clinical presentation and the reticulogranular appearance of chest roentgenograms as follows (67):

> HMD was diagnosed from the clinical signs of tachypnea, dyspnea, retractions, grunting and cyanosis corrected by oxygen administration, and radiographic signs of air bronchograms and a diffuse reticulogranular pattern in the pulmonary parenchyma.
>
> TTN was characterized by tachypnea and mild dyspnea, and radiographic features of interstitial pulmonary edema with no reticulogranular patterns or air bronchogram.

The classification of mild HMD or HMD was a subjective decision based on the severity of the case. Respiratory problems secondary to sepsis or cardiac anomaly were not included in the classification.

Estimation of the infants gestational age was made by the attending pediatrician at birth utilizing the Dubowitz technique (68). The gestational age of the fetus at the time of amniocentesis was calculated by subtracting the intervening period.

To analyze the clinical significance of the lamellar body phospholipid content, lecithin/sphingomyelin ratio, phosphatidyl glycerol content, and rapid surfactant test in their prediction of fetal pulmonary maturity, the sensitivity (per cent of disease states with a positive value), specificity (per cent of nondisease states with a negative value), predictive value of an immature value, predictive value of a mature value, and efficiency of each test was calculated(69). A positive value is an indication of fetal lung immaturity and HMD while a negative value indicates fetal lung maturity (disease-free condition). Efficiency is the percentage of results which are true positives and true negatives.

D. ASSAY PROCEDURES

1. Lamellar Body Phospholipid Assay

The method for determining the lamellar body phospholipid (LB-PL) content of amniotic fluid was essentially that of Duck-Chong (66) with a few modifications to adapt the procedure for use in the clinical chemistry laboratory. Using a 25ul SMI pipet, four 100 ul samples of whole amniotic fluid were layered over 75 ul of 15% Ficoll 70

(w/v in 0.9% NaCl) in 5x20 mm cellulose nitrate tubes and centrifuged for 20 minutes at 28 psi (115,000 x g) in the Beckman Airfuge. Two 100 ul samples of a pooled amniotic fluid control specimen were centrifuged with each patient sample.

Using a Pasteur pipet as much of the supernatant was discarded as possible without disrupting the lamellar body fraction which forms a distinct band at the interface of the two solutions. With a clean Pasteur pipet the lamellar body fraction was removed taking only enough Ficoll to ensure that all of the visible band was removed and transferred to 4 ml chloroform-methanol (2:1, v/v) in a 20 x 125 mm screw cap borosilicate glass test tube. The LB fractions from two centrifuge tubes were combined in a 4 ml portion of chloroform-methanol (2:1) for each sample determination. Thus, the patient samples were run in duplicate and the control sample once. After mixing, 0.8 ml of 0.9% NaCl was added to each tube and vortexed for 10 seconds. Separation was aided by centrifuging for 2-5 minutes at 150 x g. The aqueous phase and any material at the interface was removed with a Pasteur pipet and discarded. The chloroform layer was washed with 0.6 ml chloroform-methanol-0.9% NaCl (3:48:47, by vol) by vortexing, centrifuging and removing the aqueous phase as in the previous step. The organic phase was transferred to 13 x 100mm borosilicate glass test tubes and evaporated under a nitrogen airflow at 45°C.

To quantitate the LB-PL, a phosphorous determination was utilized. After evaporation of the organic solvent, 0.33 ml of 70% perchloric acid was added to each tube and the phospholipid residue was digested

at 160 ± 5°C. for 60-90 minutes in a dry heating block. To each tube 2.9 ml deionized water, 0.4 ml 2.5% ammonium molybdate (w/v), and 0.4 ml of 10% ascorbic acid were added, mixing after each reagent. Standards ranging from 0-4 ug phosphorus (as KH_2PO_4) which were not digested contained 0.3 ml 70% perchloric acid. The optical density was determined at 720 nm in a Gilford Stasar III spectrophotometer after specimens and standards were incubated at 50°C. for 20 minutes. The lamellar body phospholipid content of the amniotic fluid was calculated in mg/dl by multiplying the phospholipid phosphorus value as determined from the standard curve by 12.5. This factor takes into account the concentration expression and the phosphorus content of the surfactant phospholipid (4%) (66).

Specimens contaminated with blood or meconium were analyzed in the same manner as uncontaminated samples. Uncontaminated specimens were either processed as soon as possible or frozen at -20°C., while contaminated samples were extracted and analyzed as soon as possible. Duck-Chong (66) has demonstrated that freezing and thawing once does not significantly affect the LB-PL content of amniotic fluid.

Analysis time for this assay is approximately 2.5 hours.

2. Lecithin/Sphingomyelin Ratio

The method for determination of the lecithin/sphingomyelin ratio (L/S ratio) is a modification of the method recommended by Gluck and associates (27, 31). A 5 ml aliquot of amniotic fluid was initially centrifuged at 1000 x g for 5 minutes to remove any cells and debris present. The supernatant was removed and the L/S ratio

run in triplicate using 0.5 ml of supernatant plus 0.5 ml 0.9% NaCl. Lipids were extracted by mixing first with 1 ml of absolute methanol for 10 seconds followed by mixing with 2 ml of chloroform for 1 minute. Samples were centrifuged at 1000 x g for 10 minutes to separate the chloroform layer which was removed with a Pasteur pipet and transferred to a 12 x 75 mm borosilicate glass test tube. After evaporation of the chloroform at 45°C. under a nitrogen stream, the tubes were chilled in ice for 1 minute. Ten drops of acetone chilled to approximately 0°C. were added to each test tube while swirling the tubes in the ice and chilled for an additional minute to precipitate the saturated phospholipids. Acetone was decanted and the precipitate washed with another 10 drops of acetone. The precipitate was then dried under a nitrogen flow keeping the tubes in the ice bath.

The remaining residue was dissolved in chloroform and spotted on ITLC-SG paper. If the rapid surfactant test was immature or intermediate, the precipitate was dissolved in 6 ul of chloroform and spotted, but if the rapid surfactant test was mature the precipitate was dissolved in 12 ul of chloroform with only 3 ul being spotted on the chromatography paper. A pooled amniotic fluid control was processed with each patient sample, and a L/S standard (Sigma L/S-30) was spotted with each run. A solvent consisting of dichloromethane-ethanol-deionized water (120:24:2.3, by vol) was used for migration of the phospholipids.

For detection, the ITLC-SG paper was sprayed with Ziminski's reagent and dried at 215°C. for 10 minutes in an oven. Lecithin and

sphingomyelin spots were scanned and quantitated by transmission densitometry.

If the L/S ratio could not be determined immediately the supernatant was frozen at -20°C. Specimens containing greater than 0.5 ml of packed red blood cells/2ml of amniotic fluid were unsatisfactory for analysis, as were samples contaminated with meconium and mucous(38).

3. Phosphatidyl Glycerol Assay

The method used for detection of phosphatidyl glycerol (PG) was a modification of the Tsai and Marshall method (43). A 0.5 ml aliquot of the supernatant obtained from the initial centrifugation of the sample for the L/S ratio was extracted with 1 ml of methanol and 2 ml of chloroform, and evaporated to dryness as the three aliquots were for the L/S ratio. The test tube was chilled in the ice bath but the phospholipid extract was not treated with acetone. Six microliters of chloroform was used to dissolve the phospholipid residue and spot the sample on ITLC-SG paper. The PG was developed and detected by the same procedure used for the L/S ratio. If the rapid surfactant test was mature, the phospholipid residue was dissolved with 12 ul of chloroform and 3 ul were spotted. A phosphatidyl glycerol standard (Sigma P-0514) was spotted corresponding to 1 ug/ml, 2 ug/ml, and 4 ug/ml and run with each specimen.

PG was considered present if a well defined spot migrated to the same position as the standard. When the full extract was spotted, a positive PG was semi-quantitated by visually comparing spot density to the standards and reported as less than 1 ug/ml, 1-2 ug/ml, 2-4

ug/ml, or greater than 4 ug/ml. If 1/4 of the full extract was used, a positive PG was semi-quantitated by comparing spot density to the 1 ug/ml standard and reported as less than 4 ug/ml or greater than 4 ug/ml.

4. Rapid Surfactant Test

Amniotic fluids were tested for the ability of pulmonary surfactant to generate stable foam in the presence of ethanol according to the method of Clements, et. al. (40) using a 1:1 dilution and a 1:2 dilution. Well-mixed whole amniotic fluid (0.5 ml) was diluted 1:1 with 95% absolute ethanol and diluted 1:2 with 95% absolute ethanol after mixing 0.5 ml of amniotic fluid with 0.5 ml of 0.9% NaCl in 13 x 100 mm borosilicate glass test tubes. Both tubes were capped with rubber stoppers, shaken vigorously by hand for 15 seconds, and interpreted after 15 minutes.

If there were enough bubbles to form a complete ring in each test tube the result was recorded as mature. Bubbles forming a complete ring in the 1:1 dilution but not in the 1:2 dilution were reported as intermediate. An immature result was reported if there were not enough bubbles to form a ring in either tube.

The rapid surfactant test (RST) was performed immediately or refrigerated at 4-6°C. until the test could be performed that same day. Amniotic fluid containing blood, meconium or mucus is unsatisfactory for the RST since each substance will contribute to foam-stability (40,41). Many samples that were visibly contaminated with blood, meconium or mucus were not analyzed due to immature and intermediate

samples giving falsely mature results in the presence of the contamination.

E. ANALYTICAL AND SPECIAL STUDIES

1. Linearity of the Standard Curve

A phosphorus standard solution containing 13.3 ug phosphorus/ml (as KH_2PO_4) was added in triplicate to 13 x 100 mm test tubes in varying amounts to check the linearity of the standard curve. Standards containing 0.067, 0.13, 0.33, 0.67, 1.33, 2.67, 4.0, and 5.32 ug P were made using a 10 ul and a 100 ul Hamilton syringe according to the previously described procedure (see LB-PL assay), and the optical density read against a zero microgram phosphorus blank at 720 nm. The absorbance readings were averaged and plotted versus micrograms of phosphorus.

2. Digestion Procedure

A known amount of dipalmitoyl phosphatidyl choline (Sigma P-6267) was dissolved in chloroform for use as a standard solution (0.95 mg DPPC/ml $CHCl_3$). Aliquots of the DPPC solution containing the equivalent of 4 ug phosphorus were digested at 160 ± 5°C. for varying periods of time. The samples run in duplicate were digested for 0, 5, 10, 20, 35, 60 or 90 minutes in 0.33 ml 70% perchloric acid after evaporation and quantitated for phosphorus according to the LB-PL assay procedure. The results reported as ug P were plotted versus time.

To compare the digestion of the DPPC standard to that of the lamellar body fraction of amniotic fluid, aliquots of a sample of amniotic fluid were digested for different periods of time. Samples run in duplicate which had been centrifuged at 115,000 x g for 20 minutes, and extracted and washed according to the LB-PL assay procedure were digested for 0, 10, 30, 60, or 90 minutes in 0.33 ml 70% perchloric acid at 160 ± 5°C. and quantitated for phosphorus as previously described. The results were plotted with the digestion of the DPPC standard solution.

3. Reagent Stability

To determine the stability of 2.5% ammonium molybdate and 10% ascorbic acid when stored in amber bottles at 4-6°C. the optical density of a 1.33 ug P, 2.67 ug P, and 4.0 ug P standard made according to the previously described procedure (see LB-PL assay) was recorded at approximately weekly intervals for seven weeks. Absorbance readings were plotted versus time.

4. Accuracy and Extraction Efficiency

To check the accuracy of the LB-PL assay the DPPC standard solution was used. Ten replicates of a 4 ug phospholipid phosphorus sample and ten replicates of a 1 ug phospholipid phosphorus sample of the standard solution in 13 x 100 mm test tubes were evaporated, digested in 70% perchloric acid, and quantitated for phosphorus using the LB-PL procedure. The mean, standard deviation, and per cent recovery were calculated for both sample sizes.

To determine the efficiency of extraction of phosphatidyl choline by the LB-PL procedure aliquots of the DPPC standard solution were assayed. Ten replicates of a 4 ug phospholipid phosphorus sample and ten replicates of a 1 ug phospholipid phosphorus sample were added to 4 ml portions of chloroform-methanol (2:1, v/v) in screw cap borosilicate glass test tubes, extracted and washed, evaporated, digested in 70% perchloric acid, and quantitated for phosphorus according to the LB-PL procedure. The mean, standard deviation, and per cent recovery were calculated for each sample size.

4. Precision

Within-run variation and day-to-day variation was assessed by assaying samples of whole amniotic fluid. To determine within-run variation ten replicates of an amniotic fluid were assayed on the same day for LB-PL content according to the procedure previously described. Three different amniotic fluids were analyzed in this manner. The mean, standard deviation, and coefficient of variation were calculated for each sample.

For determining day-to-day variation, five samples of amniotic fluid were separated into five aliquots and one sample of amniotic fluid was separated into ten aliquots and assayed for LB-PL content on different days. The mean, standard deviation, and coefficient of variation were calculated for each sample.

To study and control variations in the LB-PL method, a pooled amniotic fluid (daily control) was assayed with each patient sample.

Two 100 ul portions of the daily control were centrifuged, extracted, digested, and quantitated for lamellar body phospholipid phosphorus along with the patient amniotic fluid sample. During the period in which patient samples were analyzed two different daily control samples stored at -20°C. were used, each for a period of five months. The LB-PL content of the control pools were chosen to be in the low normal to high abnormal range. The mean, standard deviation, and coefficient of variation were calculated for each pooled control.

In addition to the daily control, another pooled amniotic fluid control (long term control) was set up to determine the effect of storage at -20°C. for an extended period of time. At approximately one month intervals for an eight-month period, one long-term control sample was analyzed in duplicate for LB-PL content according to the procedure described above. The mean, standard deviation, and coefficient of variation were calculated for the long term control.

6. Contamination

To determine the effect of blood contamination on the LB-PL content of amniotic fluid aliquots of eight amniotic fluids were contaminated with varying concentrations of heparinized whole blood. Aliquots contained 0, 1.0%, 2.0%, 3.0%, 5.0%, or 9.0% contamination. All levels of contamination were visible with a 1.0% contamination appearing slight, 2.0% moderate, 3.0% and 5.0% heavy, and 9.0% gross.

Samples were centrifuged at 140 x g for 5 minutes to remove any erythrocytes and fragments present to examine only the effect

of plasma lipids and phosphorus. The supernatant was assayed for LB-PL content according to the procedure outlined above. LB-PL values were corrected for dilution and the average difference from the uncontaminated value was calculated. The one-tailed Wilcoxin matched-pairs test was used to determine whether there was a significant difference between the uncontaminated and the contaminated values.

To evaluate the effect of meconium contamination on the LB-PL content of amniotic fluid varying amounts of a 10% saline suspension of fresh meconium was added to aliquots of seven amniotic fluids. Samples contained 0, 0.1%, 0.5%, 1.0%, 1.5%, or 2.0% contamination. Each level of contamination was visible with 0.1% and 0.5% appearing slight, 1.0% moderate, 1.5% heavy, and 2.0% gross. The LB-PL values (average of two determinations) were corrected for dilution and the average difference from the uncontaminated value was calculated. The two-tailed Wilcoxin matched-pairs test was used to determine the level of significant difference between the uncontaminated and the contaminated values.

