

Ultrasonic and Jet Aerosolization of Phospholipids and the Effects on Surface Activity

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Summary

Surface active aerosols were produced from aqueous dispersions of mixed lipids (CLL), extracted from bovine lung lavage. Particle size distributions were measured as a function of humidity for two types of aerosol generators: ultrasonic and jet. Lipid dispersions before aerosolization were prepared by sonication in an ice bath and by mechanical vortexing. Over a range of high humidity greater than 60–70%, ultrasonic nebulization gave CLL aerosols with mass median aerodynamic diameters (MMAD) of $1.4 \pm 0.1 \mu\text{m}$, compatible with predicted alveolar deposition fractions of 0.2–0.3 according to current deposition models. For humidities of 30–95%, jet nebulization gave MMAD values of 0.4–0.5 μm , which have lower predicted alveolar deposition. The surface pressure-time ($\pi - t$) adsorption characteristics at $35 \pm 2^\circ\text{C}$ of CLL dispersions prepared initially by vortexing or sonication were not significantly affected by ultrasonic nebulization over a 1–2 h time period. In addition, the dynamic surface tension lowering of both kinds of CLL dispersion was not affected by ultrasonic nebulization (minimum surface tension $<1 \text{ dyne/cm}$ at 37°C and 100% humidity). Current interest in the treatment of the respiratory distress syndrome (RDS) with exogenous surfactant replacement has focused largely on the delivery of surfactants to infants by tracheal instillation at birth. However, the ability to form multicomponent surfactant aerosols with appreciable alveolar deposition fractions and high surface activity may help to expand the utility of replacement therapy to patients with aerated lungs.

Abbreviations

CLL, calf lung lipids
DPPC, dipalmitoyl phosphatidylcholine
DPPG, dipalmitoyl phosphatidylglycerol
egg-PG, egg phosphatidylglycerol
LPC, lyso-phosphatidylcholine
MMAD, mass median aerodynamic diameter
 π , surface pressure
 $\pi - t$, surface pressure-time
PC, phosphatidylcholine
RDS, respiratory distress syndrome
S_i, sonication in an ice bath.
TLC, thin layer chromatography
V, mechanical vortexing at room temperature

Much of the present interest in exogenous surfactant replacement therapy for neonatal RDS is centered on the delivery of surfactant mixtures to the respiratory system by direct airway instillation (18, 22, 23). In animal model experiments for such therapy, the most effective time for surfactant instillation has been found to be before, or at least very close to, the onset of breathing (22). Current research (e.g., 10) also indicates that a single pre-ventilatory dose of exogenous surfactant material can have an initial beneficial effect on lung function, which then decreases with time after instillation. The possibility thus exists that repeti-

tive dose therapy might be beneficial in the treatment of RDS, and this in turn raises the question of what method might be most appropriate for the delivery of surfactants to aerated lungs.

Although tracheal instillation may be a preferred method for lungs in the initial stage of ventilation, a useful technique for surfactant delivery to aerated alveoli is by surface active aerosols. Studies by several groups of investigators, Brain *et al.* (2) and Watson *et al.* (26), have indicated that aerosolization gives a more uniform distribution of alveolar deposition than that found with intratracheal instillation of various kinds of particles.

The use of surfactant aerosols in RDS therapy has received attention in the past. As reviewed elsewhere (18, 19), aerosols of pure DPPC have been used in several studies on lung surfactant replacement over a decade ago. More recently, Ivey *et al.* (9) reported a limited clinical study with an aerosol of 9:1 DPPC:DPPG. But it is complex multicomponent mixtures of naturally derived lung surfactant material that have thus far proved most useful for RDS therapy in animal model instillation studies (19, 22, 23), and aerosols of such mixtures have received little investigation. Ikegami *et al.* (8) studied the effects of natural lung surfactant aerosols on excised lung pressure-volume characteristics. However, detailed descriptions of the aerosols in terms of particle size distribution, humidity dependence, or intrinsic surface activity at physiologic conditions were not reported.

The present study is concerned with the aerosolization of dispersions of mixed lipids (CLL) extracted from bovine lung lavage. The CLL preparation studied has been shown to be effective in improving pulmonary function and gas exchange in premature lambs (3, 4, 17). The primary focus of the work is to characterize multicomponent phospholipid aerosols in terms of surface activity and particle size distribution for nebulization by both ultrasonic and jet type aerosol generators. Ultrasonic nebulization is the technique that has generally been used in previous clinical studies with DPPC aerosols. A potential disadvantage of the ultrasonic nebulizer is that it subjects the solution to be aerosolized to significant vibrational energy. Phospholipid systems in aqueous dispersion can be sensitive to such effects (19), and it is possible that an ultrasonic nebulizer might alter the surface properties or biochemical composition of therapeutic surfactant mixtures. In addition to surface property effects, another variable studied is the humidity dependence of the particle size distribution of CLL aerosols by ultrasonic and jet nebulization. Humidity dependence is characterized because of its importance for particles entering the humid airways and its effect on alveolar deposition.

