Evidence of Lung Surfactant Abnormality in Respiratory Failure

STUDY OF BRONCHOALVEOLAR LAVAGE PHOSPHOLIPIDS, SURFACE ACTIVITY, PHOSPHOLIPASE ACTIVITY, AND PLASMA MYOINOSITOL

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ABSTRACT Autopsy findings suggest that lung surfactant is damaged in the adult respiratory distress syndrome. In the present study 225 bronchoalveolar lavage specimens (78 from 36 patients, 1–78 yr old with respiratory failure, 135 from another 128 patients with other respiratory disease, and 12 from healthy controls) were assayed for the lung profile [lecithin/sphingomyelin (L/S) ratio, saturated lecithin, phosphatidylinositol, and phosphatidylglycerol]. Bronchoalveolar lavage fluid was further analyzed for phospholipids and for phosphatidic acid phosphohydrolase, phospholipase A_2, and phosphatidylinositol phosphodiesterase activities. A lipid-protein complex was isolated and analyzed for surface activity, and plasma was measured for myoinositol.

There were only small differences seen in the recovery of total phospholipid between respiratory failure patients and normal controls. However, in respiratory failure, phospholipids in bronchoalveolar lavage were qualitatively different from those recovered either from normal controls or from patients with other lung disease: the L/S ratio, phosphatidylglycerol, and disaturated lecithin were low, whereas sphingomyelin and phosphatidylserine were prominent. These abnormalities were present early in respiratory failure and tended to normalize during recovery. Low L/S ratio (<2), and low phosphatidylglycerol (1% or less of glycerophospholipids) in bronchoalveolar lavage was always associated with respiratory failure. Abnormal lavage phospholipids were not due to plasma contamination. The phospholipase studies revealed little evidence of increased catabolism of phospholipids.

In respiratory failure, the lipid-protein complexes from lung lavage were not surface active, whereas that from healthy controls had surface properties similar to lung surfactant.

Phospholipids from patients with respiratory failure were similar to those from respiratory distress syndrome in the newborn. However, the latter condition is characterized by fast recovery of surfactant deficiency and by high plasma myoinositol that suppresses the synthesis of surfactant phosphatidylglycerol and increases phosphatidylinositol (*Pediatr. Res.* 1981. **15:** 720.). On the other hand, in adult respiratory distress syndrome, the abnormality in surfactant phospholipids may last for weeks and in most cases is associated with low phosphatidylinositol, low phosphatidylglycerol, and low plasma myoinositol.

INTRODUCTION

Adult respiratory distress syndrome (ARDS),[1] acute respiratory failure with generalized lung involvement,

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[1] *Abbreviations used in this paper*: ARDS, adult respiratory distress syndrome; BAL, bronchoalveolar lavage; L/S ratio, lecithin/sphingomyelin ratio; RDS, respiratory distress syndrome of the newborn.

carries a mortality rate of 50% or more despite recent advances in respiratory care (1–3). Although ARDS is a heterogeneous syndrome associated with sepsis, aspiration, toxins, emboli, circulatory collapse, and metabolic, neurogenic, or hematologic disorders, among others, the resulting abnormalities in lung function are similar (4, 5).

Ashbaugh et al. have suggested that the surfactant system is damaged in ARDS (6). However, reports on surfactant in ARDS are scanty, and involve characterization of surface activity and phospholipids post mortem (7, 8) Although these studies demonstrate evidence of surfactant deficiency, it may be due to terminal or post-mortem changes and thus not represent mechanisms that lead to respiratory failure. In addition, there is evidence based upon animal studies, that the surfactant-producing type II alveolar cells are more resistant to acute alveolar injury than are other cells of the alveolocapillary unit, and that surfactant secretion may increase rather than decrease following lung injury (9, 10). However, it is uncertain whether the mechanism and severity of lung damage in these model studies are similar to those in ARDS.

Respiratory distress syndrome of the newborn (RDS) bears similarities to ARDS both in symptoms and in radiologic findings. In RDS, a primary cause of the disease is a deficiency in surfactant. This is caused by immaturity in the alveolar epithelium resulting in deficient biosynthesis and secretion of surfactant (11–13). Surfactant from developing fetal lung accumulates in amniotic fluid. It may be sampled, allowing evaluation of lung maturity. Postponing delivery until the surfactant system has matured prevents RDS. In addition, lung aspirates obtained during suctioning of airways can be analyzed for the biochemical diagnosis of RDS and for monitoring the recovery of the surfactant system (14, 15).

Surfactant complex from normal adults contains, other than lecithins (~70% of total lecithin is disaturated, containing predominantly palmitate), phosphatidylglycerol in uniquely high concentration, phosphatidylethanolamine, phosphatidylinositol, cholesterol, small amounts of some other lipids, and, at the least one surfactant-specific protein that seems to possess phosphatidate phosphohydrolase activity (16–19). However, during fetal development, before the final maturation of the surfactant system, the complex contains no phosphatidylglycerol, but instead very high levels of phosphatidylinositol (15, 19).

Owing to complexities in surfactant function and the possibility that a part rather than the whole complex is abnormal, it is preferable to monitor several surfactant components and to measure surface activity. The lung profile, which includes measurements of lecithin/sphingomyelin (L/S) ratio, saturated lecithin, phosphatidylglycerol, and phosphatidylinositol, is an accurate method of measuring lung maturity during the perinatal period (20–21).