7. Preparative Centrifugation

To determine the effect of centrifuging amniotic fluid to remove cells and debris on the LB-PL content, aliquots of nine amniotic fluids were centrifuged at different relative centrifugal forces (RCF) prior to analysis for the LB-PL content. Aliquots of each sample were centrifuged for 5 minutes at 0, 150, 300, 500, or 1000 x g and the supernatant assayed for LB-PL content according to the

procedure described above. For each RCF the average per cent LB-PL content remaining was calculated, and the LB-PL content was plotted versus RCF. Differences between values for uncentrifuged aliquots and those centrifuged at the designated RCF were analyzed statistically by the Wilcoxin matched-pairs test (one-tailed).

8. Electron Microscopy

Ten 100 ul samples of amniotic fluid were centrifuged at 115,000 x g for 20 minutes in the Beckman Airfuge and examined by electron microscopy to verify the presence of lamellar bodies at the interface between the Ficoll solution and whole amniotic fluid. After centrifugation, the samples were separated into three fractions and diluted 1:10 with 1/2s Brenner's Broth + 2.5% glutaraldehyde. The fractions which were pooled from the ten samples prior to dilution with the buffered solution consisted of the supernatant, the lamellar body band, and the remaining Ficoll and pellet. Fractions were post-fixed in 1% osmium tetroxide buffered with veronal acetate (70) and processed according to routine procedures for electron microscopy.

RESULTS

A. ANALYTICAL AND SPECIAL STUDIES

1. Linearity of the Standard Curve

Figure 1 demonstrates that the standard curve for the phosphorus assay is linear in the range from 0.07 ug P to 5.32 ug P. This is equivalent to lamellar body phospholipid content of 0.88 mg/dl to 66.5 mg/dl. All samples analyzed in the study were within this range.

2. Digestion Procedure

Digestion of the standard solution of dipalmitoyl phosphatidyl choline and digestion of the lamellar body fraction of amniotic fluid measured by phosphorus content as a function of time is plotted in Figure 2. Oxidation of DPPC in 70% perchloric acid at 160 ± 5°C. was 93% complete after 60 minutes and was 95% complete after 90 minutes of digestion time. The time course for digestion of the sample of amniotic fluid follows a similar pattern for that of the DPPC standard solution. Since the DPPC concentration used for oxidation is equal to 50.0 mg/dl a digestion time of 60 to 90 minutes at 160 ± 5°C. is adequate for digestion.

3. Reagent Stability

The color developed by the reagents at three different concentrations of phosphorus was consistent over a period of seven weeks

(Figure 3). All reagents used were shown to be stable for the testing period.

4. Accuracy and Extraction Efficiency

The accuracy of quantifying phospholipid phosphorus by the LB-PL assay is demonstrated in Table I. For the 4 ug phospholipid phosphorus sample, a mean of 3.89 ug P was detected while a mean of 0.95 ug P was detected in the 1 ug phospholipid phosphorus sample tested. Respectively, 97.3% and 94.1% of the phospholipid phosphorus added was detected.

Table II demonstrates the extraction efficiency of the DPPC standard solution at the two concentration levels tested. Means of 3.67 ug P and 0.93 ug P from the 4 ug phospholipid phosphorus and 1 ug phospholipid phosphorus samples were extracted and quantitated by the LB-PL method. The mean per cent recovered was 91.8% and 92.1% respectively.

A 4 ug P sample is equivalent to 50.0 mg DPPC/dl and a 1 ug P sample is equivalent to 12.5 mg DPPC/dl.

5. Precision

Results of the determination of within-run variation and day-to-day variation for the lamellar body assay are in Table III. Coefficients of variation (CV) for within-run variation were 6.0%, 3.1%, and 6.7%, while the CV's for day-to-day variation of samples containing little or no vernix caseosa were 4.5%, 5.4%, 9.6%, 9.7%, and 11.7%.

The one sample tested for precision containing a large amount of vernix caseosa had a CV of 18.9%.

The mean (± 1 standard deviation) for the daily controls was 4.90 ± 0.39 (n=170) and 3.59 ± 0.24 (n=103) with CV's of 8.0% and 6.7% respectively. The mean (± 1 standard deviation) for the long-term control used to assess the effects of variation associated with prolonged storage at -20°C. was 7.97 ± 0.54 (n=9) with a CV of 6.8%.

The precision of the L/S ratio judged on the basis of 75 duplicate determinations of a pooled control processed with each sample assayed had a CV of 16.7% (mean ± 1 SD=1.08 ± 0.18).

6. Contamination

Table IV shows the effect of meconium on the LB-PL content of amniotic fluid. Calculation of the mean difference depicts a slight decrease at the 0.1%, 0.5%, and 1.0% levels of contamination, and a slight increase at the 1.5% and 2.0% levels of contamination. A statistically significant difference is not indicated at all levels of contamination.

The interference from blood contamination is demonstrated in Table V which indicates a statistically significant increase in the determination of LB-PL content at all levels of contamination.

7. Preparative Centrifugation

The effect of relative centrifugal force on the LB-PL content of nine amniotic fluids is depicted in Figure 4. The mean per cent

(± 1 SD) of the original lamellar body phospholipid content remaining at 150, 300, 500, and 1000 RCF for the fluids is 80 ± 8.9%, 73 ± 9.9%, 61 ± 11.8%, and 46 ± 9.4% respectively. LB-PL values obtained after centrifugation were statistically different from uncentrifuged values ($p < 0.005$) at all RCF tested.

8. Electron Microscopy

There were no lamellar bodies found in the supernatant of the amniotic fluid centrifuged in the ultracentrifuge. Lamellar bodies were demonstrated at the interface of the amniotic fluid and the Ficoll solution (Photograph 1) while cells and debris were found in the pellet (Photograph 2) of amniotic fluid centrifuged according to the routine procedure for collection of lamellar bodies. As previously reported by Duck-Chong (66), cell fragments and whole cells were also found in the LB fraction.

B. CLINICAL STUDIES

1. Sample Population

Amniotic fluid samples used in the study were collected from a number of high-risk pregnancies (Table VI). Of the 166 pregnancies from which amniotic fluid was obtained 46 (28%) were delivered prematurely (i.e., less than or equal to 36 weeks gestational age). One hundred one uncontaminated samples from 100 pregnancies (one case of twin pregnancy) were obtained within three days of delivery.

Twenty-five of these pregnancies were delivered at 36 weeks or less gestational age.

Visible blood contamination was present in 14 samples analyzed and 7 samples contaminated with meconium were analyzed.

One hundred nine (65.7%) of the pregnancies were delivered by cesarean section. Of pregnancies in which samples were analyzed within three days of delivery, 78% were delivered by cesarean section.

2. Lamellar Body Phospholipid Content and Gestational Age

Figure 5 shows the relationship of amniotic fluid lamellar body phospholipid content and gestational age. The mean and standard error of the mean for gestational ages of 33 weeks through 40 weeks were calculated and linear regression analysis performed. The correlation coefficient (r) of 0.46 is significant to $P < 0.001$. Uncontaminated samples obtained at cesarean section or by transabdominal amniocentesis from uncomplicated and high-risk pregnancies were used for the calculations.

Pregnancies from which three or more samples of amniotic fluid were obtained are plotted in Figure 6 to demonstrate the variability in the onset and rate of increase of LB-PL content. Case C received a treatment course of betamethasone and treatment with β-adrenergics at 32 weeks gestational age for premature labor. Cases F and G also received β-adrenergics at 33 and 35 weeks gestational age respectively for premature labor.

3. Lamellar Body Phospholipid Content and the L/S Ratio

The LB-PL content and L/S ratio of 208 uncontaminated samples of amniotic fluid (including 4 vaginal drainage samples) are plotted in Figure 7. Regression analysis by the power model ($y=ax^b$) provides the line of best fit with the coefficient of determination (r^2) equal to 0.62.

Using 1.8 as the L/S ratio indicating pulmonary maturity and 4.0 mg/dl as the level of maturity for the LB-PL assay based on the performance of each assay in predicting the development of hyaline membrane disease described below the two assays can be compared for agreement in assessing maturity (Figure 8). Forty-nine per cent of the samples were indicated to be mature by both tests while 33% were indicated as immature by both tests for agreement in 82% of the uncontaminated samples examined. Disagreement in assessing maturity was found in 18% of the samples. Two per cent of the samples were determined to be mature by the L/S ratio and immature by the LB-PL assay. Mature LB-PL values and immature L/S ratios were found in 16% of the samples.

4. Clinical Evaluation

The lamellar body phospholipid assay, lecithin/sphingomyelin ratio, phosphatidyl glycerol assay, and the rapid surfactant test were each correlated with the respiratory status of neonates to determine the effectiveness of each method for assessing pulmonary maturity and the risk of hyaline membrane disease. The respiratory

status assessed according to the criteria outlined above (see materials and methods section) of samples obtained within three days of delivery is plotted for each assay (Figures 9, 10, 11, 12). There were five cases of HMD, two cases of mild HMD, and two cases of TTN in 101 samples of amniotic fluid obtained within three days of delivery. The phosphatidyl glycerol content was semi-quantitated in only 77 of the 101 amniotic fluids, while the RST was performed in 96 of the 101 samples processed.

Pertinent data from the nine pregnancies which developed respiratory problems can be found in Table VII. Using a LB-PL value of 4 mg/dl as the level indicating maturity there were 5 of 7 cases of hyaline membrane disease with immature values, and no cases of TTN with values indicating lung immaturity. L/S ratios were less than 1.8 in 6 of 7 cases of hyaline membrane disease and less than 1.8 in 1 of 2 cases of TTN. By the phosphatidyl glycerol method employed, 4 of 7 cases of hyaline membrane disease were positive for PG, and both cases of TTN had detectable levels of PG. The RST predicted immaturity in 5 of 7 of the cases of respiratory disease but predicted maturity in both cases of TTN.

Cases 7 and 9 were complicated by abruptio placentae and were contaminated with hemoglobin pigments, and case 8 was slightly contaminated with blood. All other specimens were uncontaminated.

Five of the pregnancies with infants that developed respiratory problems had received glucocorticoids (betamethasone) or β-adrenergic agents (terbutaline or ritodrine) in an attempt to enhance lecithin

biosyntheseis as part of the management of premature labor (71-73). Cases 1, 3, and 6 received treatment within twenty-four hours of delivery with β-adrenergics, cases 1 and 5 were treated with betamethasone within twenty-four hours of delivery, and case 8 received betamethasone and β-adrenergics four weeks prior to delivery. Amniotic fluid was obtained for analysis either one day prior to delivery or on the day of delivery in all cases receiving treatment to hasten lung maturity.

Evaluation of the methods for determining fetal pulmonary maturity in terms of sensitivity, specificity, predictive value, and efficiency for samples of amniotic fluid analyzed within three days of delivery are in Table VIII. The L/S ratio and PG assay demonstrate the highest sensitivity (85.7%), but the LB-PL assay had the highest specificity (83.7%). The predictive value of an immature test was low for all assays with the LB-PL assay having the highest predictability (25.0%). Predictive value of a mature result was high for each of the tests(96.6% - 98.7%). The LB-PL assay and the L/S ratio demonstrated the highest efficiency (82.8% and 75.8% respectively).

5. Complicating and Interfering Factors

Results of 15 samples visibly contaminated with blood are in Table IX. Included are three samples from pregnancies complicated by abruptio placentae which were not visibly contaminated with erythrocytes (no erythrocytes in pellet after centrifugation either)

but were discolored from hemoglobin pigments. Two of the cases contaminated with hemoglobin pigments are included in Table VII as noted above.

The three infants developing respiratory problems (Table IX) had immature L/S ratios and were positive for PG, but one sample had a mature LB-PL value. Seven pregnancies that did not develop respiratory problems in which fluid was analyzed within 3 days of delivery all had mature LB-PL values, while 6 cases had mature L/S values with one sample unable to be analyzed due to the degree of contamination. The PG content in these seven cases was mature in four (greater than or equal to 1 ug/ml), immature in two and not performed in one. The three pregnancies which developed HMD had immature L/S ratios, immature PG values in 2 cases, and immature LB-PL values in two cases.

Seven samples were visibly contaminated with meconium (Table X). The RST could not be performed in all seven samples, the PG assay could not be performed in four of the samples, and it was not possible to obtain an L/S ratio in two of the samples due to the presence of meconium. However, it was possible to obtain a LB-PL value for each specimen. All pregnancies delivered infants which developed no respiratory problem. The LB-PL values in all cases indicated maturity with 3 of 5 L/S ratios predicting maturity.

Sixteen cases of pregnancy complicated by diabetes mellitus that was either pre-existing or gestational were included in the study. The laboratory results and the status of the cases can be

found in Table XI. No cases in which samples were obtained within three days of delivery developed respiratory problems. Of ten cases sampled within three days of delivery that did not develop respiratory problems, eight cases had LB-PL values predicting maturity, 5 of 10 had L/S ratios indicating maturity, and 6 and 10 were positive for PG.

Data from four pregnancies were obtained from samples collected vaginally due to the premature rupture of membranes (Table XII). The LB-PL value, L/S ratio, PG assay, and RST were all in agreement in their prediction of fetal pulmonary status. None of the cases developed any respiratory problem although only one pregnancy was predicted to be mature.

The presence of vernix caseosa was indicated to increase the variability in the LB-PL results (Table III, sample 3 day-to-day variation). Vernix caseosa was found in significant quantities (i.e., visible) in 46 samples examined. The L/S ratio was mature ($\geq$ 1.8) in 43 of the samples while the LB-PL value was mature ($\geq$ 4.0 mg/dl) in 44 of the samples. The immature results were obtained from vaginal drainage samples.

DISCUSSION

A. ANALYTICAL AND SPECIAL STUDIES

1. Analytical Parameters

The LB-PL method used for this study proved to be an acceptable method for quantitating phospholipid phosphorus. The phosphorus determination demonstrated the necessary sensitivity and linearity for the range of phospholipid levels tested (Figure 1). Extraction and oxidation of phospholipids were shown to be adequate by the procedure employed for use in the clinical laboratory as demonstrated in Figure 2 and Tables I and II. The use of a standard solution of DPPC in chloroform represents the best possible performance for quantitation due to matrix differences between the standard solution and amniotic fluids. The lack of an accurate standard in a more appropriate matrix due to solubility problems precludes the use of a matrix that closely approximates amniotic fluid. The smallest sample size (i.e., 1 ug P equal to 12.5 mg DPPC/dl), although not close to the medical decision level of 4 mg/dl, is adequate to judge the extraction efficiency and accuracy of the LB-PL method. The use of a more dilute or concentrated standard solution would not necessarily be a judge of the accuracy of the method but would be influenced by pipetting and weighing accuracy.

Reagents (10% ascorbic acid and 2.5% ammonium molybdate) were stable for the period of seven weeks which confirms a similar report by Duck-Chong (66). The color development and stability has been

reported to be stable by both Chen, et. al. (75) and Duck-Chong (66). This means it is unnecessary to make fresh reagents for each sample determination.

Precision of the LB-PL assay using whole amniotic fluid (Table III) for demonstrating within-run variation and day-to-day variation is acceptable for clinical use. A range of values including immature as well as mature samples had fairly consistent coefficients of determination demonstrating acceptable random analytical error. The one case with poor precision was the sample containing a large amount of vernix caseosa. When vernix is present a more diffuse LB band is obtained after centrifugation due to the uneven and inconsistent distribution of vernix in the centrifuge tube. This presents a problem for removing the supernatant from the tube without disrupting the LB band. As a consequence the precision of the assay is decreased as demonstrated in Table III with sample number 3 of the day-to-day variation determination.

The presence of vernix caseosa does not contribute to the amount of phosphorus detected due to vernix consisting primarily of non-polar lipids and only trace amount of phospholipid (1). Since mature samples are associated with the presence of vernix caseosa (76) it is likely that the presence of this substance will not present a significant problem for determining the LB-PL content of a sample, although it should be kept in mind that the presence of vernix may increase the error associated with the value obtained. As noted in the results section all fluids obtained by transabdominal amniocentesis

or at cesarean section containing vernix has mature L/S ratio and LB-PL values. Vaginal drainage samples which were noted as containing vernix may have contained mucus instead.