MATERIALS AND METHODS

Lipids. CLL were obtained by chloroform-methanol extraction of 0.15 M NaCl bovine lung lavage as described previously (4, 12, 20). The lipids were analyzed by phosphorus determination and TLC to be 91% by weight phospholipid, with 73% phosphatidylcholine, 8% phosphatidylglycerol, 6.8% phosphatidylethanolamine, 5.3% phosphatidylinositol, 3.8% sphingomyelin and 3.1% phosphatidylserine. The CLL contained 6% cholesterol and cholesterol esters, and of order 1 weight % protein.

The composition of CLL was re-analyzed by TLC after 1-h and 2-h exposures to ultrasonic nebulization to rule out the possibility of significant lipid hydrolysis with this method of aerosolization. TLC analysis with the solvent system of Touchstone *et al.* (25), and with the system chloroform, methanol, 7 M ammonium hydroxide 60:35:5, showed no significant compositional changes in CLL after ultrasonic nebulization. Furthermore, to determine specific conversion of PC to LPC, [^{14}C] dipalmitate-labeled DPPC was mixed with CLL and nebulized for 2 h in the closed chamber of the ultrasonic nebulizer. Conversion of PC to LPC was $\approx 2\%$ after 2 h of continuous ultrasonic nebulization and reflux.

Aerosol generators. Although there are a number of different aerosol generator types, two which have been applied widely for clinical use because of their reliability and simplicity are ultrasonic and jet type nebulizers (13).

Ultrasonic nebulizer. These experiments used a Heyer Ultrasonic Nebulizer (USE 77) with an operating frequency of 2.5–2.8 megaHertz. An insert (medication cup) was used to minimize the volume of the solution to be nebulized. This cup was partially submerged in a liquid bath in the aerosol generator. In an ultrasonic nebulizer, vibrations are generated by a piezoelectric crystal at the convex base of the bath, and are transmitted through the bath to the solution. The ultrasonic vibrations cause the formation of capillary waves at the solution surface and these give rise to aerosol droplets. The capillary wavelength (λ) between crests for an ultrasonic nebulizer is given by (11):

$$\lambda = (8\pi\gamma/\rho f^2)^{1/3} \quad (1)$$

where: γ , surface tension; ρ , density of the solution; and f , vibrational frequency. The capillary wavelength (λ) in Equation (1) has been shown to have a strong association with the droplet size leaving the surface of the solution being nebulized (11). If surface tension approaches zero, Equation (1) predicts that capillary waves will not form. Although γ does approach zero for lung surfactant films, this is only true under dynamic compression. In the absence of dynamic compression to monolayer collapse, γ is non-zero for lung surfactant component films, and ultrasonic nebulization of CLL dispersions occurs readily as shown in "Results." For the ultrasonic nebulizer, the humidity of the aerosol leaving the generator is initially high, generally $>80\%$.

Jet nebulizer. Jet experiments were done with a Dautrebande D-30 nebulizer. In a jet-type nebulizer, gas passes through a nozzle at high velocity. This causes a low pressure that entrains liquid droplets from the solution in the nebulizer reservoir. Many of these recruited (or primary) droplets are recycled back into the reservoir solution as they impact on structures within the nebulizer. For the D-30 jet nebulizer used in this study, a network of small capillary holes ($D = 1\text{--}2\text{mm}$) was present to cause the impaction and reflux of large aerosol droplets. Because of the complex geometry and flow patterns that affect particle size distributions from jet nebulizers, no generally applicable correlation is available for *a priori* prediction of aerosol size distribution (13). For jet type nebulizers, the humidity of the aerosol initially generated is low, of order 40–45%.

Particle size distributions. The continuous flow system used to measure aerosol particle size distribution as a function of humidity is described in detail by Marks *et al.* (12). In brief, compressed filtered air was delivered to a given nebulizer to generate an aerosol stream. This aerosol was then mixed with a diluting air stream of variable humidity. The humidity of the final aerosol stream was a function of the flow rates and humidities of the two air streams. The final aerosol stream was allowed to equilibrate at a given humidity by passage through a tubing system, which allowed aerosol residence times of up to 15 sec. This is several orders of magnitude larger than the times found necessary for size equilibration of saline aerosols at a given humidity (1, 6).