In this study, we have characterized the biochemical and physicochemical properties of phospholipids recovered from bronchoalveolar lavage (BAL), and attempted to clarify mechanisms that lead to altered phospholipids in respiratory failure. The methods described allow monitoring of the peripheral airway lining during the course of ARDS.

METHODS

Study population

The number of patients and the number of specimens studied are shown in Table I. Subjects ranged in age from 1 to 78 yr: 117 were males, 59 females. Without knowledge of the biochemical analyses, patients were classified into diagnostic categories after review of the hospital record. These categories were defined as follows:

Respiratory failure. (*a*) Requirement for mechanical ventilation for more than 24 h, (*b*) requirement for 50% or more inspiratory oxygen, and (*c*) panlobar infiltrates on the

TABLE I
Source of BAL

Patients	Number of Individuals	Number of Specimens
Respiratory failure	36	78
ARDS	26	65
Burns	3	10
Drowning	3	13
Sepsis	4	11
Trauma	2	4
Other*	14	27
Respiratory failure, no ARDS‡	10	13
Pneumonia	19	22
Malignant tumor	15	16
Chronic obstructive pulmonary disease	19	19
Pulmonary alveolar proteinosis	5	5
Other respiratory disease§	70	73
Normal control	12	12
Total	176	225

* Multiple disorders 11 (23) (sepsis 7, aspiration 4, hypovolemic shock 5, central nervous system disease 5, malignant tumor 4, pancreatitis 1, collagen disease 1, uremia 1, liver disease 2, diabetes 4), aspiration of gastric content 1 (2), central nervous system disease 1, hypovolemic shock 1.

‡ Multiple disorders that include cardiac failure, collagen disease, chronic obstructive pulmonary disease, myasthenia gravis, uremia, sepsis, central nervous system disease, malignant tumor, diabetes.

§ Multiple disorders 19, trauma 14 (16), burns 8 (9), lung node or lung infiltrate 20, atelectasis 3, pulmonary fibrosis 3, hemoptysis 2, pleural effusion 1.

chest radiographs (22). A subgroup of respiratory failure was defined ARDS according to the following criteria: acute respiratory failure that developed within 7 d of the insult that was associated with pulmonary artery wedge pressure of <18 mmHg. 10 patients with respiratory failure differed from ARDS owing to the nonacute development of respiratory failure (nine cases), or else had cardiac failure (three cases).

Pneumonia. Infiltrates on the chest radiograph, isolation of an infectious organism or consistent lung biopsy, or a consistent clinical course.

Malignant tumors. Primary lung carcinoma (seven patients), or lung metastasis (eight patients).

Chronic obstructive pulmonary disease. Presence of asthma, chronic bronchitis, or emphysema.

Pulmonary alveolar proteinosis. This was demonstrated by open lung biopsy.

Other pulmonary disease. See Table I.

Normal controls. Seven subjects were nonsmoking adults, with normal arterial blood gases, physical examinations, and chest radiographs. In five others who underwent diagnostic bronchoscopy, the lung disease was considered to be inactive. BAL phospholipids (or the other analyses) from these five individuals were not significantly different from those from healthy volunteers.

Technique of obtaining BAL during bronchoscopy

Informed consent was obtained in each case before the procedure. The bronchoscope was wedged in a lower lobe segment (in most cases). 20 ml of normal saline was injected via the bronchoscope, and immediately suctioned into a Lukens trap attached directly to the bronchoscope. Among the children that were studied, 2–4 ml, instead of 20 ml of saline was used. In pulmonary alveolar proteinosis the specimens were obtained during therapeutic lavage.

Treatment of BAL

Half of each aspirate was available for the present study. BAL was centrifuged at 140 *g* for 5 min and the supernatant was used for analysis of lipids and enzymatic activities, unless otherwise indicated. The lipid-protein complex for the measurement of surface activity was isolated at 4°C as follows: BAL supernatant was centrifuged at 10,000 *g* for 2 h. The pellet was suspended in normal saline and layered on top of a discontinuous density gradient containing the following layers (4 ml each): 1.3 M sucrose, 10 mM Tris–Cl, 100 mM NaCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$ (pH 7.4); 0.7 M sucrose/Tris/NaCl/$MgCl_2$/$CaCl_2$; 0.2 M sucrose/Tris/NaCl/$MgCl_2$/$CaCl_2$, and centrifuged (model L5-65 ultracentrifuge, Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) in a SW-27 rotor for 2 h at 100,000 g_{av}. Following centrifugation, the material between 0.2 and 0.7 M sucrose, and 0.7 and 1.3 M sucrose, were collected by aspiration. The two fractions were diluted with excess normal saline and centrifuged at 15,000 *g* for 2 h. The pellets represented the lipid-protein complexes that were studied for surface activity.

Lipid analysis

The methods used have been described previously (20, 23). Briefly, the lipids were extracted according to the method of Bligh and Dyer (24), followed by the diagnostic test, the lung profile (20). Surface active phospholipids were concentrated by precipitation with cold acetone. The acetone precipitable and acetone soluble fractions were analyzed for phospholipids by two-dimensional thin-layer chromatography, the quantity of individual phospholipids was analyzed by reflectance densitometry (20). Whenever sufficient amounts of lipids were available, the acetone precipitable and acetone soluble fractions were combined, and following analyses were performed: two-dimensional thin-layer chromatography of phospholipids that were quantified by measuring the phosphorus content of individual lipids (23). Disaturated lecithin was analyzed according to Mason et al. (25) using OsO_4. The recovery of disaturated lecithin was calculated using [^{3}H]dipalmitoyl lecithin (Applied Science Laboratories, State College, PA). Fatty acids of phosphatidylcholine were analyzed as methyl esters by gas-liquid chromatography (model 3920, Perkin-Elmer Corp., Norwalk, CT) as described previously (23). The columns were packed with 10% Silar 10C on 100/120 mesh, and developed at 180°C using N_2 as a carrier. The individual peaks were quantified by integrator (Hewlett-Packard Co., Palo Alto, CA).