The use of pooled samples for controls to be assayed with patient specimens provides another means of assessing the variability of the LB-PL assay. The coefficients of variation for the two different daily controls used for the LB-PL method again demonstrated adequate precision (8.0% and 6.7%). This variability was much less than that demonstrated for the L/S ratio using a pooled sample as a control to be run with patient samples in the same manner as the LB-PL method (CV=16.7%).

Storage at -20°C. for up to eight months did not seem to affect the precision of the assay as demonstrated by the CV of the long term control (6.8%). Prolonged storage at this temperature therefore appears to have little effect on the LB-PL content of the sample. This supplements the finding of Duck-Chong (66) that the LB-PL content of amniotic fluid is not affected by freezing and thawing once. However, Duck-Chong, et. al. (60) subsequently reported that freezing and thawing may affect the lamellar body content of samples that were in the region of borderline maturity but published no supporting data.

Specimens in this study were analyzed fresh when possible or stored frozen at -20°C. The effect of freezing could aid the disruption of lamellar bodies thus lowering the amount of LB-PL collected in the LB band or could fragment cells present in amniotic fluid

resulting in portions of membranes which contain phospholipids to be collected in the LB band. The significance of this factor concerning the LB-PL value and its clinical effect needs to be investigated further.

2. Contamination

The contamination of amniotic fluid with blood as demonstrated in Table V increases the LB-PL content due to the presence of plasma phospholipids and plasma phosphorus. Any erythrocytes present in the fluid will pass through the Ficoll solution due to their greater bouyant density (1.081) (77) than lamellar bodies (1.055) (66), while phospholipids (density of lecithin = 1.0305) would remain in the supernatant. Since a small portion of the supernatant is removed with the lamellar body fraction, amniotic fluid that is contaminated with blood will show a slight increase in LB-PL content. Based on the data in Table V, slight contamination with blood (up to 1.0%) should not clinically affect the results. Because the matrix of the contaminated samples used in the study was slightly different from the uncontaminated sample due to the dilution factor, it is important to examine the clinical outcome of specimens contaminated with blood. Table IX as previously described lists all samples contaminated with blood that were analyzed in this study. Of the three cases that developed respiratory problems one specimen had a mature LB-PL value but an immature L/S ratio and PG content. The other two cases were indicated to be immature by each assay. It is

possible since the case with the elevated LB-PL value was from a pregnancy complicated by abruptio placentae that erythrocyte fragments present in the specimen could have been included in the LB fraction increasing the amount of phospholipid present. If so, this would be a complicating factor in the analysis of samples from such pregnancies.

Contamination of amniotic fluid by blood has been shown to interfere with the other tests of fetal pulmonary maturity (37,38,41). Wagstaff, et. al. (37) have demonstrated an increase in the L/S ratio in immature samples with as little as 1.2% contamination and noted that contamination would significantly affect the L/S ratio of values close to the critical value ratio of 2.0. Buhi and Spellacy (38) demonstrated an increase of the L/S ratio of immature samples and a decrease in the L/S ratio of mature samples when contaminated with fetal or maternal serum due to a L/S ratio present in serum of 1.31-1.46. This effect was confirmed by Torday, et. al. (58).

On the basis of the analytical and clinical data presented in this study for the LB-PL content of amniotic fluid contaminated with blood, specimens that have LB-PL values near the value indicating fetal maturity should be judged with caution due to the increase resulting from such contamination. Samples with values clearly indicating maturity or immaturity even with large amounts of contamination present can safely be judged as such.

The detection of PG in samples contaminated by serum or erythrocytes did not result in the detection of PG in the two-dimensional thin-layer chromatographic procedure utilized by Strassner, et. al.(46).

Although the effect of blood on the determination of PG by the method employed in this study has not been examined, it is reasonable to assume that the presence of blood contamination would not interfere in the determination of PG in amniotic fluid.

As demonstrated in Table IV contamination of amniotic fluid with meconium does not consistently alter the LB-PL content. The fluctuations seen in the LB-PL values are most likely due to the nature of the meconium solution used to contaminate the aliquots of amniotic fluid. The solution contained some particulate material due to the insolubility of meconium which caused the formation of a more diffuse LB band upon centrifugation. This more diffuse band may have affected the precision of the assay as did the presence of vernix caseosa. The presence of meconium should not directly contribute to the amount of phosphorus detected since meconium has a total phospholipid phosphorus content of approximately 1 umol of phosphorus per gram (78). All specimens received for analysis contained no particulate material.

Again due to the slight difference in matrix of the samples contaminated with meconium and the uncontaminated sample, it is important to consider the clinical outcome of contaminated samples. As indicated there were no cases of respiratory problems in any of the seven cases that were contaminated. The ability to perform the LB-PL assay on all specimens is a clear advantage since it has been demonstrated that the LB-PL content is not influenced by meconium. This is in aggreement with Duck-Chong, et. al. (60) who observed

that samples contaminated with meconium still provided useful clinical information.

This conclusion is in contrast to the other fetal maturity assays used in this study. The presence of meconium is known to affect the interpretation of chromatograms due to streaking and the presence of lyso-lecithin. Wagstaff, et. al. (37) and Torday, et. al. (58) demonstrated that an immature L/S ratio increased in the presence of meconium. It has also been demonstrated that a mature L/S ratio is decreased in the presence of meconium (38, 58). In any case, it is safe to assume that the presence of meconium will alter the determination of the L/S ratio. The RST is known to give falsely mature results in the presence of contamination by meconium (37,40,41).

3. Preparative Centrifugation

Although the effect of centrifugation of amniotic fluid on LB-PL content is variable significant amounts of lamellar bodies are lost even when samples are centrifuged at low relative centrifugal forces such as 150 x g for 5 minutes. The findings demonstrated in Figure 4 are in agreement with the decrease in total phospholipid with increasing RCF (77) and the results of Oulton (35) showing the increase in lamellar bodies precipitated as reflected in total precipitable phospholipids with increasing g forces. Erythrocytes and other cells present in amniotic fluid have bouyant densities greater than that of lamellar bodies (66) plus the fact that lamellar bodies are precipitated at low g forces, it is unnecessary

to centrifuge amniotic fluid to remove cells and debris prior to centrifugation in the ultracentrifuge. Oulton (35) has demonstrated that cells present in amniotic fluid contribute only 6-9% to the total precipitable phospholipids at all gestational ages.

The RCF used for centrifugation of amniotic fluid for the L/S ratio has been demonstrated to be a significant factor in the determination of the L/S ratio especially at gestational ages of 34 to 37 weeks (34,35). By increasing the RCF for preparative centrifugation the number of false immature L/S ratios will increase while the number of false mature L/S ratios will increase if the RCF is low. The force of 1000 x g is approximately in the midrange of centrifugal forces recommended by other authors (34). Since lamellar bodies provide the phospholipids of surfactant, removal of these structures would be expected to influence the L/S ratio performed on the supernatant. Use of whole amniotic fluid for the LB-PL assay averts this problem.

4. Electron Microscopy

The electron microscopy scans of the LB band and pellet demonstrate the effectiveness in separating and concentrating the LB from a sample of amniotic fluid. It is not surprising to find fragments of cells in the LB fraction; however, the presence of cell fragments and debris would contribute to the amount of phospholipid phosphorus detected in the LB fraction thus increasing the LB-PL content. The extent of this problem needs to be investigated further. Samples

used for analysis had been frozen and thawed once prior to quantitation; therefore, it is possible that this would aid the destruction of cells. This may be a factor in the note of caution by Duck-Chong, et. al. (60) that the LB-PL content of amniotic fluid may be affected in immature samples that have been frozen prior to analysis.

B. CLINICAL STUDIES

1. Lamellar Body Phospholipid Content and Gestational Age.

Although the coefficient of determination for LB-PL content as a function of gestational age is not high (r^2=0.21), the correlation coefficient is statistically significant to a high degree ($p<0.001$). Lack of a better correlation may be attributed to the large degree of variation of LB-PL content for each gestational age due to variation in the onset of increased synthesis of lecithin. The degree of variability in the Dubowitz score (95% confidence limits of 2.0 weeks) (68) may also be a contributing factor. Samples from uncomplicated as well as high-risk pregnancies were used in the analysis which may be a cause for additional variability in the LB-PL content with gestational age due to accelerated maturation occuring in pregnancies complicated by some classes of diabetes, prolonged rupture of membranes, and severe hypertension (45).

The increase in LB-PL content with gestational age (Figures 7 and 8) is similar to that noted by Duck-Chong, et. al. (59,60) who found the onset for the increase in LB-PL content from a basal concentration of less than 3 mg/dl taking place between the 29th

and 40th week of gestation. The one case (E) in Figure 6 in which the LB-PL content decreased as gestational age increased demonstrates the variability in the assay since the sample contained large amounts of vernix caseosa. The L/S ratio of this sample increased as a function of time.

Glucocorticoids and β-adrenergics were used in only three of the cases with serial samples in Figure 8 so no conclusion can be drawn from this study as to the effects of these compounds on the LB-PL concentration.

The expected increase of LB-PL content with gestational age is similar to the increase in L/S ratio with gestational age that has been demonstrated by many investigators (27,79,80).

2. Lamellar Body Phospholipid Content and the L/S Ratio

The relationship between LB-PL content and the L/S ratio is best described by a curvilinear relationship (Figure 9). The variability of the LB-PL content at each L/S ratio value and the inherent variability of the L/S ratio due to densitometric quantitation and variability with gestational age explain the lack of a better correlation ($r=0.79$) of these two tests. A disagreement in the prediction of fetal pulmonary status of 18% (Figure 10) is in close agreement to the study of Duck-Chong, et. al. (59) who found a disagreement in 25% of the samples in which both L/S ratio value and LB-PL content value were obtained.

3. Clinical Evaluation

Duck-Chong, et.al. (60) assayed 451 samples collected within 2 days of delivery for LB-PL content and related this to respiratory status of the infant. Using 3.5 mg/dl as the critical value indicating fetal pulmonary maturity 11 out of 13 cases of HMD had a LB-PL content less than 3.5 mg/dl. The predictive value of an immature result (<3.5 mg/dl) was reported to be 72.7%, the predictive value of a mature result (≥3.5 mg/dl) 99.5%, with an overall efficiency 98.4%. Duck-Chong, et. al. (60) also reported the absence of respiratory problems with a LB-PL value greater than or equal to 5.0 mg/dl. In an earlier study done by Duck-Chong, et. al. (59), the LB-PL content was immature in all seven cases that developed HMD while the L/S ratio was immature (<2.0) in 6 out of 7 of the cases.

With so few cases of HMD encountered in this study (seven), one case of HMD not detected by an assay results in an appreciable difference in the performance of the test. As shown in Table VIII, the predictive value of an immature LB-PL (<4.0 mg/dl) value in this study was only 25.0%, but the predictive value of a mature result (≥4.0 mg/dl) was comparable at 98.6%. The efficiency, however, was lower (87.8%) due to the low predictive value of an immature value. There were two cases of hyaline membrane disease encountered with LB-PL values greater than 5.0 mg/dl. One case which developed mild HMD was predicted to be mature by all the assays employed.

The case of HMD with a mature LB-PL value, immature L/S ratio, and immature PG content was contaminated with hemolyzed blood from a pregnancy complicated by placentae abruptio as explained above.

The predictive value of an immature result for all fetal maturity tests used in this study was low (Table VIII), while the predictive value of mature results was high for each test. The overall efficiency of the tests is therefore low due to the poor reliability of an immature value. This is a problem that has been characteristic of fetal maturity assays used in the past and the reason for the continued search for an assay which is a better predictor of fetal pulmonary maturity and the development of HMD.

The L/S ratio when mature has been associated with a low incidence (1-2%) of respiratory distress syndrome (1,2, 39). Immature L/S ratios have not been as accurate in predicting the occurence of respiratory problems with incidence reports varying widely (2,58,81,39).

A number of authors using both one-dimensional as well as two-dimensional thin-layer chromatography have indicated the absence of RDS when PG is detected but the absence of PG is not invariably associated with the development of RDS (43,82,83). PG was detected in 4 of 7 cases which developed HMD in this study indicating that the presence of PG is not a guarantee that HMD will not develop. For the PG method employed when a level of PG greater than 1 ug/ml is used as the point indicating maturity, the performance of the PG assay is similar to the other assays used in this study and similar

to the performance of other assays for PG which either simply detect its presence or calculate the per cent of PG of total phospholipids chromatographed (42). This latter method is a time consuming and technically demanding procedure.

A mature RST has been associated with mature fetal pulmonary function (40,41,84), but this test is also inadequate in predicting respiratory porblems when either an intermediate or immature result are obtained. For example, failure to obtain a mature RST results in an RDS occurence rate of approximately 24% (2). Results of the RST from this study are consistent with this trend. The number of affected infants were detected by the other chemical analyses performed with fewer false immature results (Table VIII).

Kulovich, et. al. (39,45) have used a combination of fetal pulmonary assays including the L/S ratio, disaturated lecithin, per cent phosphatidyl inositol, and per cent PG for determining a lung profile used to predict the pulmonary maturity of the fetus. This technique has been shown to be 93% accurate in determining maturity when the L/S ratio is less than 2.0. The number of assays performed, the technical requirements, and the interpretive knowledge necessary to implement this series of tests make this approach unacceptable for use in the routine clinical laboratory.

Based on the limited number of infants developing respiratory problems in the study, it is difficult to determine with any degree of certainty a cut-off point indicating maturity for the LB-PL assay

and the PG assay. This is not necessarily the case with the L/S ratio and RST which have been used more extensively in the clinical laboratory. The clinical evaluation of the L/S ratio and the RST in this study are in fair agreement with the work of other authors cited above. With more data it may be possible to make a better estimate concerning the usefulness of the LB-PL assay for use on a routine basis. As it now stands the LB-PL assay appears to provide an advantage when samples are contaminated with meconium but is probably not any better than the L/S ratio when samples are contaminated with blood, although the effect of blood contamination is consistent in that the LB-PL content will always be higher in the presence of blood. To better evaluate the performance of the LB-PL assay, L/S ratio, and PG assay in predicting the development of respiratory problems in the newborn, more immature samples will have to be analyzed before implementation of any assay can be recommended over that of the assays examined.

4. Complicating and Interfering Factors

For pregnancies complicated by diabetes mellitus the LB-PL assay was the most accurate in assessing fetal lung maturity (Table XI). However, this subsample of the population analyzed did not include any cases which developed respiratory problems. To more accurately assess the performance of the LB-PL assay more immature samples from pregnancies complicated by diabetes mellitus will have to be assayed. This same finding and conclusion has been reached by Duck-Chong, et. al. (60).

Some authors have found a higher incidence of RDS in pregnancies of diabetic mothers which have mature L/S ratios (85,86), while others have found no increase in incidence (87,88). Kulovich and Gluck (45) have demonstrated a delay in the appearance of PG in Class A diabetics but that the L/S ratio according to gestational age did not differ significantly from other classes of diabetes or from a normal uncomplicated pregnancy. The problem of false mature L/S ratios, false mature LB-PL values, and false mature PG values in diabetic pregnancies was not encountered in this study. More data will have to be collected from diabetic pregnancies which develop respiratory problems before anything definitive can be stated concerning the LB-PL assay and the PG method used.