After equilibration, the particle size distribution of the aerosol was measured with a seven stage cascade impactor as described by Mercer *et al.* (14). In this type of apparatus, the aerosol flows through small holes past a series of stages. The air passes around the stages but inertial effects cause the particles to impact on glass

plates that sit on each stage. The orifice size for flow between each stage of the impactor decreases continuously, and the aerosol particles impact on the stages according to their aerodynamic diameter. The critical orifice at the last stage of the impactor determines the flow rate through the system.

In the experiments here, the flow rate through the impactor was 0.92 liter/min, which was provided by a piston pump (Terracon Corp, Type R 506). The impactor was designed to measure particles with aerodynamic diameters ranging from 0.166–5.3 μm . A number of other kinds of diameter such as geometric and count median diameters may be defined for an aerosol in addition to the aerodynamic diameter (13); however, for clinical purposes, the aerodynamic diameter is probably the most useful because it relates directly to the pulmonary deposition pattern of the aerosol. For the experiments here, the aerodynamic diameter measured is the activity median aerodynamic diameter, equivalent to the MMAD.

The mass of "dry" material (NaCl and CLL) deposited on each impactor stage was measured from the activity of a [$^{99\text{m}}\text{Tc}$] label which was used to mark the aqueous phase. This label then remained with the dry particles on the impactor stages after water evaporation. Since the Tc activity of the solution in the aerosol generator was known, the mass of NaCl (or CLL) on a given stage could be found as:

Mass of NaCl on stage X

$$= \left[\frac{\text{Tc activity, stage X}}{\text{Tc activity per volume in nebulizer solution}} \right] \times \left[\frac{\text{Mass of NaCl in solution}}{\text{Volume of solution}} \right]$$

Because the CLL being nebulized was a highly multicomponent amphipathic mixture, it was necessary to verify that it was nebulized proportionally with the aqueous phase by the aerosol generator. This was studied in a double label experiment with [^{14}C] DPPC, which is the major component of CLL (and also of lung surfactant). The [^{14}C] DPPC was mixed with CLL in chloroform, where the lipids are totally soluble. The chloroform was then evaporated under nitrogen, and the [^{14}C] labeled lipids dispersed in 0.15 M NaCl by S_i or V. The aqueous dispersions were then labeled with [$^{99\text{m}}\text{Tc}$], and nebulized in the jet and ultrasonic generators. These double-label experiments reported in detail by Marks *et al.* (12) indicated that neither CLL nor NaCl were aerosolized preferentially, and that lipid aerosol particle size distributions could be accurately measured by an aqueous phase radiolabel [$^{99\text{m}}\text{Tc}$].

Adsorption. $\pi - t$ adsorption characteristics were measured at $35 \pm 2^\circ\text{C}$ as described previously by Notter *et al.* (20). For experiments involving the adsorption of lipid dispersions after exposure to ultrasonic nebulization, the procedure was as follows. Initial dispersion of 5 mg of CLL per ml of 0.15 M NaCl was accomplished by V at room temperature ($22\text{--}23^\circ\text{C}$) or by S_i . These dispersions were then placed in the Heyer Generator and the output port was capped so that the nebulization compartment was closed. Nebulization was then started; the resultant aerosol droplets coalesced and refluxed back into the bulk solution. Because of the convection currents from the vigorous agitation present in the ultrasonic nebulization process, the bulk solution in the nebulizer cup was well-mixed. After a given time of continuous nebulization, 1 ml aliquots were removed from the cup solution. In $\pi - t$ adsorption experiments, each 1-ml aliquot was injected beneath the surface of a stirred subphase of 70 ml of 0.15 M NaCl, and π was then monitored as a function of time by measurement of the force on a sandblasted platinum slide dipped in the surface (20).

Adsorption experiments of this type were done for CLL dispersions subjected to continuous aerosolization and reflux in the closed nebulizer cup for times of 5 min to 2 h. For this relatively long time scale of nebulization and reflux (min to h), it is reasonable to assume that the final nebulizer cup solution approximates a collection of aerosol droplets, *i.e.*, that the majority of the CLL

dispersion has gone through the actual nebulization process one or more times.