Enzyme activities

For measurement of the enzyme activities, the BAL was used either fresh, or kept at −20°C for no longer than 2 wk. Storage did not significantly change the enzyme activities.

Phosphatidic acid phosphohydrolase (EC 3.1.3.4.) was assayed essentially as described by Johnston et al. (26). The reaction mixture contained 0.2% Triton X-100, 0.1 M Tris-maleate (pH 6.8), 0.4 mM [^{32}P]phosphatidic acid, 19,000–32,000 cpm/assay, and 8–12 μg of BAL protein in 75 μl. The incubation with the radioactive isotope took place for 10 min, followed by lipid extraction and counting of the nonlipid layer for radioactivity. Blank activity was measured in the absence of BAL. Radioactive phosphatidic acid was prepared as follows: the membrane fraction derived from *Escherichia coli* (27), heated for 5 min at 100°C, was incubated in the presence of 0.9 mM 1,2-dipalmitoyl-*sn*-glycerol, 0.5% cutscum, 40 mM sodium phosphate (pH 7.0), 0.4 mM adenosine 5′-[γ-^{32}P]triphosphate (Amersham Corp., Arlington Heights, IL), 50 mM $MgSO_4$. Following incubation, the lipids were extracted (24) and radioactive phosphatidic acid was isolated using two-dimensional thin-layer chromatography. Phosphatidic acid carrier was purchased from Supelco Inc., Belefonte, PA.

Phospholipase A_2 activity (EC 3.1.1.4) was measured in two different media. Each assay was run in duplicate. The incubation medium contained either 50 mM Tris–HCl, 0.1 mM EDTA, 10 mM $CaCl_2$ (pH 8.0), or 50 mM Tris-maleate (pH 6.5). The reaction mixture additionally contained 5 × 10^4 cpm of [9,10-^{3}H]2-palmitoyl L-α-dipalmitoyl lecithin (Applied Science), 0.5 mM L-α-dipalmitoyl lecithin, and 16–25 μg of BAL protein in 0.1 ml. Liposomes of lecithin were prepared by sonication in 0.5 M Tris-maleate or 0.5 M Tris–HCl. After incubation for 1 h at 37°C, the reaction was terminated by extraction of the lipids. The lipid fraction, together with the palmitate carrier, was applied to silica gel H plates, and developed using petroleum ether/ethyl ether/acetic acid (160:40:2 vol/vol/vol). The palmitate-containing lipid spot was counted for radioactivity. The amount of lecithin present in BAL was taken into account when the activity was calculated. Blank activity was measured in the absence of BAL.

Phosphatidylinositol phosphodiesterase (EC 3.1.4.10) activity was measured as follows: two duplicate assays in the presence of 0.5 mM [^{32}P]phosphatidylinositol (7,200–14,500 cpm/assay) and 5 mM Tris-maleate (pH 7.0) were per-

formed: one with 2 mM $CaCl_2$, the other without $CaCl_2$. [^{32}P]Phosphatidylinositol was suspended by sonication in 0.5 M Tris-maleate. BAL protein content was 16–25 μg in a final volume of 0.1 ml. After incubation at 37°C for 20 min, the reaction was stopped by addition of chloroform (2 ml), and methanol (1 ml). Following the addition of 0.9 ml of 10 mM citrate (pH 2.0) the lipids were extracted according to Bligh and Dyer. The layer containing no lipid was counted for radioactivity, and the enzyme activity was calculated on the basis of the radioactivity released to the nonlipid layer minus the blank radioactivity with boiled BAL. [^{32}P]Phosphatidylinositol was prepared by incubating lung slices in Krebs-Ringer-bicarbonate medium containing 0.5 mM myoinositol and 10 mCi/ml of ^{32}P-phosphate (23). [^{32}P]Phosphatidylinositol was isolated by two-dimensional thin-layer chromatography followed by silicic acid column chromatography (28). Carrier phosphatidylinositol was purchased from Supelco Inc.

Plasma myoinositol

This was measured as a trimethylsilyl ether derivative by gas-liquid chromatography (7620A research chromatograph with integrator, Hewlett-Packard Co.) using α-methylmannoside as an internal standard. 3 vol of 5% $ZnSO_4$ and 3 vol of 0.3 N $Ba(OH)_2$ were added to 1 vol of serum (50–100 μl), the mixture centrifuged at 900 *g* for 10 min and the supernatant freeze dried. 5 vol of the silylating mixture containing trimethylchlorosilane-hexamethyldisilazane-pyridine (1:3:9 vol/vol/vol, Sigma Chemical Co., St. Louis, MO) was added. After 60–90 min the mixture was evaporated under nitrogen, taken up in CS_2, and analyzed on a 180-cm column of 3% SE-30 Supelcoport (80/100 mesh, Supelco Inc.).