It has been demonstrated that the L/S ratio of amniotic fluid collected vaginally is not altered (89) and that the L/S ratio in amniotic fluid obtained vaginally can effectively be used to assess the maturity of the fetus (90). Vaginally collected amniotic fluid can successfully be used for determining the PG content for pregnancies complicated by premature rupture of membranes (91). The effect of vaginal and cervical secretions on the LB-PL content of amniotic fluid has not been determined.

Of the four vaginal samples collected in this study (Table XII) only one was assessed as mature (all assays reported mature values) even though no respiratory problems developed (Table XII). Since cervical mucus contains only a small amount of lipid material (92)

and the LB-PL values were apparently not elevated when collected vaginally, the assessment of fetal maturity using such specimens seems reasonable. The most significant problem may be the effect of mucus and other secretions on the precision of the assay. Further work concerning the use of vaginal drainage samples is needed.

It is common practice to administer glucocorticoids and β-adrenergic agents to enhance the synthesis and release of surfactant by the type II epithelial cell in an attempt to avoid the development of HMD in premature deliveries (1,73,93,94). Recently, it has been reported that glucocorticoid administration may impair the acceleration of surfactant production due to a resultant hyperinsulinemia which has an inhibitory effect on lipolysis (95). However, Kulovich and Gluck (39) note that the effect of insulin to inhibit lecithin synthesis has not clinically been demonstrated and note that diabetic patients often have higher L/S ratios and higher concentrations of disaturated lecithin.

Of the cases developing respiratory problems (Table VII) cases 1 and 5 received glucocorticoid treatment less than 24 hours before delivery. This is most likely not enough time for there to be any significant effect noted (71,96), besides both fluids were analyzed on the same day as delivery (case 5 a cesarean section) and should accurately reflect the status of the lungs. Case 8 received glucocorticoids 4 weeks prior to delivery; therefore, the results should be an accurate reflection of lung status. Cases 1, 3, and 6 received β-adrenergic agents less than 24 hours before delivery

which should not significantly affect the laboratory data for these cases.

Whether the mode of delivery is an additional risk factor for the development of RDS is a controversial subject (2,86,97). Although 5 of 7 cases (71%) that developed HMD were delivered by cesarean section, the per cent of cesarean section deliveries was high (65.7%) in the population studied as a result of this being a group at risk for premature or complicated delivery.

The volume of amniotic fluid during gestation is subject to variation (98) and, therefore, must be a consideration when the concentration of a substance is measured. No problems of false assessment of maturity can be attributed to oligohydramnios or polyhydramnios. According to Bhagwanani, et. al. (47) and Falconer, et. al. (98) the prognostic significance of concentration results are not greatly altered unless variations are quite large and the value is in the borderline range of maturity.

It should be noted that the site of amniocentesis may be an additional factor in the result of fetal lung maturity tests. Worthington, et. al. (99) have shown a difference of up to 0.7 in the L/S ratio depending upon whether the site of amniocentesis was at the caudal pole or cephalic pole of the fetus. This may also be significant factor for the LB-PL concentration especially when the result is near the critical cut-off point indicating maturity. These results indicate that the rate of mixing of amniotic fluid does not account for an entirely homogenous composition.

SUMMARY AND CONCLUSIONS

A method for determining lamellar body phospholipid content was investigated and compared to the L/S ratio, PG content, and RST for the assessment of fetal pulmonary maturity and the prediction of hyaline membrane disease in complicated pregnancies which are at risk of premature delivery. The LB-PL assay and the L/S ratio were demonstrated to have comparable sensitivities, specificities, predictive values, and efficiency ratings for assessing maturity in the population studied. LB-PL content of amniotic fluid demonstrated appropriate correlations with the gestational age of the fetus and with the L/S ratio currently in use as the primary procedure for assessing fetal pulmonary maturity. It is necessary that more immature deliveries resulting in respiratory problems including pregnancies complicated by diabetes mellitus be analyzed before a definitive statement can be made concerning the efficacy of the procedures used in this comparative study.

The LB-PL method as employed demonstrated adequate sensitivity, accuracy, and precision for use in the clinical laboratory. A valid assessment of lung maturity can be obtained in the presence of meconium contamination, but contamination by blood results in an elevated assessment of lamellar body phospholipid content. Significant amounts of lamellar body phospholipid are removed even when low relative centrifugal forces are used for preparative centrifugation of amniotic fluid to remove cells and debris prior to analysis. The effects of freezing on the LB-PL content determined by the method needs to be investigated further.

REFERENCES

1. Biezenski, J.J. Pulmonary surfactant and amniotic fluid lipids. Obstet. Gynecol. Ann., 1976. 5, 71-102.

2. Farrell, P., and Avery, M.E. Hyaline membrane disease. Am. Rev. Resp. Dis., 1975. 111, 657-688.

3. Morgan, T.E. Pulmonary surfactant. N. Engl. J. Med., 1971. 284, 1185-1193.

4. Avery, M.E., and Mead, J. Surface properties in relation to atelectasis and hyaline membrane disease. Am. J. Dis. Child., 1959. 97, 517-523.

5. King, R.J. The surfactant system of the lung. Fed. Proc., 1974. 33, 2238-2247.

6. Strang, L.B. Growth and development of the lung: Fetal and postnatal. Ann. Rev. Physiol., 1977. 39, 253-276.

7. Pfleger, R.C., Henderson, R.F., and Waide, J. Phosphatidyl glycerol-a major component of pulmonary surfactant. Chem. Phys. Lipids, 1972. 9, 51-68.

8. King, R.J., and Clements, J.A. Surface active material from dog lung. I. Method of isolation. Am. J. Physiol., 1972. 223, 707-714.

9. King, R.J., Klass, D.J., Gikas, E.G., and Clements, J.A. Isolation of apoproteins from canine surface active material. Am. J. Physiol., 1973. 224, 788-795.

10. King, R.J., Martin, H., Mitts, D., and Holmstrom, F.M. Metabolism of the apoproteins in pulmonary surfactant. J. Appl. Physiol., 1977. 42, 483-491.

11. Kikkawa, Y., Motoyama, E.K., and Gluck, L. Study of the lungs of fetal and newborn rabbits: Morphological, biochemical, and surface physical development. Am. J. Pathol., 1968. 52, 177-209.

12. Kikkawa, Y., Yoneda, K., Smith, F., Packard, B., and Suzuki, K. The type II epithelial cells of the lung. II. Chemical composition and phospholipid synthesis. Lab. Invest., 1975. 32, 295-302.

13. Kennedy, E.P., and Weiss, S.B. The function of cytidine coenzymes in the biosynthesis of phospholipids. J. Biol. Chem., 1956. 222, 193-214.

14. Tierney, D.F. Lung metabolism and biochemistry. Ann. Rev. Physiol., 1974. 36, 209-231.

15. Goerke, J. Lung surfactant. Biophys. Biochim. Acta, 1974. 344, 241-261.

16. Hallman, M., and Gluck, L. Phosphatidylglycerol in lung surfactant. Biochim. Biophys. Acta, 1975. 409, 172-191.

17. Ryan, U.S., Ryan, J.W., and Smith, D.S. Alveolar type II cells: Studies on the mode of release of lamellar bodies. Tissue Cell, 1975. 7, 587-599.

18. Williams, M.C. Conversion of lamellar body membranes into tubular myelin in alveoli of fetal rat lungs. J. Cell Biol., 1977. 72, 260-277.

19. Williams, M.C. Freeze-fracture studies of tubular myelin and lamellar bodies in fetal and adult rat lungs. J. Ultrastruct. Res., 1978. 64, 352-361.

20. Stratton, C.J. The periodicity and architecture of lipid surfactant and its origin from multilamellar bodies. Tissue Cell, 1977. 9, 301-316.

21. Sanders, R.L., Hassett, R.J., and Vatter, A.E. Isolation of lung lamellar bodies and their conversion to tubular myelin figures in vitro. Anat. Rec., 1980. 198, 485-501.

22. Henderson, R.F., and Pfleger, R.C. Surface tension studies of phosphatidyl glycerol isolated from the lungs of beagle dogs. Lipids, 1972. 7, 492-494.

23. Godinez, R.I., Sanders, R.L., and Longmore, W.J. Phosphatidyl glycerol in rat lung. I. Identification as a metabolically active phospholipid in isolated perfused rat lung. Biochem., 1975. 14, 830-834.

24. Brumley, G.W., Hodson, W.A., and Avery, M.E. Lung phospholipids and surface tension correlations in infants with and without hyaline membrane disease and in adults. Pediatrics, 1967. 40, 13-19.

25. Biezenski, J.J., Pomerance, W., and Goodman, J. Studies of the origin of amniotic fluid lipids. I. Normal composition. Am. J. Obstet. Gynecol., 1968. 102, 853-861.

26. Nelson, G.H. Amniotic fluid phospholipid patterns in normal and abnormal pregnancies - term. Am. J. Obstet. Gynecol., 1969. 105, 1072-1077.

27. Gluck, L., Kulovich, M.V., Borer, R.C., Brenner, P.H., Anderson, G.G., and Spellacy, W.N. Diagnosis of the respiratory distress syndrome by amniocentesis. Am. J. Obstet. Gynecol., 1971. 109, 440-445.

28. Novy, M.J., Portman, O.W., and Bell, M. Evidence for pulmonary and other sources of amniotic fluid phospholipids in the rhesus monkey. In Villee, C.A., Villee, D.B., and Zuckerman, J. (Ed.) Respiratory Distress Syndrome. New York, N.Y., Academic Press Inc., 1973. pp. 205-218.

29. Novy, M.J., Portman, O.W., Alexander, M., and Bell, M. Dynamics of amniotic fluid phospholipids: A model in the rhesus monkey. Clin. Res., 1973. 21, 321 (Abstract).

30. Gluck, L., Chez, R.A., Kulovich, W.V., Hutchinson, D.L., and Niemann, W.H. Comparison of phospholipid indicators of fetal lung maturity in the amniotic fluid of the monkey (Macaca mulatta) and baboon (Papio papio). Am. J. Obstet. Gynecol., 1974. 120, 524-530.

31. Gluck, L., and Kulovich, M.V. Lecithin/sphingomyelin ratios in amniotic fluid in normal and abnormal pregnancy. Am. J. Obstet. Gynecol., 1973. 115, 539-546.

32. Gluck, L., Kulovich, M.V., Borer, R.C., and Keidel, W.N. The interpretation and significance of the lecithin/sphingomyelin ratio in amniotic fluid. Am. J. Obstet. Gynecol., 1974. 120, 142-155.

33. Penny, L.L., Hagerman, D.D., and Sei, C.A. Specificity and reproducibility of acetone precipitation in identifying surface-active phosphatidylcholine in amniotic fluid. Clin. Chem., 1976. 22, 681-682.

34. Cherayil, G.D., Wilkinson, E.J., and Borkowf, H.I. Amniotic fluid lecithin/sphingomyelin ratio changes related to centrifugal force. Obstet. Gynecol., 1977. 50, 682-688.

35. Oulton, M. The role of centrifugation in the measurement of surfactant in amniotic fluid. Am. J. Obstet. Gynecol., 1979. 135, 337-343.

36. Olson, E.B., and Graven, S.N. Comparison of visualization methods used to measure the lecithin/sphingomyelin ratio in amniotic fluid. Clin. Chem., 1974. 20, 1408-1415.

37. Wagstaff, T.I., Whyley, G.A., and Freedman, G. Factors influencing the measurement of the lecithin/sphingomyelin ratio in amniotic fluid. J. Obstet. Gynecol. Br. Commonw., 1974. 81, 264-277.

38. Buhi, W.C., and Spellacy, W.N. Effects of blood or meconium on the determination of the amniotic fluid lecithin/sphingomyelin ratio. Am. J. Obstet. Gynecol., 1975. 121, 321-323.

39. Kulovich, M.V., Hallman, M.B., and Gluck, L. The lung profile. I. Normal pregnancy. Am. J. Obstet. Gynecol., 1979. 135, 57-63.

40. Clements, J.A., Platzker, A.C.G., Tierney, D.F., Hobel, C.J., Creasy, R.K., Margolis, A.J., Thibeault, D.W., Tooley, W.H., and Oh, W. Assessment of the risk of the respiratory-distress syndrome by a rapid test for surfactant in amniotic fluid. N. Engl. J. Med., 1972. 286, 1077-1081.

41. Keniston, R.C., Noland, G.L., and Pernall, M.L. The effect of blood, meconium, and temperature on the rapid surfactant test. Obstet. Gynecol., 1976. 48, 442-446.

42. Hallman, M., Kulovich, M., Kirkpatrick, E., Sugarman, R.G., and Gluck, L. Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: Indices of lung maturity. Am. J. Obstet. Gynecol., 1976. 125, 613-617.

43. Tsai, M.Y., and Marshall, J.G. Phosphatidylglycerol in 261 samples of amniotic fluid from normal and diabetic pregnancies, as measured by one-dimensional thin-layer chromatography. Clin. Chem., 1979. 25, 682-685.

44. Gotelli, G.R., Stanfill, R.E., Kabra, P.M., Farina, F.A., and Marton, L.J. Simultaneous determination of phosphatidylglycerol and the lecithin/sphingomyelin ratio in amniotic fluid. Clin. Chem., 1978. 24, 1144-1146.

45. Kulovich, M.V., and Gluck, L. The lung profile. II. Complicated pregnancy. Am. J. Obstet. Gynecol., 1979. 135, 64-70.

46. Strassner, H.T., Golde, S.H., Mosley, G.H., and Platt, L.D. Effect of blood in amniotic fluid on the detection of phosphatidylglycerol. Am. J. Obstet. Gynecol., 1980. 138, 697-702.

47. Bhagwanani, S.G., Fahney, D., and Turnbull, A.C. Prediction of neonatal respiratory distress by estimation of amniotic fluid lecithin. Lancet, 1972. 1, 159-162.

48. Nelson, G.H. Relationship between amniotic fluid lecithin concentration and respiratory distress syndrome. Am. J. Obstet. Gynecol., 1972. 112, 827-833.

49. Biezenski, J.J. Amniotic fluid phospholipids in early gestation. Obstet. Gynecol., 1973. 41, 825-828.

50. Russell, P.T., Miller, W.J., and McLain, C.R. Palmitic acid content of amniotic fluid lecithin as an index to fetal lung maturity. Clin. Chem., 1974. 20, 1431-1434.

51. Warren, C., Holton, J.B., and Allen, J.T. Assessment of fetal lung maturity by estimation of amniotic fluid palmitic acid. Br. Med. J., 1974. 19, 94-96.

52. King, R., Ruch, J., Gikas, E., Platzker, A., and Creasy, R. Appearance of apoproteins of pulmonary surfactant in human amniotic fluid. J. Appl. Physiol., 1975. 39, 735-741.

53. Herbert, W.N.P., Johnston, J.M., MacDonald, P.C., and Jimenez, J.M. Fetal lung maturation. Human amniotic fluid phosphatidate phosphohydrolase activity through normal gestation and its relation to the lecithin/sphingomyelin ratio. Am. J. Obstet. Gynecol., 1978. 132, 373-379.

54. Brocklehurst, D., and Wilde, C.E. Amniotic fluid alkaline phosphatase, γ-glutamyltransferase, 5'nucleotidase activity from 13 to 40 weeks gestation, and alkaline phosphatase as an index of fetal lung maturity. Clin. Chem., 1980. 26, 588-591.

55. Shinitzky, M., Goldfisher, A., Bruck, A., Goldman, B., Stern, E., Barkai, G., Mashiach, S., and Serr, D.M. A new method for assessment of fetal lung maturity. Br. J. Obstet. Gynecol., 1976. 83, 838-844.