Dynamic surface tension lowering. The effect of nebulization on the magnitude of surface tension lowering achieved by CLL dispersions under dynamic compression was measured at 37°C and 100% humidity with an oscillating bubble apparatus described in detail by Enhorning (5). Experiments involving dynamic surface pressure were done on CLL dispersions (5 mg/ml) subjected to ultrasonic nebulization for various times as described above under adsorption studies.

RESULTS

CLL—SALINE AEROSOL PARTICLE SIZE DISTRIBUTIONS

Ultrasonic nebulization. The particle size distributions for aerosols produced by ultrasonic nebulization of lipid dispersions are given as a function of humidity in Table 1. Results are presented in terms of the MMAD and the geometric standard deviation, σ_g . A major feature of the data in Table 1 is that the MMAD of lipid aerosols produced by ultrasonic nebulization is very stable at about 1.4 μm over a range of high humidity (*i.e.*, relative humidity > 60–70%). This MMAD stability for lipid aerosols (27) is quite different from the behavior of pure saline aerosols, which show a continuous decrease in median particle size as humidity decreases from 100% (24). Possible biophysical mechanisms for CLL aerosol stability are discussed in detail by Marks *et al.* (12). For the present paper, the existence of MMAD values of 1.3–1.5 μm for relative humidity > 60–70% is most important in terms of its ramifications for pulmonary deposition as discussed later.

Jet nebulization. Particle size distribution results for CLL nebulized by the jet-generator are given in Table 2 for the cases of initial dispersion by S_i and V. The results show that jet nebulized CLL particles are essentially constant in diameter as humidity is varied over the range of 30–95%. Equilibration times of 0.08–14 sec had no effect in terms of particle size measurements. The constant MMAD behavior found for CLL jet nebulization is again different from that found by jet nebulization of pure saline

Table 2. Particle size distributions of aerosols produced from CLL dispersions by jet nebulization

Relative humidity (%)	Mass median aerodynamic diameter (μm)	Geometric standard deviation σ_g	CLL initially dispersed by
≥95	0.43 ¹ ($n = 3$)	1.5 ¹	S_i
90–91	0.46 (2)	1.4	S_i
78–80	0.48 (2)	1.5	S_i
70–74	0.43 (4)	1.5	S_i
60–66	0.45 (3)	1.6	S_i
52	0.42 (1)	1.7	S_i
36–38	0.44 (2)	1.8	S_i
30	0.40 (1)	1.7	S_i
≥95	0.41 (2)	1.5	V
60	0.45 (4)	1.5	V
30–35	0.39 (2)	1.5	V

¹ Values for MMAD and σ_g are averages of n experiments (in parentheses) within the stated humidity range.

Table 3. Dynamic surface tension lowering of lipids exposed to ultrasonic nebulization

Time in nebulizer (min)	Minimum surface tension ¹ (dyne/cm)	Maximum surface tension (dyne/cm)	Initial dispersion method
0	<1	30	V
5	<1	34	V
10	<1	33	V
30	<1	34	V
60	<1	34	V
0	<1	33	S_i
5	<1	34	S_i
10	<1	33	S_i
30	<1	32	S_i
60	<1	34	S_i

¹ Dynamic surface tension lowering measured at 37°C and 100% humidity with oscillating bubble. Cycle rate was 20 cycles/min. Measurements made on 20 μL aliquots (5 mg CLL/ml) subjected to ultrasonic nebulization in Heyer generator for various times. See text for details.

solutions, which typically show a rapid rise in particle size when humidity reaches a value called the deliquescent humidity (21). Possible mechanistic explanations for the lack of deliquescence found for CLL aerosols from the jet nebulizer are discussed by Marks *et al.* (12).

Effects of nebulization on surface activity. The question of whether ultrasonic nebulization changes the surface properties of CLL mixtures was addressed by looking at the dynamic surface tension lowering and adsorption characteristics of CLL dispersions after they had been subjected to ultrasonic nebulization for various periods of time (see "Materials and Methods").

The dynamic surface tension lowering characteristics of CLL dispersions were measured at 37°C and 100% humidity in an oscillating bubble apparatus described by Enhorning (5). The results presented in Table 3 are expressed as minimum and maximum surface tensions of 20- μL aliquots of CLL dispersions (initially dispersed by V or S_i) compressed and expanded between 100% area and 50% area at a rate of 20 cycles/min in the oscillating bubble. Initial dispersion concentration was 5 mg CLL/ml. The time values in Table 3 represent the length of time the CLL dispersions were subjected to continuous ultrasonic nebulization (and reflux) in the cup of the Heyer generator when an aliquot was taken for surface tension measurement. As shown in Table 3, ultrasonic nebulization for times as long as 1 h did not change the minimum surface tension values achieved upon dynamic compression of CLL dispersions.