Surface activity

The surface tension was measured in a Wilhelmy balance with a tight fitting Teflon barrier (29). The maximum surface area was 444 cm^2, and the minimum 40 cm^2. The balance was housed within a double-walled thermostated (37±0.4°C) plastic bath. Air within the chamber was moistened with wet blotter pads. To avoid leaks at low surface tension, the inside walls of the trough were treated with $LaCl_3$-disaturated lecithin mixture (29). The complex containing 300 nmol phospholipid was applied to the surface in <30 μl of isopropanol/water/chloroform (2:1:0.5 vol/vol/vol). 10 min after the application, the surface area was compressed, and then expanded at a constant rate of 5 $cm^2 \times s^{-1}$. Surface tension vs. area isotherms were recorded using an X-Y recorder.

The collapse rate of the film, K_m, held at minimum surface area was calculated as follows:

$$K_m = \frac{1}{\gamma_{eq} - \gamma_0} \times \left(\frac{d\gamma}{dt}\right)_0,$$

where γ_{eq} = equilibrium surface tension; γ_0 = initial surface tension; $d\gamma/dt$ = rate of increase in surface tension. Surface tension and time were continuously recorded during the measurement of the collapse rate.

Compressibility, C_m, was calculated from the isotherms as follows: $C_m = 1/A \times dA/d\gamma$, where A = surface area.

Other assays

Protein was measured according to Lowry et al. (30). The statistical significance was evaluated using analysis of variance, Wilcoxon rank test, or χ^2 test. The results were expressed as the mean±SEM, or as the median (range).

RESULTS

Quantity of phospholipids. There was a large variability in the recovery of BAL phospholipids (cf. reference 14). In seven cases, the specimen contained <200 nmol of phospholipids (i.e., insufficient amount for quantitative analysis). The recovery of phospholipids (half of the BAL return) in ARDS (median 0.6 μmol, range 0.2–2.2) was not significantly different from that of the normal controls (median 0.6 μmol, range 0.2–1.3), pneumonia (median 0.8 μmol, range 0.5–2.5), or carcinoma (median 0.4 μmol, range 0.1–0.9). However, in chronic obstructive pulmonary disease the recovery of phospholipids (mean 0.3 μmol, range 0.1–0.6) was smaller than in normal controls ($P < 0.002$).

There was no significant difference in the concentration of total phospholipid in BAL as compared amongst cases of ARDS, normal controls, chronic obstructive lung disease, pneumonia, and carcinoma (data not shown).

In six cases, two segments of the lung were lavaged (each with 20 ml of saline), during a single bronchoscopy, and the lavage returns were analyzed separately. Table II shows the measurements of the total phospholipid recovery, the concentration of total phospholipid, the L/S ratio and phosphatidylglycerol in these six cases. Furthermore, the difference between the lavage pairs are shown. The L/S ratio and phosphatidylglycerol revealed less difference between the pairs than either the total phospholipid ($P < 0.02$) or the total phospholipid concentration ($P < 0.05$).

Quality of phospholipids. Table III illustrates the lung profile from BAL. The specimens are divided into three groups: respiratory failure, normal controls, and other lung disease. In respiratory failure the phospholipids were strikingly different from the two other groups, particularly the L/S ratio and phosphatidylglycerol, which were low.

Fig. 1 illustrates the lung profile from respiratory failure at the height of the disease and from normal controls. The regression lines included in the figure represent the sequential changes in the lung effluent phospholipids that take place during the last 3 mo of fetal development (20). In respiratory failure, BAL phospholipids revealed similarities to phospholipids from immature lung, except for phosphatidylinositol, which was inappropriately low. In five cases phosphatidylglycerol was detectable (i.e., >1% of phospholipids). However, the phospholipids of lung effluent from healthy controls resembled those recovered from fetuses at term.

TABLE II

Comparison between the Quantity and Quality of Phospholipids Recovered by BAL from Two Different Segments of the Lung

Case	Source	Analysis	Recovery of total phospholipid	Difference*	Concentration of total phospholipid	Difference*	L/S ratio	Difference*	Phosphatidylglycerol: Acetone-precipitated lipids	Phosphatidylglycerol: Difference*
			μmol		*μmol/ml*				%	
1	RML‡	A	0.53	0.56	0.35	0.49	3.0	0.13	0	0.00
	RLL	B	0.15		0.12		2.3		0	
2	RLL	A	0.35	0.07	0.21	0.21	2.8	0.11	9	0.14
	LLL§	B	0.40		0.32		3.5		12	
3	RML	A	0.41	0.34	0.30	0.25	2.4	0.02	10	0.18
	RLL	B	0.20		0.50		2.5		7	
4	RML	A	0.55	0.23	0.29	0.27	2.1	0.14	11	0.00
	RLL		0.88		0.50		2.8		11	
5	RML	A	0.20	0.27	0.20	0.01	3.1	0.05	15	0.06
	RLL	B	0.35		0.25		2.8		17	
6	LLL	A	0.15	0.70	0.25	0.41	2.0	0.05	NM	
	RLL	B	0.85		0.60		2.2		0	

* (A − B)/(A + B)

‡ RML, right middle lobe.

§ LLL, left lower lobe. NM, not measurable.

Table IV illustrates a detailed analysis of phospholipids in BAL. Owing to the scarcity of phospholipids available, all specimens could not be analyzed fully. However, the results shown in Table IV are representative, since the lung profiles were not significantly different, as compared with those in Table III and with those that were analyzed only for the lung profile. Phosphatidylserine and sphingomyelin tended to be higher, and lecithin lower in ARDS. Similar, though less remarkable changes, were frequently seen in other lung diseases too.