56. Blumenfeld, T.A., Stark, R.I., James, L.S., George, J.D., Dyrenfurth, I., Freda, V.J., and Shinitzky, M. Determination of fetal lung maturity by fluorescence polarization of amniotic fluid. Am. J. Obstet. Gynecol., 1978. 130, 782-787.

57. Sbarra, A.J., Michlewitz, H., Selvaraj, R., Mitchell, G.W., Cetrulo, C.L., Kelley, E.C., Kennedy, J.L., Herschel, M.J., Paul, B.B., and Louis, F. Relation between optical density at 650 nm and L/S ratios. Obstet. Gynecol., 1977. 50, 723-724.

58. Torday, J., Carson, L., and Lawson, E.E. Saturated phosphatidylcholine in amniotic fluid and prediction of the respiratory-distress syndrome. N. Engl. Med., 1979. 301, 1013-1018.

59. Duck-Chong, C.G., Gupta, J.M., Storey, G.N.B., and Houghton, C.R.S. Lamellar body phospholipid content of amniotic fluid and L/S ratio compared in assessing fetal lung maturity. Clin. Chem., 1980. 26, 766-769.

60. Duck-Chong, C.G., Henderson-Smart, D.J., Gupta, J.M., and Hensley, W.J. Lamellar body phospholipid in amniotic fluid: A method for assessing fetal lung maturity. Unpublished manuscript, 1981.

61. Hook, G.E.R., Gilmore, L.B., Tombropoulos, E.G., and Fabro, S.E. Fetal lung lamellar bodies in human amniotic fluid. Am. Rev. Resp. Dis., 1978. 117, 541-550.

62. Duck-Chong, C.G. The isolation of lamellar bodies and their membranous content from rat lung, lamb tracheal fluid, and human amniotic fluid. Life Sci., 1978. 22, 2025-2030.

63. Lee, W., Bell, M., and Novy, M.J. Pulmonary lamellar bodies in human amniotic fluid: Their relationship to fetal age and the lecithin/sphingomyelin ratio. Am. J. Obstet. Gynecol., 1980. 136, 60-66.

64. Snyder, J.M., Mendelson, C.R., Gallaspy, S.E., and Johnston, J.M. Morphologic studies of human fetal lung tissue in organ culture. Presented at the 26th Annual Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 21-24, 1979. p. 113 (Abstract)

65. Paul, G.W., Hassett, R.J., and Reiss, O.K. Formation of lung surfactant films from intact lamellar bodies. Proc. Natl. Acad. Sci. U.S.A., 1977. 74, 3617-3620.

66. Duck-Chong, C.G. Lamellar body content of amniotic fluid: A possible index of fetal lung maturity. Ann. Clin. Biochem., 1979. 16, 191-196.

67. Reynolds, J.W. Personal Communication. 1981.

68. Dubowitz, L.M.S., Dubowitz, V., and Goldberg, C. Clinical assessment of gestational age in the newborn infant. J. Pediatr., 1970. 77, 1-10.

69. Galen, R.S., and Gambino, S.R. Beyond normality. New York: John Wiley and Sons, 1975. pp. 9-14, 30-36.

70. Palade, G.E. A study of fixation for electron microscopy. J. Exp. Med., 1952. 95, 285-298.

71. Liggins, G.C., and Howie, R.N. A controlled trial of antepartum glucocorticoid treatment for the prevention of the respiratory distress syndrome in premature infants. Pediatrics, 1972. 50, 515-525.

72. Iddlekinge, B. Van, and Hughes, E.A. The effect of intrauterine fetal transfusion and a β-sympathomimetic substance on the L/S ratio in human amniotic fluid. Br. J. Obstet. Gynecol., 1977. 84, 669-673.

73. Hitchcock, K.R. Lung development and the pulmonary surfactant system: Hormonal influences. Anat. Rec., 1980. 198, 13-34.

74. White, P. Pregnancy and diabetes, medical aspects. Med. Clin. North Am., 1965. 49, 1015-1024.

75. Chen, P.S., Toribara, T.Y., and Warner, H. Microdetermination of phosphorus. Anal. Chem., 1956. 28, 1756-1758.

76. McLaughlan, J., and Chang, A. L/S ratio and presence of vernix in amniotic fluid. Br. Med. J., 1977. 2, 1544-1545.

77. Leif, R.C., and Vinogard, J. The distribution of bouyant density of human erythrocytes in bovine albumin solutions. Proc. Nat. Acad. Sci., 1964. 51, 520-528.

78. Voorst, E. Effects of centrifugation, storage, and contamination of amniotic fluid on its total phospholipid content. Clin. Chem., 1980. 26, 232-234.

79. Bryson, M.J., Gobert, H.A., and Stenchever, M.A. Amniotic fluid lecithin/sphingomyelin ratio as an assessment of fetal pulmonary maturity. Am. J. Obstet. Gynecol., 1972. 114, 208-212.

80. Schreyer, P., Tanier, I., Bukovsky, I., Weinraub, Z., and Caspi, E. Amniotic fluid total phospholipids versus lecithin/sphingomyelin ratio in the evaluation of fetal lung maturity. Am. J. Obstet. Gynecol., 1974. 120, 909-917.

81. Doran, T.A., Ford, J.A., Allen, L.C., Wong, P.Y., and Benzie, R.J. Amniotic fluid lecithin/sphingomyelin ratio, palmitic acid, palmitic acid/stearic acid ratio, total cortisol, creatinine, and percentage of lipid-positive cells in assessment of fetal maturity and fetal pulmonary maturity: A comparison. Am. J. Obstet. Gynecol., 1979. 133, 302-307.

82. Obladen, M., Merritt, T.A., and Gluck, L. Acceleration of pulmonary surfactant maturation in stressed pregnancies: A study of neonatal lung effluent. Am. J. Obstet. Gynecol., 1979. 135, 1079-1085.

83. Hallman, M., Feldman, B.H., Kirkpatrick, E., and Gluck, L. Absence of phosphatidylglycerol (PG) in respiratory distress syndrome in the newborn. Pediat. Res., 1977. 11, 714-720.

84. Cowett, R.M., Unsworth, E.J., Hokanson, D.O., Williams, J.R., and Oh, W. Foam stability test on gastric aspirate and the diagnosis of respiratory-distress syndrome. N. Engl. J. Med., 1975. 293, 413-416.

85. Dahlenburg, G.W., Martin, F.I., Jeffery, P.E., and Horacek, I. Amniotic fluid lecithin/sphingomyelin ratio in pregnancy complicated by diabetes. Br. J. Obstet. Gynecol., 1977. 84, 294-299.

86. Muellar-Heubach, E., Caritis, S., Edelstone, D., and Turner, J.H. Lecithin/sphingomyelin ratio in amniotic fluid and its value for the prediction of neonatal respiratory distress syndrome in pregnant diabetic women. Am. J. Obstet. Gynecol., 1978. 130, 28-34.

87. Gabbe, S.G., Lowensohn, R.I., Mestman, J.H., Freeman, R.K., and Goebelsmann, U. Lecithin/sphingomyelin ratio in pregnancies complicated by diabetes mellitus. Am. J. Obstet. Gynecol., 1977. 128, 757-760.

88. Curet, L., Olson, R., Schneider, J., and Zachman, R. Effects of diabetes mellitus on amniotic fluid lecithin/sphingomyelin ratio and respiratory distress syndrome. Am. J. Obstet. Gynecol., 1979. 135, 10-13.

89. Sbarra, A.J., Blake, G., Cetrulo, C.L., Selvaraj, R.J., Herschel, M.J., Delise, C., Kennedy, J.L., and Mitchell, G.W. The effect of cervical/vaginal secretions on measurements of lecithin/sphingomyelin ratio and optical density at 650 nm. Am. J. Obstet. Gynecol., 1981. 139, 214-216.

90. Goldstein, A.S., Mangurten, H.H., Libretti, J.V., and Berman, A.M. Lecithin/sphingomyelin ratio in amniotic fluid obtained vaginally. Am. J. Obstet. Gynecol., 1980. 138, 232-233.

91. Stedman, C.M., Crawford, S., Staten, E., and Cherny, W.B. Management of preterm rupture of membranes: Assessing amniotic fluid in the vagina for phosphatidylglycerol. Am. J. Obstet. Gynecol., 1981. 140, 34-38.

92. Creeth, J.M. Constituents of mucus and their separation. Br. Med. Bull., 1978. 34, 17-24.

93. Farrell, P.M. Fetal lung development and the influence of glucocorticoids on pulmonary surfactant. J. Steroid Biochem., 1977. 8, 463-470.

94. Arias, F., Pineda, J., and Johnson, L.W. Changes in human amniotic fluid lecithin/sphingomyelin ratio and dipalmitoyl lecithin associated with maternal betamethasone therapy. Am. J. Obstet. Gynecol., 1979. 133, 394-898.

95. Beck, J.C., Johnson, J.W.C., Mitzner, W., Lee, P.A., London, W.T., and Sly, D.L. Glucocorticoids, hyperinsulinemia, and fetal lung maturation. Am. J. Obstet. Gynecol., 1981. 139, 465-470.

96. Thornfeldt, R.E., Franklin, R.W., Pickering, N.A., Thornfeldt, C.R., and Ancell, G. The effects of glucocorticoids on the maturation of premature lung membranes. Am. J. Obstet. Gynecol., 1978. 131, 143-148.

97. Callen, P., Goldsworthy, S., Graves, L., Harvey, D., Mellows, H., and Parkinson, C. Mode of delivery and the lecithin/sphingomyelin ratio. Br. J. Obstet. Gynecol., 1979. 86, 965-968.

98. Falconer, G.F., Hodge, J.S., and Gadd, R.L. Influences of amniotic fluid volume on lecithin estimation in prediction of respiratory distress. Br. Med. J., 1973. 2, 689-691.

99. Worthington, D., and Smith, B. The site of amniocentesis and the L/S ratio. Obstet. Gynecol., 1978. 52, 552-554.

Table I. Accuracy of the Lamellar Body Assay. Aliquots of a solution of dipalmitoyl phosphatidyl choline were evaporated, digested in $HClO_4$, and the phospholipid phosphorus quantitated (see text for method description).

Sample size:	4 ug P	1 ug P
Replicates :	10	10
Mean Detected :	3.89ug P	0.95ug P
Standard Deviation :	0.09	0.02
% Detected :	97.3	94.1

Table II. Extraction of Phosphatidyl Choline. Aliquots of a solution of dipalmitoyl phosphatidyl choline were added to $CHCl_3$-MeOH (2:1), extracted and washed, evaporated, digested in $HClO_4$, and quantitated for phosphorus (see test for method description).

Sample size:	4 ug P	1 ug P
Replicates :	10	10
Mean Detected :	3.67ug P	0.93ug P
Standard Deviation :	0.11	0.02
% Detected :	91.8	92.1

Table III. Precision of the Lamellar Body Assay using Amniotic Fluid.

Within-Day Variation

Sample	1	2	3
Replicates	10	10	10
Mean	3.01	10.25	4.96
Standard Deviation	0.18	0.32	0.33
Coefficient of Variation:	6.0%	3.1%	6.7%

Day-to-Day Variation

Sample	1	2	3*	4	5	6
Replicates	5	5	5	5	5	10
Mean	2.14	2.92	6.40	8.30	5.58	4.67
Standard Deviation	0.25	0.13	1.21	0.45	0.54	0.45
Coefficient of Variation:	11.7%	4.5%	18.9%	5.4%	9.7%	9.6%

* sample contained a large amount of vernix caseosa

Table IV. Effect of Meconium on the LB-PL Content of Amniotic Fluid. Aliquots of seven amniotic fluids were contaminated with varying concentrations of meconium and analyzed as whole amniotic fluid (see text for method description). LB-PL values (mg/dl) were corrected for dilution and the average difference from the uncontaminated value was calculated. Data was evaluated statistically by the Wilcoxin matched-pairs test.

	Percent Contamination					
Sample	0	0.1%	0.5%	1.0%	1.5%	2.0%
1	10.3	10.2	10.1	9.3	9.8	10.3
2	13.3	12.8	13.3	13.7	13.0	13.8
3	12.1	12.0	11.6	11.1	12.0	12.5
4	4.4	4.2	4.3	4.4	4.8	5.0
5	1.9	1.9	1.7	1.7	2.2	2.2
6	2.9	2.8	2.6	2.9	3.8	3.8
7	4.8	4.8	4.3	4.7	5.4	5.9
Average Difference*		0.14	0.26	0.27	-0.19	-0.54
Standard Deviation		0.17	0.19	0.53	0.50	0.37
P value		0.02	0.02	0.1	NS+	0.02

* negative value indicates an increased concentration compared to uncontaminated aliquot

\+ not significant

Table V. Effect of Blood on the LB-PL Content of Amniotic Fluid. Aliquots of eight amniotic fluids were contaminated with varying concentrations of heparinized blood, centrifuged at 150 x g for 5 minutes to remove the erythrocytes, and assayed for LB-PL content (see text for method description). LB-PL values (mg/dl) were corrected for dilution and the average difference from the uncontaminated value was calculated. Data was evaluated statistically by the Wilcoxin matched-pairs test.

	Percent Contamination					
Sample	0	1.0%	2.0%	3.0%	5.0%	9.0%
1	7.4	7.8	8.4	8.5	9.3	10.8
2	5.3	5.5	6.0	6.1	6.9	7.9
3	4.4	4.9	5.1	5.3	5.9	6.4
4	7.3	7.9	8.8	10.0	11.4	13.1
5	7.8	8.3	8.9	9.3	9.9	12.4
6	9.4	10.6	10.8	11.1	11.3	----
7	6.3	6.9	7.3	7.8	8.0	8.6
8	2.1	2.5	2.6	2.9	3.0	3.8
Average Difference*		-0.55	-0.99	-1.38	-1.96	-3.20
Standard Deviation		0.29	0.35	0.64	0.94	1.51
P value		.005	.005	.005	.005	.005

* negative value indicates an increased concentration compared to uncontaminated aliquot

Table VI. Number of Complications in Pregnancies from which Amniotic Fluid was Analyzed.*

Complications	Gestational Age+ ≤ 36	≥ 37
Uncomplicated	--	75
Premature labor	26	--
Pre-eclampsia	4	5
Pregnancy induced hypertension	3	5
Chronic hypertension	--	2
Diabetes mellitus (pre-existing)	3	6
Diabetes mellitus (gestational)	1	6
Abruptio placentae	7	1
Placenta previa	2	1
Intrauterine growth retardation	--	2
Postmaturity, uncertain dates	--	4
Rh sensitization	--	2
Polyhydramnios	1	3
Other	3	10

Total number of pregnancies = 166

* some pregnancies with more than one complication

+ gestational age in weeks

Table VII. Results of Fetal Maturity Assays from Pregnancies Resulting in Respiratory Problems.

Case	Respiratory Problem	GA* (weeks)	LB-PL (mg/dl)	L/S Ratio	PG° (ug/ml)	RST#	Mode of Delivery	Maternal Condition
1	TTN	31	14.0	1.2	+	M	vaginal	Premature labor
2	TTN	38	10.6	2.9	< 4	M	cesarean	Elective cesarean section
3	Mild HMD	28	1.8	1.2	=	I	vaginal	Premature labor
4	Mild HMD	38	11.9	2.3	< 4	M	cesarean	Cephalopelvic disproportion
5	HMD	27	1.5	0.6	=	I	cesarean	Premature labor
6	HMD	32	1.4	1.5	< 1	I	vaginal	Premature labor
7	HMD	28	1.9	1.1	< 1	I	cesarean	Pre-eclampsia Abruptio placentae
8	HMD	30	3.6	1.6	=	I	cesarean	Premature labor
9	HMD	31	7.4	0.8	< 1	M	cesarean	Chronic abruptio placentae

* GA = geatational age
° + is PG present but not semi-quantitated
= is PG not present
\# M = mature
I = immature

Table VIII. Evaluation of Fetal Maturity Assays. The clinical performance of each test is evaluated for its determination of lung maturity and the development of hyaline membrane disease. See text for description of terms.