Table 1. Particle size distributions of aerosols produced from CLL dispersions by ultrasonic nebulization

Relative humidity (%)	Mass median aerodynamic diameter (μm)	Geometric standard deviation ¹ σ_g	CLL initially dispersed by
≥99	1.48 ² ($n = 2$)	2.0	S_i
95	1.33 (2)	1.8	S_i
87–90	1.33 (2)	1.8	S_i
83	1.45 (1)	1.9	S_i
77–80	1.36 (3)	1.8	S_i
71–75	1.48 (2)	2.0	S_i
60–63	1.32 (3)	2.0	S_i
55–56	0.89 (2)	1.9	S_i
45	0.54 (1)	1.7	S_i
38	0.38 (1)	1.8	S_i
95	1.38 ($n = 2$)	1.8	V
90–91	1.34 (2)	1.8	V
80–86	1.39 (4)	1.8	V
73	1.44 (2)	1.7	V
68	0.70 (1)	1.7	V
59–62	0.72 (3)	1.9	V
25–31	0.49 (4)	1.7	V

¹ For a log-normal aerosol size distribution, D_{50} and D_{84} can be defined as the diameter at which 50% and 84%, respectively, of the total mass of aerosol particles can be accounted for by particles of diameter $D < D_{50}$ or $D < D_{84}$. The geometric standard deviation σ_g is defined as D_{84}/D_{50} .

² Values expressed are averages of n experiments (in parentheses) over the humidity range given. Because of mechanical factors it was not possible to duplicate a given humidity exactly in repetitive experiments.

The $\pi - t$ adsorption behavior of vortexed CLL dispersions subjected to ultrasonic nebulization for various times is shown in Figure 1. The results indicate that the adsorption of vortexed CLL dispersions is not significantly affected by ultrasonic nebulization for times as long as 2 h. This is true in terms of both the surface pressure magnitude achieved and its time dependence. The lack of effect of ultrasonic nebulization on the adsorption of vortexed CLL dispersions is critical because such dispersions have been shown to improve lung function in premature lambs (3, 4, 17), as noted earlier.

The effect of ultrasonic nebulization on the adsorption characteristics of sonicated CLL dispersions was also studied. The $\pi - t$ behavior of CLL (S_i) dispersions in Figure 2 shows that the mixture adsorption properties are not significantly affected by ultrasonic nebulization for times as long as 1 h. In all cases in Figure 2, the $\pi - t$ adsorption of CLL (S_i) dispersions to surface pressure values of 45 dynes/cm is rapid, and occurs over a shorter time-scale than that found for vortexed CLL dispersions at the dilute lipid concentration used (0.07 mg/ml). The $\pi - t$ behavior in Figure 2A before nebulization is the same as that found previously by Notter *et al.* (20) for sonicated dispersions of another CLL preparation. The results in Figures 2B and 2C show that this behavior persists after ultrasonic nebulization (28).

DISCUSSION

The finding that ultrasonic nebulization does not adversely affect the surface tension lowering behavior of CLL dispersions is