Table V shows the fatty acid structure of BAL lecithins. In respiratory failure the fatty acids were significantly less saturated (low palmitate in particular)

TABLE III

Lung Profiles of BAL

	A	B	C	A vs. B	A vs. C	B vs. C
	ARDS/respiratory failure	Other lung disease*	Normal control			
n	70	123	12		*P*	
L/S ratio in acetone-precipitated fraction	1.9±0.1	3.3±0.1	6.1±0.8	<0.0005	<0.0005	<0.0005
Acetone-precipitable lecithin (*% of total lecithin*)	54±1	62±1	64±2	<0.0025	<0.0005	
Phosphatidylinositol‡	9.0±0.5	10.3±0.3	8.6±1.1			
Phosphatidylglycerol‡	0.0(0–24.0)	11.0±0.4	21.0±1.5	<0.0005	<0.0005	<0.0005

* Includes cases with no respiratory failure: pneumonia (22 specimens), carcinoma (16 specimens), chronic obstructive pulmonary disease (15 specimens), other pulmonary disease (70 specimens).

‡ Percentage of glycerophospholipids (lecithin + phosphatidylglycerol + phosphatidylinositol + phosphatidylethanolamine + phosphatidylserine) in acetone-precipitated fraction.

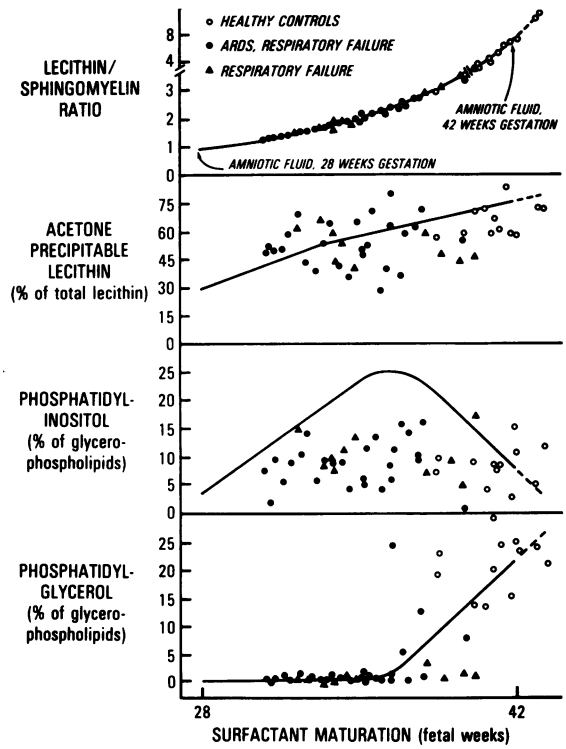

FIGURE 1 The lung profile for 12 normal controls and 36 patients with respiratory failure. Regression lines represent normal amniotic fluid values found between the 28th and 42nd wk of gestation. Control and patient L/S values have been placed on the L/S ratio regression line, thus determining a degree of surfactant "maturity". Using this degree of maturity, values for the remaining components of the lung profile are compared with the normal regression lines.

and there was less disaturated lecithin than in healthy controls or in lung diseases with no respiratory failure.

Predictability of the L/S ratio and phosphatidylglycerol. The accuracy of the L/S ratio and phosphatidylglycerol in differentiating between respiratory failure and no respiratory failure, was studied. The former group included cases of respiratory failure when the symptom was evident (n = 70). The group with "no respiratory failure" included all other cases (n = 148). The overall accuracy (correct predictions in percentage of total predictions) was evaluated as a function of the L/S ratio. It was found that a cutoff point of two (no respiratory failure when L/S >2) had the best overall accuracy of 83% in predicting or excluding the respiratory failure. The corresponding figures for phosphatidylglycerol were 1% of glycerophospholipids (no respiratory failure when phosphatidylglycerol > 1%), and 82% (overall accuracy).

Table VI shows the incidence of respiratory failure on the basis of the patterns of the lung effluent phospholipids. Respiratory failure was always associated with an L/S ratio of 2 or less and phosphatidylglycerol of 1% or less.

The L/S ratio and phosphatidylglycerol during the course of ARDS. Fig. 2 shows data on the L/S ratio and phosphatidylglycerol in relation to the onset of respiratory failure. The indexes were abnormal early in the disease. In addition, there was evidence of normalization of the phospholipids during recovery.

Comparison between BAL and plasma lipids in ARDS. Considering the increased permeability of the alveolocapillary membrane, it is likely that plasma lipids contaminate BAL in ARDS. In five instances BAL and plasma lipids from ARDS were compared. Although the total L/S ratios were similar, and phosphatidylglycerol was undetectable in both plasma and BAL, several striking differences were detected: (*a*) disaturated lecithin/total lecithin ratio (BAL 0.29±0.08; serum 0.04±0.01); (*b*) phosphatidylserine (BAL 11.5±1.2% of total phospholipid; serum 1.2±0.6%); (*c*) glycolipid (BAL contains prominent glycolipid, while plasma has only traces of glycolipid,