	LB-PL Assay	L/S Ratio	PG Assay	RST
Sensitivity	71.4%	85.7%	85.7%	71.4%
Specificity	83.7%	75.0%	66.7%	64.4%
Predictive value of an immature result	25.0%	20.7%	20.7%	13.9%
Predictive value of a mature result	97.5%	98.6%	97.9%	96.6%
Efficiency	87.8%	75.8%	68.4%	64.9%
Mature value	$\geq$ 4mg/dl	$\geq$1.8	$\geq$1ug/ml	Mature

Table IX. Results of Fetal Maturity Assays from Samples Contaminated with blood.+

Case	GA° (weeks)	LB-PL (mg/dl)	L/S Ratio	PG# (ug/ml)	RST	Days to Delivery	Condition
1*	32	3.3	1.5	-	I	6	No RDS
2	41	10.0	3.3	-	NP	0	No RDS
3	40	12.0	3.2	2-4	NP	0	No RDS
4	40	11.5	NP	NP	NP	0	No RDS
5	40	8.5	1.9	< 1	NP	0	No RDS
6	36	1.4	1.1	< 1	I	27	No RDS
7	40	30.1	3.5	2-4	M	3	No RDS
8	36	2.9	1.6	< 1	INT	9	No RDS
9	42	5.5	1.9	1-2	M	0	No RDS
10	37	5.5	1.7	-	INT	22	No RDS
11	39	10.0	2.2	> 4	M	0	No RDS
12*	28	1.9	1.1	< 1	I	0	HMD
13	30	3.6	1.6	-	I	0	HMD
14*	31	7.4	0.8	< 1	M	0	HMD

+ NP = not performed, I = immature, INT = intermediate, M = mature, No RDS = no respiratory distress syndrome

* contaminated with hemoglobin pigments

° GA = gestational age

- is PG not present

Table X. Results of Fetal Maturity Assays from Samples Contaminated with Meconium.*

Case	GA° (weeks)	LB-PL (mg/dl)	L/S Ratio	PG+ (ug/ml)	RST	Days to Delivery
1	40	17.1	1.8	NP	NP	1
2	35	4.9	1.7	-	NP	0
3	36	9.4	NP	NP	NP	0
4	40	11.5	NP	NP	NP	0
5	40	19.8	1.9	> 4	NP	1
6	35	6.8	1.2	NP	NP	0
7	40	12.8	1.8	2-4	NP	2

* NP = not performed

° GA = gestational age

+ - is PG not present

Table XI. Results of Fetal Maturity Assays from Pregnancies Complicated by Maternal Diabetes.

Case	GA° (weeks)	LB-PL (mg/dl)	L/S Ratio	PG# (ug/ml)	RST+	Days to Delivery	Classi-fication≠
1*	37	6.4	1.6	+	I	0	A
	37	3.8	1.3	-	I	0	A
2	41	7.9	3.0	> 4	M	0	A
3	38	9.4	2.4	1-2	M	0	A
4	38	9.0	2.5	< 1	M	0	A
5	40	8.3	3.3	> 4	NP	0	A
6	36	3.5	1.0	-	I	1	B
7	38	5.9	1.3	-	INT	1	D
8	37	4.0	1.2	-	INT	2	C
9	37	8.0	2.3	< 1	INT	3	B
10	37	14.6	1.8	2-4	M	4	B
11	35	13.4	1.9	< 1	INT	5	C
12	36	23.8	1.3	-	INT	7	B
13	38	2.3	1.6	-	I	7	B
14	37	4.3	1.5	-	INT	11	A
15	37	3.5	1.6	-	I	12	C
16	36	2.5	1.8	< 1	I	31	A

° GA = gestational age
- is PG not present, + is PG present but not semi-quantitated
+ NP = not performed, I = immature, INT = intermediate, M = mature
≠ Classification of White (74)
* twin pregnancy

Table XII. Results of Fetal Maturity Assays Performed on Vaginal Drainage Specimens.

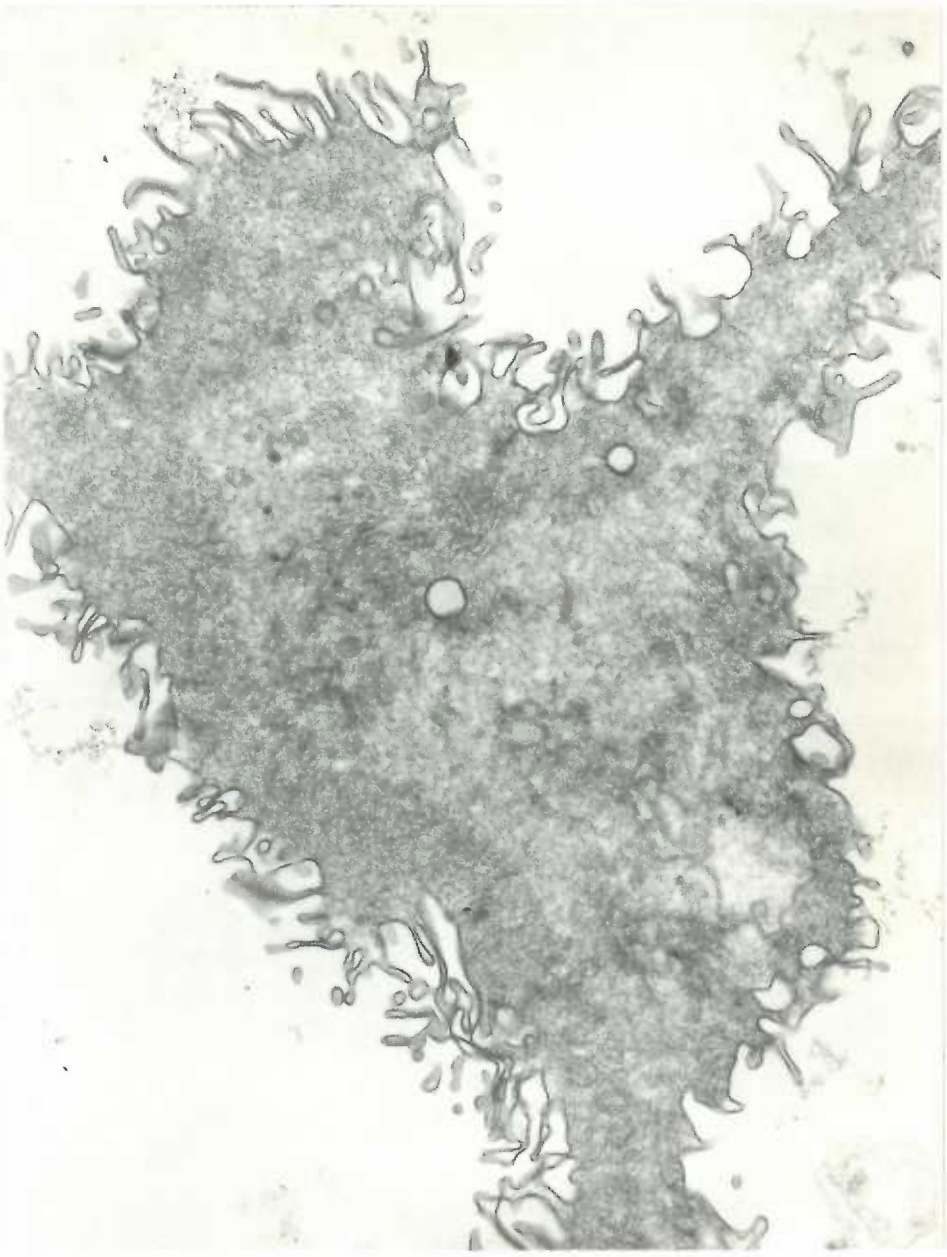
Case	GA° (weeks)	LB-PL (mg/dl)	L/S Ratio	PG+ (ug/ml)	RST*	Days to Delivery
1	34	0.6	0.4	-	I	5
2	35	2.3	1.5	< 1	NP	0
3	35	5.0	2.8	< 4	M	1
4	35	1.3	0.9	< 1	I	2

* NP = not performed, I = immature, M = mature

° GA = gestational age

+ - is PG not present

Photograph 1. Electron microscopy scan of LB band obtained after centrifugation at 115,000 x g for 20 minutes (see text for description of method). Cluster of lamellar bodies which have dissociated to form multi-laminate figures. X 17,7000.



Photograph 2. Electron microscopy scan of pellet obtained after centrifugation at 115,000 x g for 20 minutes (see text for description of method). Characteristic cell found in pellet. X 13,000.

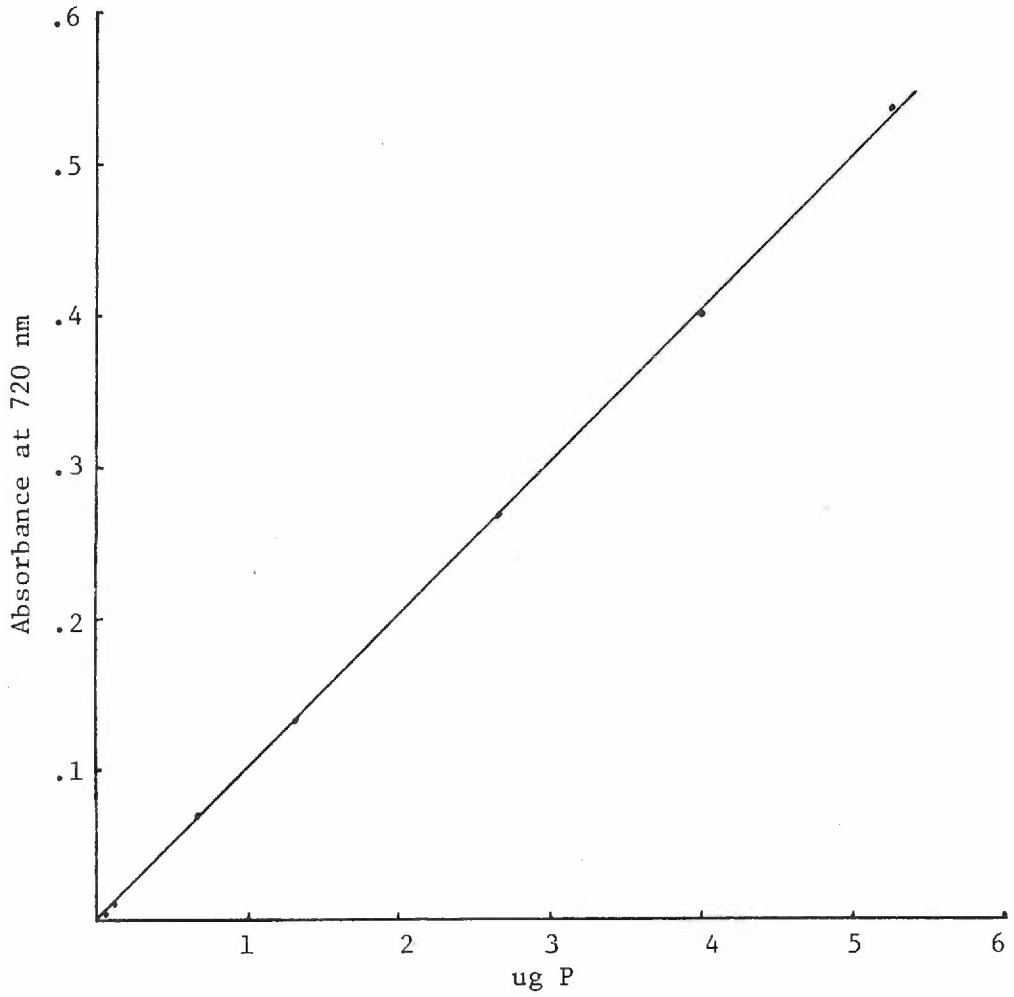


Figure 1. Linearity of standard curve. Known amounts of phosphorus were assayed in triplicate to determine the linearity of the standard curve. See text for description of the method.

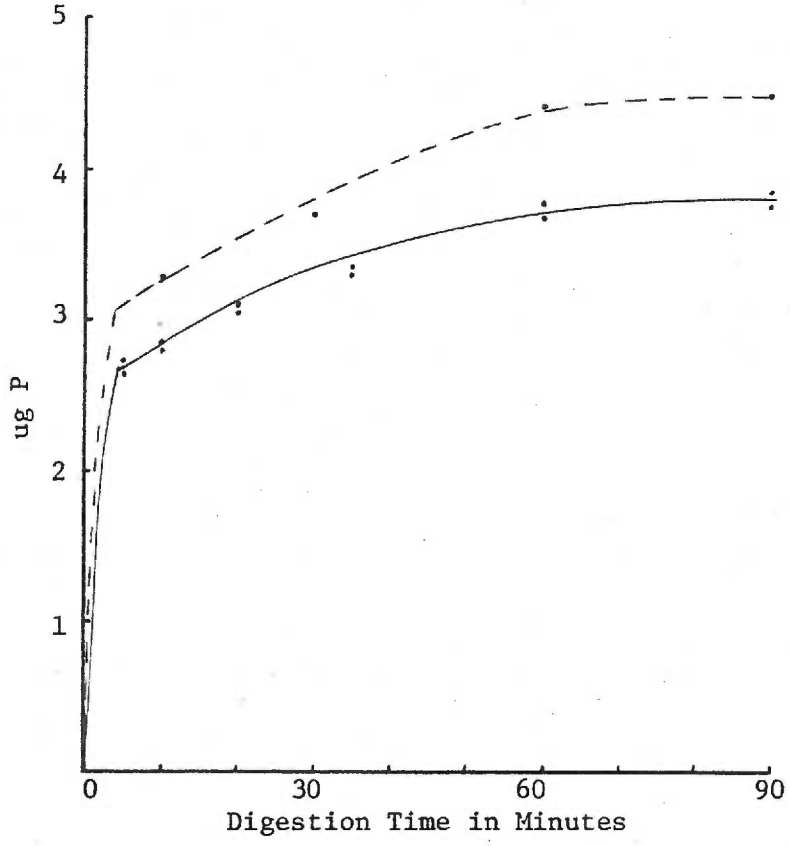


Figure 2. Digestion of phospholipids versus time. Aliquots of the DPPC standard solution (——) containing 4 ug phospholipid phosphorus were digested at 160 ± 5°C for varying periods of time. The LB fraction (---) of samples of amniotic fluid were digested at 160 ± 5°C for varying periods of time. Samples were assayed in duplicate for each time period and quantitated for phosphorus. See text for description of method.