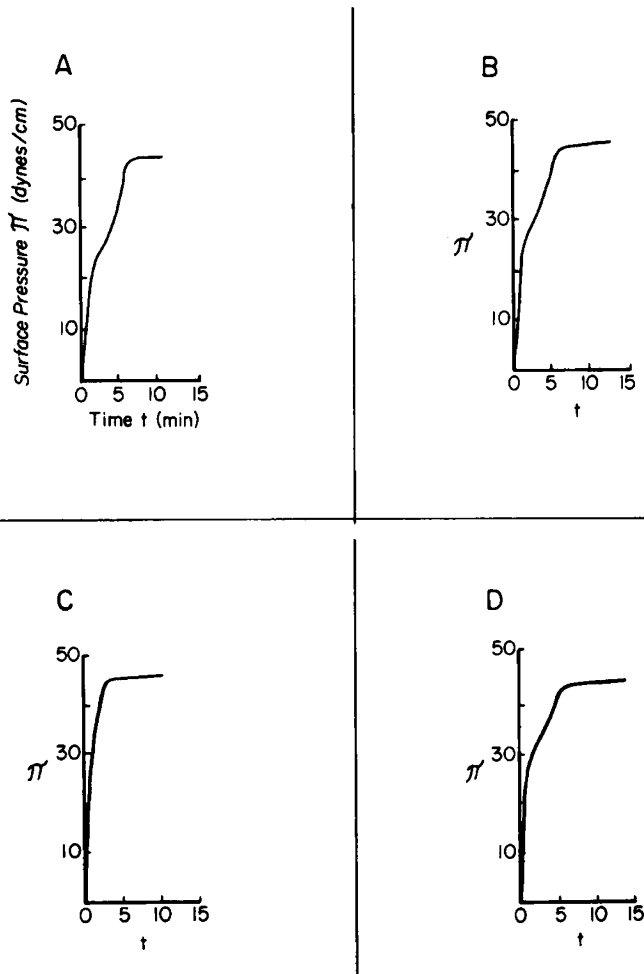


Fig. 1. Surface pressure-time ($\pi - t$) adsorption of vortexed lipid dispersions subjected to ultrasonic nebulization. See "Materials and Methods" for details. (A) CLL (V), initial; (B) CLL (V) 30 min in nebulizer; (C) CLL (V), 60 min in nebulizer; and (D) CLL (V), 120 min in nebulizer. See text for details.

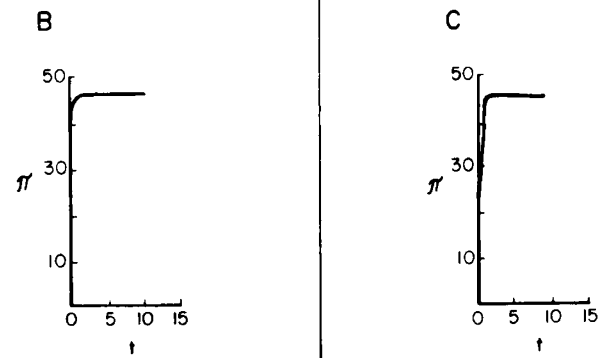
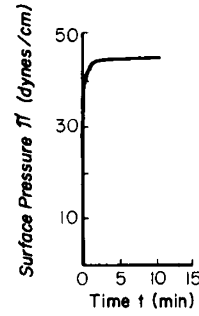


Fig. 2. Adsorption of sonicated lipid dispersions subjected to ultrasonic nebulization. See "Materials and Methods" for details. (A) CLL (S_i), initial; (B) CLL (S_i), 10 min in nebulizer; and (C) CLL (S_i), 60 min in nebulizer.

consistent with previous work (20), which showed that the adsorption of this kind of naturally derived lipid mixture is less sensitive to heating effects than phospholipid mixtures from non-pulmonary sources (e.g., DPPC-egg PG). The results of Figures 1 and 2 show that the adsorption of CLL dispersions is also relatively unaffected by exposure to a high ultrasonic energy input in the Heyer nebulizer. In addition, the data in Table 3 show that CLL dispersions are also not significantly affected in terms of dynamic surface tension lowering ability when subjected to ultrasonic nebulization.

For any surface active mixture of potential use in inhalation therapy for RDS, the pulmonary deposition characteristics of the aerosol form must be considered. The literature concerning aerosol deposition in the pulmonary system is complex, and a multitude of experimental studies and empirical correlations are available. Many of these correlations are discussed by Mercer (13), including the ICRP Task Group on Lung Dynamics model (7). According to this model, alveolar deposition fraction (including the respiratory bronchioles) peaks at about 0.3 for an aerodynamic diameter of 2 μm , and is still above 0.2 for diameters of 1 or 3 μm . Consequently, CLL aerosols produced by ultrasonic nebulization with $\text{MMAD} = 1.4 \pm 0.1$ and $\sigma_g = 1.7-1.9$ should yield alveolar depositions of more than 20% at physiologic humidity. Because of the stability of aerosol size distribution for relative humidity >70% precise control of humidity would not be required in lipid aerosol delivery systems used in a clinical setting. Humidity must be maintained at values above about 60%, however, or the aerosol droplets will evaporate to dry particles which will not easily grow back to droplets (Table 1).

The consumption of solution with the Heyer ultrasonic nebu-

lizer was found to be in the range 0.05–0.3 ml/min for air flow rates of 0.3–7.5 liter/min. These values are compatible with delivery of sufficient surface active material to cover the alveolar interface with a tight packed monolayer in a time period practical in an intensive care nursery. For example, if a value of 8 ml/kg/breath is assumed for an infant (16), a typical minute volume at 30–50 breaths/min is just below the lower range of air flow rates studied. For a solution consumption of 0.05 ml/min, a CLL solution concentration of 10 mg/ml, and an alveolar deposition fraction of 0.2, this gives 0.1 mg CLL delivered to alveoli per min. If a gas exchange surface area of 1 m²/kg is assumed, it takes 3.1 mg/kg to cover the alveolar surface with a close packed monolayer of CLL (molecular weight assumed as 750) occupying 40 Å²/molecule. This dose could be delivered to a 1-kg infant in approximately 30 min (29).