TABLE IV
Percentage of Phospholipid Composition of the BAL Fluids

Diagnosis	Respiratory failure	Pneumonia	Carcinoma	Chronic obstructive pulmonary disease	Pulmonary alveolar proteinosis	Other pulmonary disease	Normal control
n	13	10	5	5	5	9	7
Lecithin	59.5±1.9	66.5±2.5	69.1±3.4	75.0±1.3	61.9±3.0	68.3±1.5	73.0±2.3
Phosphatidylglycerol	0.3(0–7.0)*	5.4±0.6	7.4±1.2	8.8±0.8	11.3±0.0	6.5±0.6	12.4±0.6
Phosphatidylinositol	3.1±0.2	5.0±0.7	3.4±0.3	2.1±0.3	3.2±0.2	4.2±0.5	2.7±0.2
Phosphatidylethanolamine	4.3±0.2	3.4±0.2	4.0±0.2	2.9±0.6	3.4±0.3	3.7±0.2	2.6±0.3
Phosphatidylserine	13.0±1.1*	7.8±0.8	6.1±1.0	4.2±0.4	7.1±0.9	6.8±0.6	3.3±0.3
Bis(monoacyl-glycerol)phosphate	0.8±0.1	1.2±0.1	1.0±0.2	0.9±0.2	3.0±0.8	1.2±8.2	1.9±0.3
Sphingomyelin	17.5±1.6*	10.0±1.3	8.0±1.4	5.0±1.0	8.9±0.9	8.6±0.4	3.7±0.5
Lysolecithin	1.5±0.2	0.7±0.1	1.0±0.3	1.1±0.4	1.2±0.4	0.7±0.2	0.4±0.2

* $P < 0.0005$ compared with the other groups.

TABLE V

Percentage of Fatty Acid Composition of BAL Lecithins, and Percentage of Disaturated Lecithins

	A	B	C	A vs. B	A vs. C	B vs. C
	ARDS/respiratory failure	Other lung disease*	Normal control		*P*	
n	9	7	7			
		%				
Fatty acids						
Myristic (14:0)	4.4±0.8	6.0±1.2	3.6±0.8			
Palmitic (16:0)	46.7±2.6	54.0±2.6	68.0±3.0		<0.0005	<.0025
Palmitoleic (16:1)	9.5±1.3	8.8±1.6	4.6±0.7		<0.005	<.025
Stearic (18:0)	8.5±0.7	6.7±0.8	4.5±0.7		<0.005	<.05
Oleic (18:1)	11.8±1.3	13.2±2.0	10.9±1.1			
Linoleic (18:2)	8.8±1.0	5.2±0.5	3.6±0.7	<0.005	<0.0005	
Linolenic (18:3)	5.0±0.9	2.9±0.4	3.2±0.4	<0.025		
Arachidonic (20:4)	5.3±2.9	3.2±0.3	1.6±0.3	<0.05	<0.0025	<.0025
Saturated	59.6±1.0	66.7±3.1	76.1±3.1	<0.05	<0.0005	<.025
Disaturated lecithin	32.1±2.4	49.9±4.3	63.4±3.0	<0.0025	<0.0005	<.025

* Pneumonia (2 specimens), carcinoma (1 specimen), chronic obstructive pulmonary disease (1 specimen), other pulmonary disease (3 specimens).

quantitative data not shown); and (*d*) lysolecithin (BAL: 1.2±0.4% of total phospholipid; plasma: 6.7±0.8%). Although in ARDS an increase in plasma lipid contamination is likely, it cannot explain the changes in BAL.

Surface activity. In preliminary experiments, dry BAL was used for measuring surface activity. However, even dry BAL from normal subjects often failed to decrease surface tension below 10 dyn/cm, despite as much as 300 nmol phospholipid having been added to the surface. This was not surprising, since dry BAL from animals exhibits similar surface properties, suggesting that some contaminating material that inhibits the surfactant function is present in BAL. Therefore, the lipid-protein complex was isolated using sucrose density gradient centrifugation. Most of BAL material

TABLE VI

Incidence of Respiratory Failure on the Basis of the L/S Ratio and Phosphatidylglycerol in the Acetone-precipitated Lipid Fraction of the BAL

Result	Total number of cases	Respiratory failure	
		n	%
L/S ≤ 2 and phosphatidylglycerol ≤ 1% of glycerophospholipids	21	21	100
L/S ≤ 2 and phosphatidylglycerol > 1%	18	15	83
L/S > 2 and phosphatidylglycerol ≤ 1%	31	20	65
L/S > 2 and phosphatidylglycerol > 1%	148	14	10
Total	218	70	32

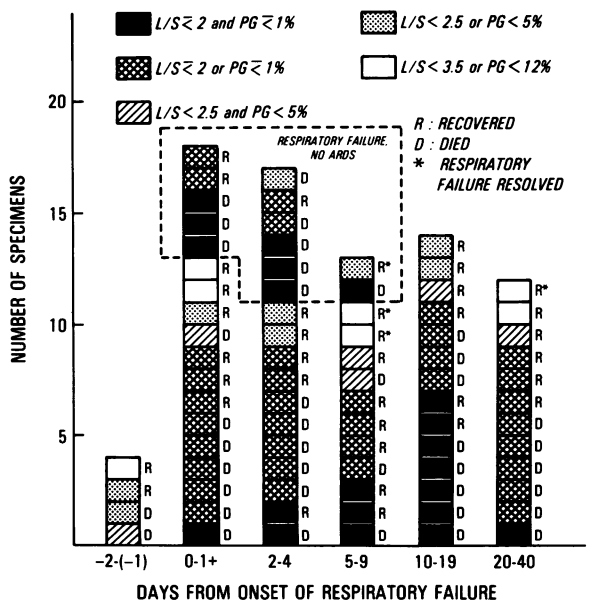

FIGURE 2 The L/S ratio and phosphatidylglycerol (PG, expressed as a percentage of acetone-precipitable glycerophospholipid) during the course of respiratory failure. Each square represents one BAL specimen. The asterisk indicates that the patient had no further respiratory failure.

from normal subjects sedimented between 0.2 and 0.7 M sucrose. However, in respiratory failure most of the phospholipids were between 0.7 and 1.3 M sucrose.