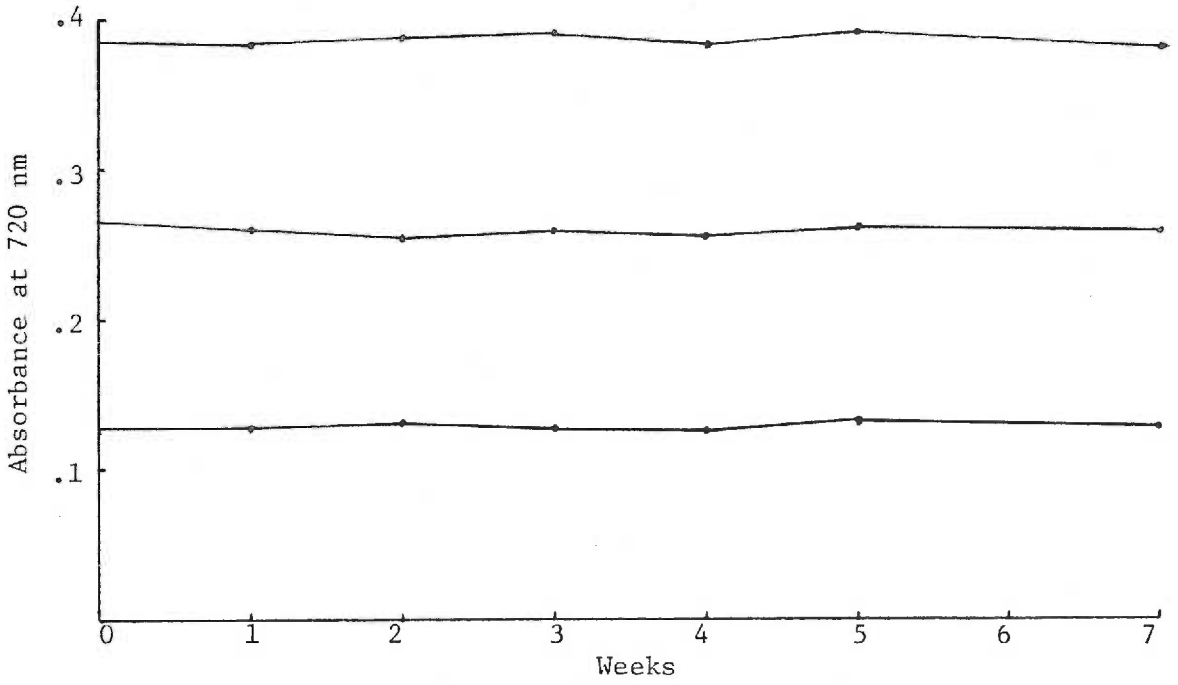


Figure 3. Stability of reagents versus time. Three phosphorus standards were used to determine the stability of reagents by checking the absorbance at weekly intervals. See text for method.

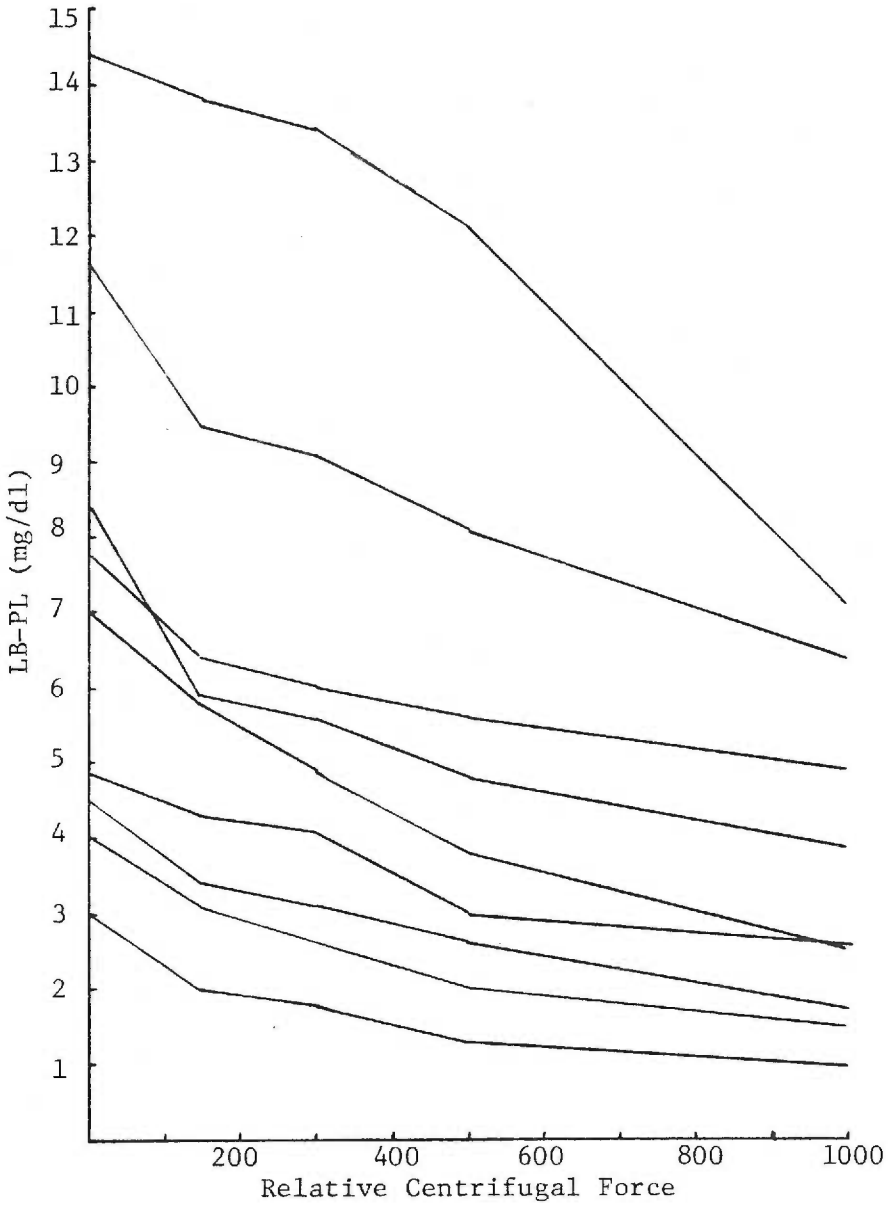


Figure 4. Effect of centrifugation on LB-PL content of amniotic fluid. Equal portions of nine amniotic fluids were centrifuged at different relative centrifugal forces and the supernatants analyzed for LB-PL content. Samples were centrifuged for 5 minutes at each RCF.

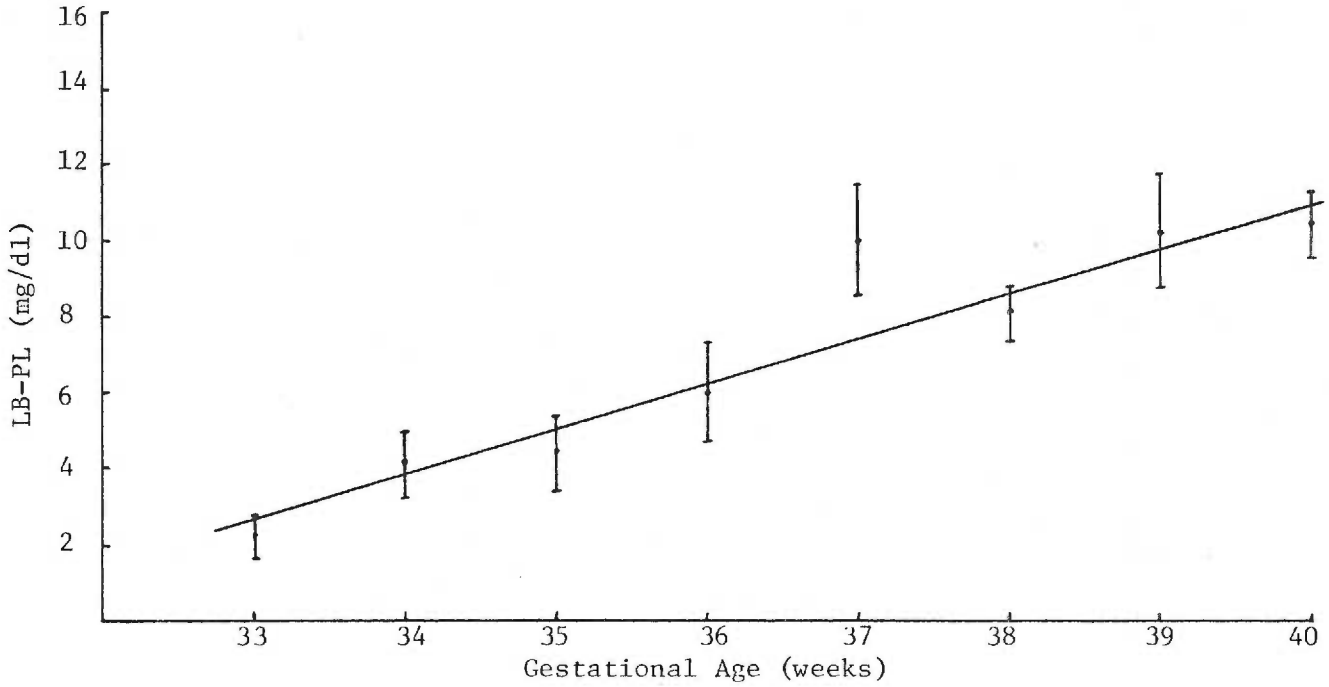


Figure 5. Lamellar body phospholipid content and gestational age. The mean LB-PL content $\pm$ standard error of the mean is plotted for each gestational age. Data was analyzed by linear regression. $y=1.16x-35.5$, $r^2=0.21$, $n=178$.

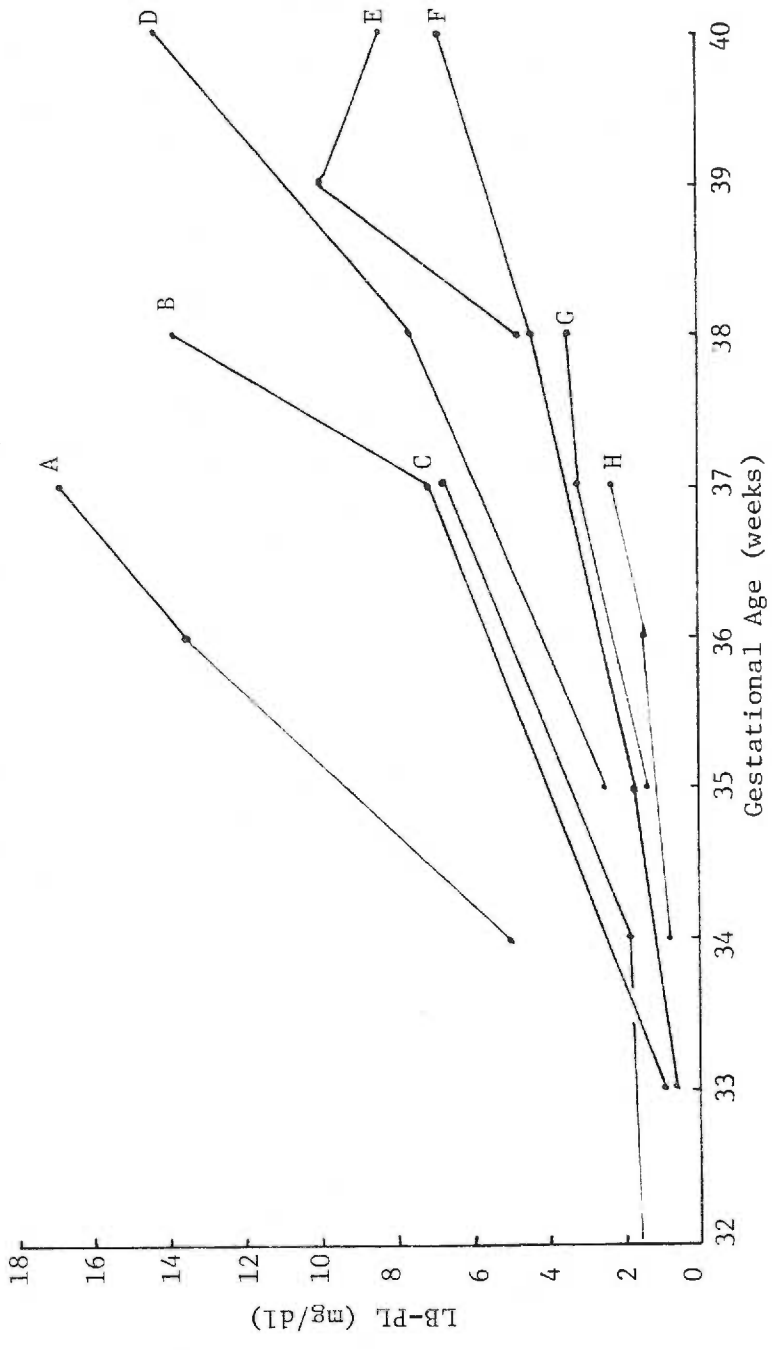


Figure 6. Lamellar body phospholipid content as a function of gestational age. Connected lines represent serial samples from eight cases in which three or more samples of amniotic fluid were analyzed.

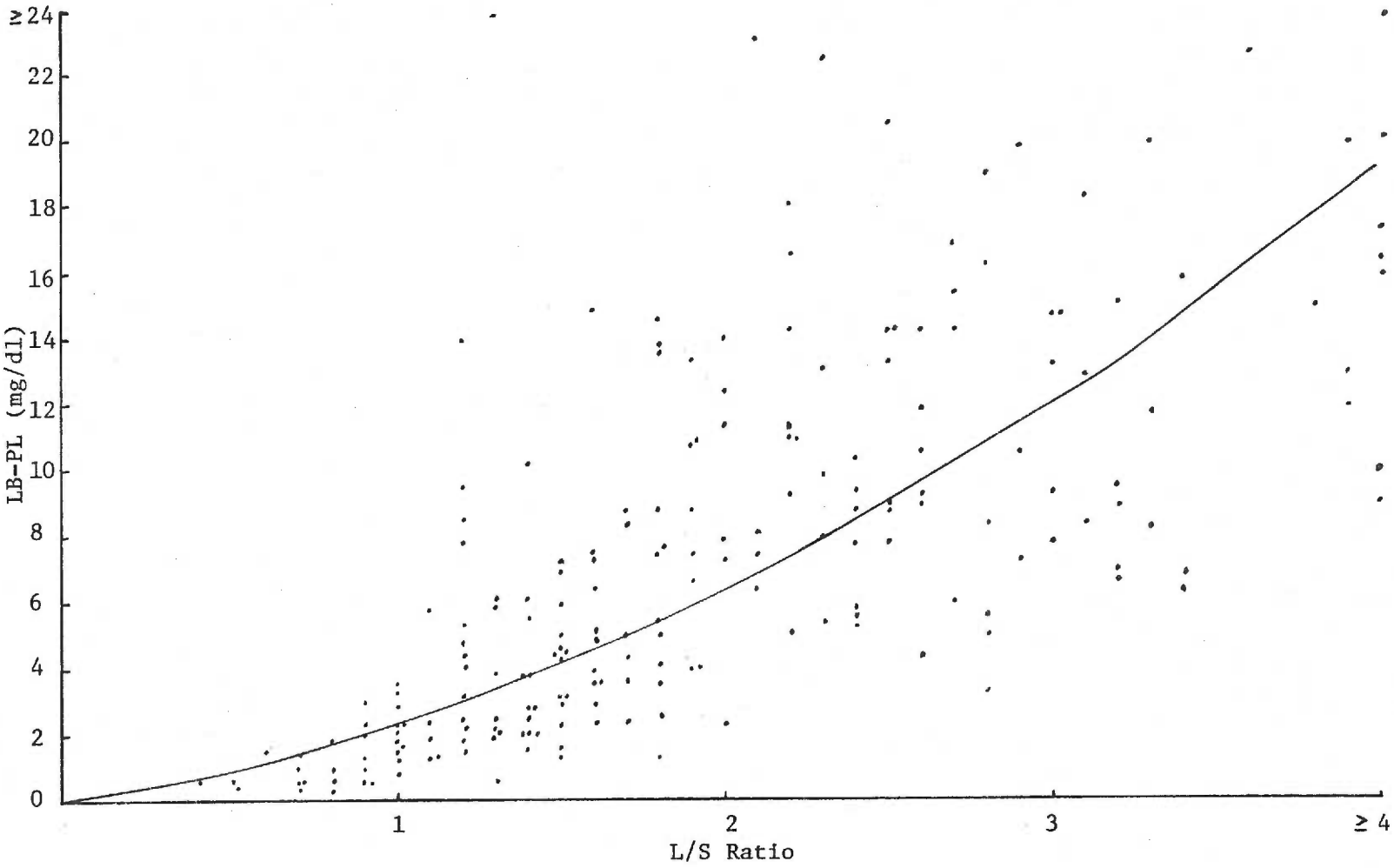


Figure 7. Comparison of lamellar body phospholipid content and lecithin/sphingomyelin ratio in 208 uncontaminated samples of amniotic fluid. Regression analysis by the power model provided the line of best fit. $y=2.06x^{1.61}$, $r^2=0.62$