In terms of the evaluation of surface active replacement mixtures for possible use in RDS therapy, research in the past decade has dealt most thoroughly with pulmonary delivery by direct tracheal instillation (22, 23). A wide variety of animal model studies have demonstrated improved lung function following the instillation of multicomponent surfactant mixtures at or before the first breath, and this is clearly the indicated therapeutic approach when feasible. However, it is also true that pre-ventilatory tracheal instillation will not be possible for all patients suffering from a primary or secondary surfactant deficiency. The relative advantages and disadvantages of multicomponent phospholipid aerosols compared to tracheal instillation therapy for such patients have not yet received a sufficiently comprehensive evaluation in terms of animal models studied, or mixtures and aerosol generation techniques used. Previous aerosol work has concentrated largely on nebulized DPPC, and the few studies involving multicomponent phospholipid mixtures (8, 9) have not included detailed characterization of intrinsic aerosol surface activity at physiologic conditions. The present results for ultrasonically nebulized CLL suggest that it is one candidate (of perhaps many) which might profitably be studied further in animal models of RDS.

The MMAD values of 0.4–0.5 μm found for jet produced CLL aerosols in Table 2 are not as optimal in terms of alveolar deposition as the larger values found in ultrasonic nebulization. The ICRP model (7, 13) does predict significant alveolar deposition fractions for 0.5 μm particles, but the magnitude is not as large as the fraction predicted for 1.5 μm particles. In addition, a jet-type nebulizer has several potential disadvantages compared to an ultrasonic nebulizer for delivery of surface active aerosols to infants. The jet nebulizer uses a relatively large amount of solution (about 0.2 ml/min) because of the large continuous air flow rate needed for jet nebulization. By contrast, an ultrasonic nebulizer can easily be used as a demand system: aerosol can be drawn off from the generator chamber only as required by any given ventilator setting.

The jet nebulizer particle size results in Table 2 are perhaps most important because of the hysteresis they show in the humidity dependence of the phospholipid aerosols. As discussed in detail by Marks *et al.* (12), the size distribution data in Tables 1 and 2 can be combined in a single plot of MMAD vs relative humidity. One feature resulting from such analysis is that CLL aerosols exposed to low humidity (<50%) will evaporate from droplets to dry particles, as noted above. If these particles are subsequently exposed to high humidity, they do not readily grow back to droplets (Table 2). The ability to form stable aerosols of phospholipid particles (as opposed to droplets) may prove useful in surfactant replacement applications involving mixtures such as 7.3 DPPC:PG, which has been used in "dry" powder form by Morley *et al.* (15).

In summary, these experiments have shown that it is feasible to form multicomponent aerosols of surface active phospholipids by ultrasonic and jet nebulization. In terms of surface property effects, it was found that the process of ultrasonic nebulization did not change the dynamic surface tension lowering effectiveness of CLL dispersions at physiologic temperature and humidity. Moreover, the character of the $\pi - t$ adsorption isotherm for CLL dispersions

prepared by mechanical vortexing or sonication was not significantly changed by ultrasonic nebulization for times as long as two hours. The particle size distributions found for both aerosol generation techniques at high humidity (relative humidity >60–70%) are compatible with some alveolar deposition. However, the alveolar deposition fraction of 0.2–0.3 predicted for CLL aerosols of MMAD 1.4 ± 0.1 μm produced from ultrasonic nebulization is greater than that predicted for the smaller particles of MMAD = 0.4–0.5 μm produced from jet nebulization. It would appear possible to produce surface active aerosols of multicomponent lipid mixtures that have potential for practical applications to neonatal RDS therapy in aerated lungs.

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- Because of the stability found for CLL containing aerosols above RH = 60–70 percent, the residence time in the aerosol flow system was varied from 0.5 seconds to 15 seconds to check for possible non-equilibrium effects. Over this residence time range, no change in particle size distribution was observed.
- There is a slight slowing in adsorption rate in Figure 2C (after 60 minutes in nebulizer) compared to Figure 2A (initial) and 2B (after 10 minutes in

nebulizer). However, the adsorption rate in Figure 2C is still more rapid than that of vortexed CLL dispersions in Figure 1 for the uniform final dispersion concentration (5 mg CLL/71 ml subphase) used (see "Materials and Methods"). This dilute concentration was chosen because it allows a discrimination between the adsorption isotherms of CLL (S_i) and CLL (V). At higher concentrations (≥ 0.25 mg/ml) vortexed CLL dispersions adsorb as rapidly as the sonicated dispersions in Figure 2.