Table VII lists the minimum surface tension, the collapse rate, the surface concentrations of the phospholipids, and the compressibility at 15 dyn/cm. The lipid-protein complex from ARDS was not surface active, regardless of whether the material was recovered between 0.2 and 0.7 M sucrose or between 0.7 and 1.3 M sucrose.

Enzyme activities in BAL. Since surfactant-associated protein contains phosphatidate phosphohydrolase activity (18), we measured this enzyme to get information of a protein component associated with surfactant. As shown in Table VIII, phosphatidate phosphohydrolase activity was lower in BAL from respiratory failure than in BAL from no respiratory failure. The result was significant even if the enzyme activity was expressed on the basis of the lavage phospholipids rather than the protein.

Considering the moderate increase in the lysolecithin fraction (Table IV), and the low phosphatidylinositol (despite low phosphatidylglycerol) the phospholipases measured in BAL were phospholipase A_2 and phosphatidylinositol phosphodiesterase. As shown in Table VIII, phospholipase A_2 activity was low. When expressed on the basis of BAL protein, it tended to be lower in respiratory failure than in the other groups. However, when the activity was expressed on the basis of total phospholipid, the normal controls had lower activity than in other groups studied.

The activities of phosphatidylinositol phosphodiesterase were low, and frequently undetectable (data not shown).

Plasma myoinositol. The rationale of measuring plasma myoinositol was based on the following considerations: (*a*) in RDS high plasma myoinositol is associated with an absence of surfactant phosphatidylglycerol; (*b*) excess dietary myoinositol in adult rabbits prevents the synthesis of surfactant phosphatidylglycerol; and (*c*) phosphatidylglycerol was undetectable in most cases of ARDS.

Despite low or absent phosphatidylglycerol, plasma myoinositol was not significantly different as compared amongst ARDS (mean plasma myoinositol 29 μM, range 10–92, $n = 20$), and normal controls (35 μM, range 16–70, $n = 8$). However, in two cases of respiratory failure serum myoinositol was high (470 μM and 395 μM), and BAL phosphatidylinositol prominent (14.4 and 17.0%). In these patients the respiratory failure was associated with renal and cardiac failure. The follow-up of the first case of respiratory failure revealed a normalization of plasma myoinositol (from 470 to 39 μM), appearance of phosphatidylglycerol, and concomitant alleviation of the respiratory symptoms. The other cases of respiratory failure (without ARDS) that were studied for plasma myoinositol did not differ from normal controls (myoinositol 37

TABLE VII
Surface Properties Measured at 37°C of the Lipid-Protein Complexes Isolated by Sucrose Density Gradient

No. of specimens	Normal control 3	ARDS 3	ARDS 4
Density (g/cm^3)	1.03–1.10	1.03–1.10	1.10–1.21
Minimum surface tension (*dyn/cm*)			
2nd compression	3.0; 12.3; 11.9	19.2; 20.8; 22.0	20.1±0.4
8th compression	5.1; 17.2; 19.5	20.3; 20.8; 22.5	20.9±0.4
Surface concentration of phospholipid ($nmol/cm^2$)*			
At 25 dyn/cm	0.58; 0.60; 0.60	0.60; 0.57; 0.62	0.59±0.01
At 15 dyn/cm	1.32; 2.80; 5.07	NM‡	NM
Collapse rate at constant surface area (*liter/min*)§	10.8; 15.9; NM	NM	NM
Lowest compressibility at 15 dyn/cm (*cm/dyn*)	0.30; 0.90; 1.15	NM	NM

* Measured during the second compression of the surface.

‡ Not measurable.

§ The collapse rate of the film at minimum surface tension was measured after the 4th compression: surface area was maintained at minimum for 5 min, and the surface tension was continuously recorded. Surface tension did not change between 3 and 5 min. After the measurement of the collapse rate, surface-tension area isotherms were further measured for a total of eight complete cycles.

TABLE VIII
Phosphatidic Acid Phosphohydrolase and Phospholipase A_2 in BAL

	A	B	C	A vs. B	A vs. C	B vs. C
	ARDS/respiratory failure	Other lung disease	Normal control		*P*	
Phosphatidate phosphohydrolase						
n	7	7*	6			
pmol/mg protein/min	30±6	125±14	555±51	<0.001	<0.001	<0.001
pmol/μmol BAL phospholipid/min	84±7	135±14	249±18	<0.01	<0.001	<0.005
Phospholipase A_2						
n	8	7‡	5			
pmol/mg protein/min						
$+Ca^{++}$, pH 8.0	27±6	86±16	62±10	<0.01	<0.02	
$-Ca^{++}$, pH 6.5	23±5	36±9	50±9		<0.025	
pmol/μmol BAL phospholipid/min						
$+Ca^{++}$, pH 8.0	65±8	130±33	29±4		<0.005	<0.02
$-Ca^{++}$, pH 6.5	57±8	56±8	24±5		<0.01	<0.01

* Pneumonia (2 specimens), carcinoma (1 specimen), other lung disease (4 specimens).
‡ Pneumonia (3 specimens), carcinoma (2 specimens), other lung disease (2 specimens).

μM, range 17–94, $n = 5$). None of these cases had renal failure.