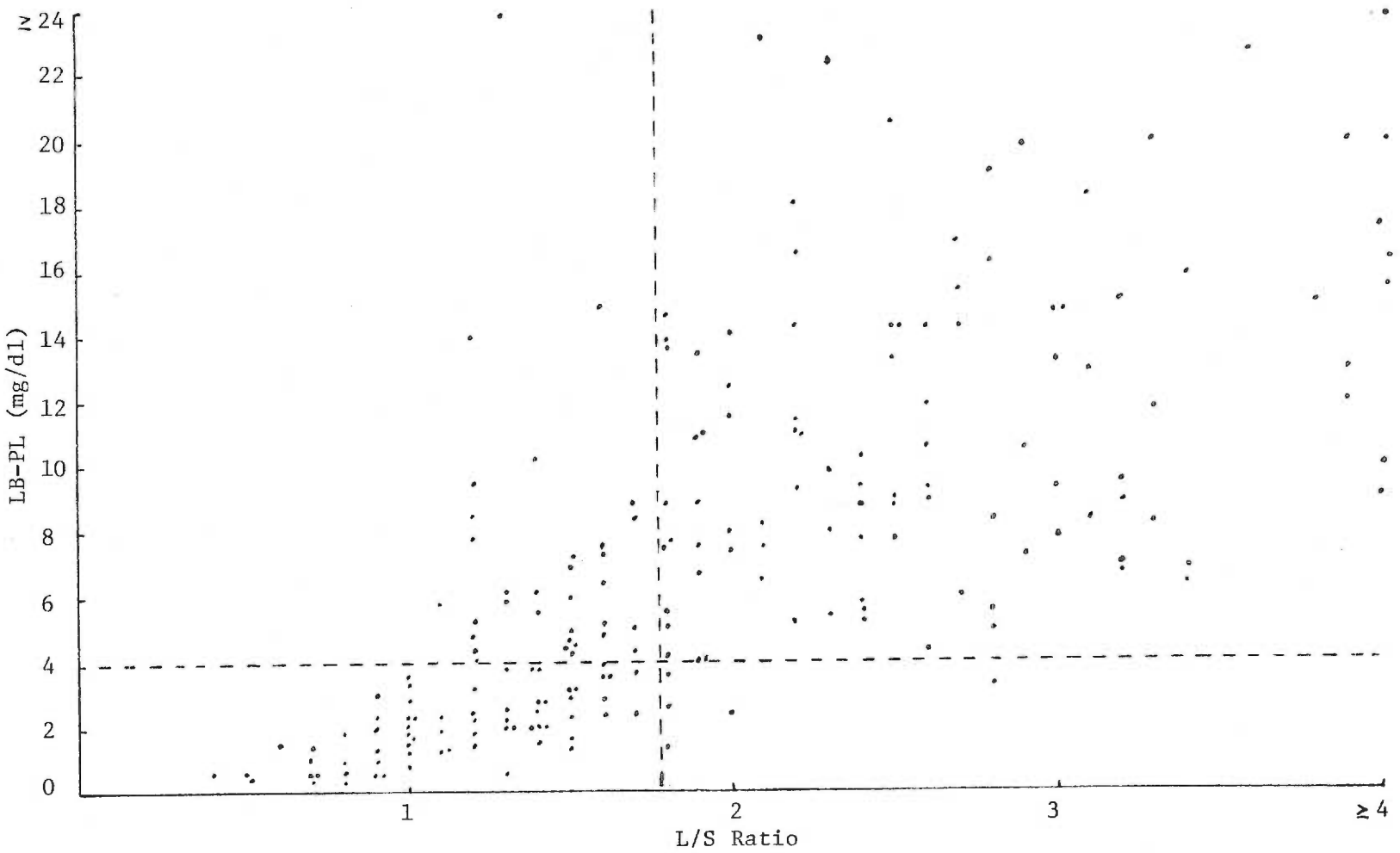


Figure 8. Comparison of lamellar body phospholipid content and lecithin/sphingomyelin ratio in 208 uncontaminated samples of amniotic fluid. Broken lines drawn at 4 mg LB-PL/dl and at a L/S ratio of 1.8 indicate the levels of maturity for each assay. Forty-nine per cent of the samples were mature by both assays, 33% immature by both assays, 16% had mature LB-PL values but immature L/S ratios, and 2% had mature L/S ratios and immature LB-PL values.

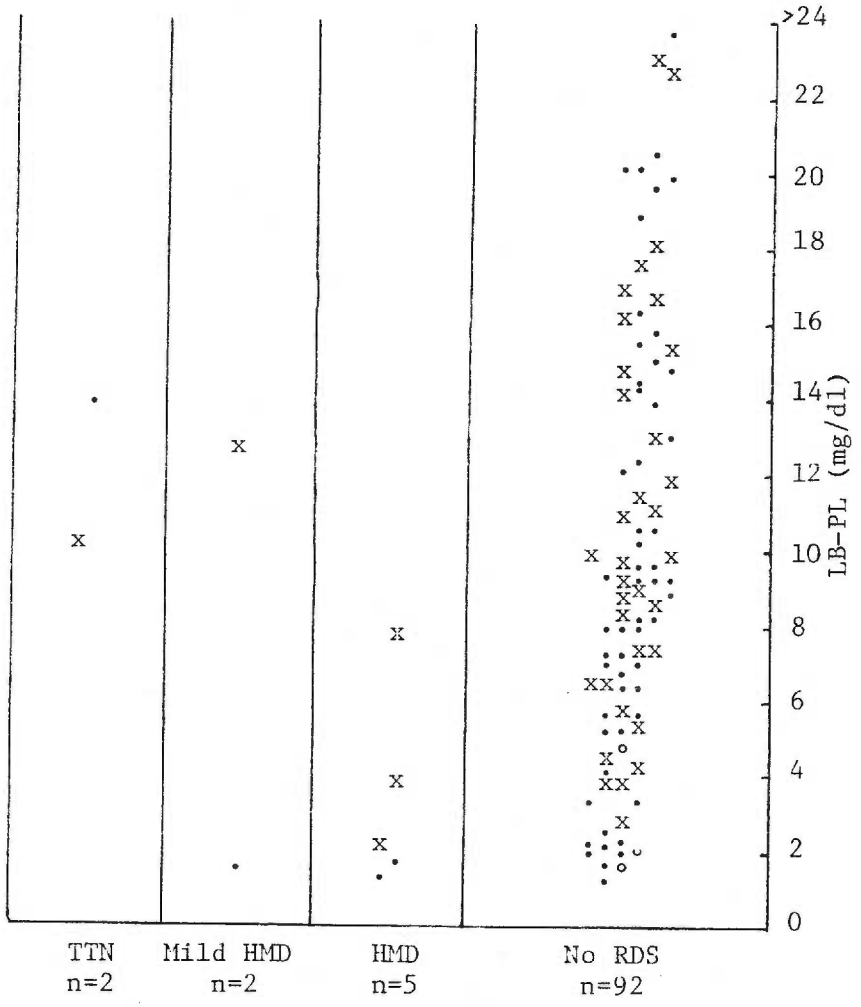


Figure 9. Amniotic fluid lamellar body phospholipid content of samples obtained within 3 days of delivery are plotted according to respiratory status. Transabdominal amniocentesis (•), cesarean section (x), and vaginal drainage (∘) samples were analyzed.

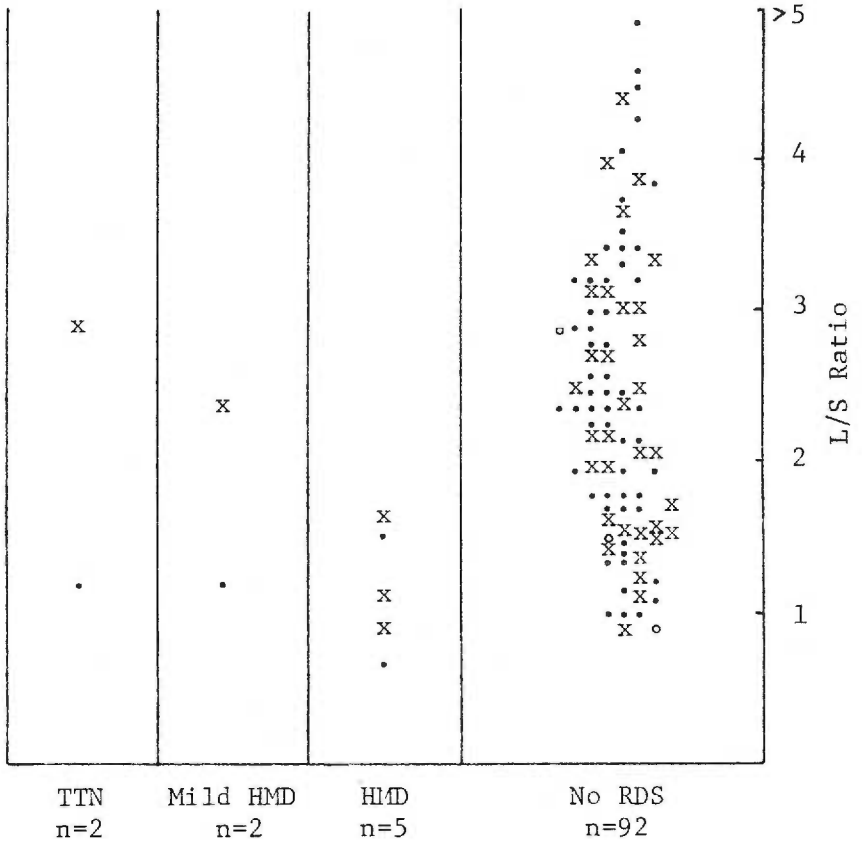


Figure 10. Amniotic fluid lecithin/sphingomyelin ratio of samples obtained within 3 days of delivery are plotted according to respiratory status. Transabdominal amniocentesis (·), cesarean section (x), and vaginal drainage (∘) samples were analyzed.

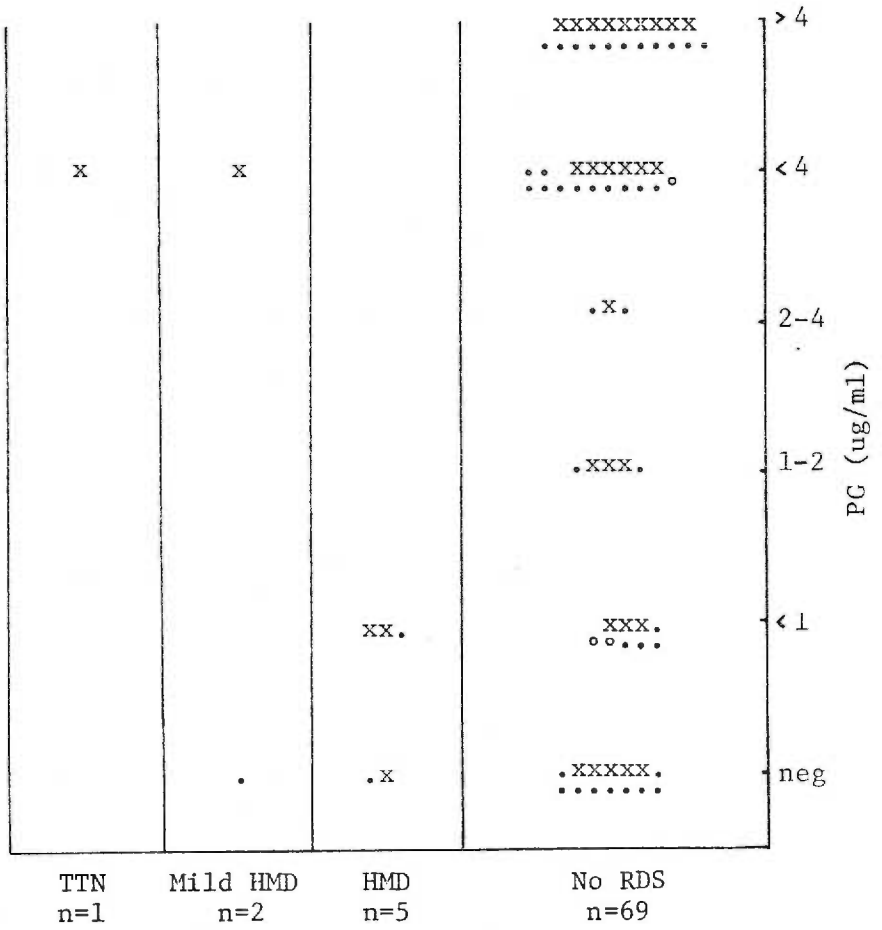


Figure 11. Amniotic fluid phosphatidyl glycerol content of samples obtained within 3 days of delivery are plotted according to respiratory status. Transabdominal amniocentesis (•), cesarean section (x), and vaginal drainage (∘) samples were analyzed.

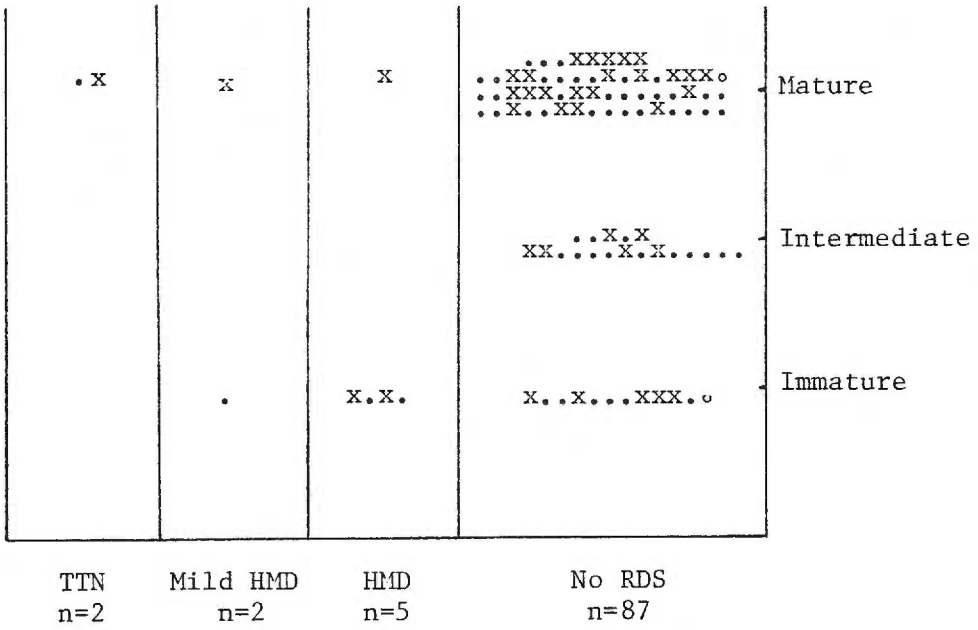


Figure 12. Rapid surfactant test result of samples of amniotic fluid obtained within 3 days of delivery are plotted according to respiratory status. Transabdominal amniocentesis (·), cesarean section (x), and vaginal drainage (∘) samples were analyzed.