29. If the assumption is made that all of the aerosolized solution (a total of 1.5 ml in 30 minutes containing 0.15 M NaCl) is retained by the infant, this would result in a sodium load of about 0.25 mEq. Note that the surfactant dose calculations here are for material which is actually deposited at the alveolar level (20% of inhaled amount). In animal studies of surfactant replacement (22, 23), typical tracheal instillation doses are 5–10 times larger than in this example calculation. However, a significant percentage of instilled surfactant probably does not

reach the alveolar interface.

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***Pseudomonas*-Infected Cystic Fibrosis Patient Sputum Inhibits the Bactericidal Activity of Normal Human Serum**

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Summary

Sputum samples were obtained from nineteen cystic fibrosis (CF) patients with respiratory tract infections due to *Pseudomonas aeruginosa* and were examined for the presence of a specific local protective or blocking factor, which might partially explain the inability of CF patients' pulmonary defense mechanisms to clear their lungs of *Pseudomonas* infection. Using an assay that measures the bactericidal activity of fresh normal human serum (FHS), eighteen of nineteen CF sputa examined were capable of protecting either autologous or heterologous strains of *P. aeruginosa* from serum bactericidal activity. Most of this protective activity was absorbable at 4°C by *Pseudomonas*, and could be reduced by dilution in phosphate buffered saline or counteracted by an increase in serum concentration. This protective activity is believed to be due to a *Pseudomonas*-specific bactericidal blocking antibody. In contrast, only two of 17 non-CF sputa were found to significantly inhibit the bactericidal activity of FHS for *P. aeruginosa*. Furthermore, three out of five CF sputa also protected *Escherichia coli* from the bactericidal activity of FHS. This protective activity was much less than that observed with *P. aeruginosa*, and was not absorbable at 4°C, suggesting the presence of a second blocking or protective factor in CF sputum. From these observations, we conclude that sputum from CF patients infected with *P. aeruginosa* contains one or more factors that interfere with the bactericidal activity present in FHS.

Abbreviations

BHI, brain heart infusion
CF, cystic fibrosis
CFU, colony forming unit
FHS, fresh normal human serum
PBS, phosphate buffered saline
PIA, pseudomonas isolation agar
slgA, secretory IgA

Chronic obstructive pulmonary infection is the major cause of morbidity and mortality in people with cystic fibrosis (CF). Although respiratory infection with *Staphylococcus aureus* often occurs, *Pseudomonas aeruginosa* (particularly the mucoid colonial form) eventually emerges as the dominant pathogen in 70–90% of CF patients, and > 90% die of progressive pulmonary insufficiency complicated by infection with *P. aeruginosa* (4).

Except for respiratory tract infections, CF children are not more susceptible to infection than normal children of comparable age. *P. aeruginosa* is known to be an opportunistic pathogen, which commonly infects the debilitated patient (25, 26), and is seldom isolated from pediatric patients with chronic respiratory disease other than CF (4, 11). These basic clinical observations, coupled with the fact that pulmonary infections rarely spread outside the respiratory tract in CF patients, strongly suggest a defect in local pulmonary defense mechanisms.

Despite the presence of elevated levels of *Pseudomonas* antibodies in CF patients' sera and pulmonary secretions (13, 15), these bacteria are not cleared by pulmonary defense mechanisms. Alveolar macrophages isolated from CF patients appear normal (23), but CF serum appears to interfere with the ability of rabbit and human alveolar macrophages to ingest and destroy *Pseudomonas* (2, 3, 5, 21, 23). This effect has been attributed to either a serum deficiency (2), or a serum inhibitory factor (3, 21, 23). Fick *et al.* (5) have recently demonstrated that IgG antibodies from CF patients are impaired in their attachment to the Fc receptors of human alveolar macrophages.

In addition to interfering with phagocytosis, CF serum has also been found to block serum bactericidal activity for *P. aeruginosa*. Whereas *P. aeruginosa* strains isolated from CF patients were more sensitive to the bactericidal activity of normal human serum than strains isolated from non-CF patients (12, and Schiller, N.L., manuscript in preparation), sera from CF patients chronically infected with *P. aeruginosa* showed a selective inability in bactericidal activity against the patient's own *P. aeruginosa* isolate,