DISCUSSION

In ARDS the phospholipids recovered by lavaging the peripheral airways were frequently abnormal; namely the L/S ratio was low and phosphatidylglycerol undetectable. The disaturated lecithin was also frequently low, whereas sphingomyelin and phosphatidylserine were elevated as compared with these phospholipids recovered from healthy subjects or patients with various lung diseases without respiratory failure. These abnormalities tended to be present early in respiratory failure, regardless of the etiology, and to normalize during recovery. Furthermore, the lipid-protein complex from ARDS was not surface active, whereas in normal controls the lavage phospholipids resembled those in lung surfactant (cf. reference 31), and the lipid-protein complex was surface active. The present data are in accordance with previous evidence on analyses of lung lavage (7), or whole lung (8) at autopsy, and suggests that surfactant deficiency is important in the pathogenesis of ARDS and respiratory failure.

In 20% of the specimens from ARDS the phospholipids did not strikingly differ from those in lung diseases without respiratory failure, but always differed from healthy controls. Besides the abnormal phospholipids, the surfactant complex may be functionally inactive owing to presence of factors that inhibit the function of surfactant complex (32, 33). On the other hand, ARDS may be associated with different precipitating events (e.g., burns, vs. drowning), and therefore variations in surfactant damage; one insult may lead to deficiency in surfactant phospholipids, another to production of surfactant inhibitors. Although the present study did not reveal differences in BAL phospholipids as compared amongst the cases of respiratory failure with different precipitating events, this possibility remains to be studied further.

Surfactant phospholipids in the airways may be deficient because of a decreased rate of synthesis and secretion, or an increased rate of catabolism and/or clearance from the airways. Since the quantity of phospholipids recovered by BAL is not a reliable measure of surfactant, using the present technique of BAL, the quality of phospholipids was analyzed. Although qualitative analysis of surfactant phospholipids has proven to be a reliable method of measuring lung maturity during perinatal period, these measurements carry a disadvantage that additional phospholipids present in the airways could "dilute" the surfactant, even if surfactant secretion was not affected per se. Contaminating material also might be deleterious to surfactant function. Nonsurfactant lipids can originate from outside the lungs or from within the lung, including the type II alveolar cells.

Since increased permeability of the alveolar lining is widely recognized as an early alteration in lung damage (34, 35), it is possible that blood components, notably plasma, enter the alveolar spaces and may alter the composition of the BAL return. Comparison of

plasma and BAL lipids revealed that although increased plasma lipid contamination in ARDS is possible, it cannot explain the dramatic alteration in BAL lipids, particularly since BAL contained disaturated lecithin (though less than that from normal controls), phosphatidylserine and glycolipid, while plasma had only traces of these lipids. It is likely that the disaturated lecithin originates from the lung. The question of whether BAL phcspholipids in ARDS represented mainly the secretory activity of type II cells, or whether they included the secretion of other airway lining cells, cellular debris, or other contaminants remains unknown. In ARDS neither the total cell counts nor the differential cell counts in BAL were significantly different from the other groups studied (data not shown).

Although the increase in contaminating lipids in peripheral airways, and increased catabolism/clearance of surfactant phospholipids may contribute to the alteration in BAL lipids, the possibility that synthesis and secretion of surfactant is deficient in ARDS appears likely. Recent evidence indicates that low myoinositol in type II cells explains the uniquely high phosphatidylglycerol content of surfactant. On the other hand, myoinositol in plasma and in type II cells can be increased by excessive dietary intake of this sugar alcohol. High myoinositol consumes cytidine diphosphate-diacylglycerol, required for both phosphatidylglycerol and phosphatidylinositol, and the latter is formed instead of phosphatidylglycerol (36). In immature fetus and in RDS of the newborn, high extracellular (and apparently also type II cell) myoinositol seems to explain the absence of surfactant phosphatidylglycerol and prominent phosphatidylinositol (37). It has additionally been suggested that absence of phosphatidylglycerol alters the surfactant function (19).

Only in two cases of respiratory failure plasma myoinositol was excessively high (apparently caused by uremia, [38]), phosphatidylglycerol absent, and phosphatidylinositol prominent. It is possible that in these cases myoinositol prevented phosphatidylglycerol synthesis, and therefore modified surfactant function. However, it is not known whether this was responsible for the respiratory failure, since the patients also had renal and cardiac failure. In the rest of the respiratory failure cases studied, both BAL phosphatidylinositol and plasma myoinositol were low (similar to healthy controls), and there was no evidence of increased breakdown of phosphatidylinositol. Therefore, the absence of phosphatidylglycerol likely was not due to high myoinositol (but to absence of surfactant), and although the phospholipids in lung effluent were similar in RDS and in ARDS, the pathobiochemistry seems different. RDS is basically a self-limiting, developmental disease, associated with high myoinositol and immature alveolar lining (37). Characteristically, surfactant secretion surges within a few days of birth (15). However, in ARDS, surfactant abnormality that is associated with low serum myoinositol may last for several weeks (Fig. 2). Interestingly, alveolar damage induced by *N*-nitroso-*N*-methylurethane in adult animals is associated with surfactant abnormality that resembles the alteration of BAL phospholipids in ARDS. According to recent evidence, administration of excess myoinositol decreases *N*-nitroso-*N*-methylurethane-induced surfactant abnormality (except the absence of phosphatidylglycerol) (39), and largely prevents the decrease in the number of type II alveolar cells (unpublished results).

The present findings are in agreement with the hypothesis that surfactant is deficient in respiratory failure, and that the abnormal lipid lining of the alveoli increases retractile forces, tends towards fluid accumulation, and decreases the compliance of the lung. Further investigation is required to elucidate the mechanism leading to surfactant deficiency, its prevention, and acceleration of recovery with drugs, diet, or by surfactant substitution.

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