Effect of recombinant SP-C surfactant in a porcine lavage model of acute lung injury

R. G. SPARRG, R. M. SMITH, K. HARRIS, J. LEWIS, D. HÄFNER, AND P. GERMANN

The Lung Surfactant System is biochemically and functionally abnormal in patients with the acute respiratory distress syndrome (ARDS) (9, 13, 22, 23). The responsible mechanisms are multiple and include abnormalities of surfactant lipid and protein production by alveolar type II cells, inhibition of surfactant function by proteins that gain access to the alveolar space of the inflamed lung, increased conversion of surfactant to inactive forms, and oxidation and nitration of surfactant phospholipids (PL) (24).

Several observations suggest that administration of exogenous surfactant may benefit patients with ARDS. First, neonates with functional deficiency of surfactant are benefited by such administration. This benefit is even experienced by neonates with established respiratory distress syndrome who exhibit evidence of coincident acute lung inflammation (15, 20). Second, exogenous surfactant provides short-term improvement in gas exchange in animal models of acute lung injury (19). Finally, clinical trials of surfactant administration to patients with ARDS suggest benefit. A recent phase II trial provided evidence that Survanta, a natural product derived from bovine lungs and containing PL, hydrophobic apoproteins, and palmitic acid, might result in improvement in both gas exchange and survival for patients with ARDS (10). Uncontrolled trials of other natural surfactants have supported this suggestion (17, 25, 27, 29). In contrast, a phase III trial of a protein-free mixture of PL, tyloxapol, and hexadecanol (Exosurf) failed to show efficacy (2). However, this study was flawed by inadequate delivery of surfactant.

As the ability to produce synthetic surfactants of defined composition is refined, it has become possible to discover which surfactant components are critical for maintenance or restoration of physiological effect. This report describes experiments testing the hypothesis that a surfactant containing recombinant surfactant apoprotein C (rSP-C) and PL, when administered in the appropriate dose and volume to pigs with acute lung injury induced by massive lavage, will result in improved gas exchange.

MATERIALS AND METHODS

Surfactant. Experiments were performed by using a rSP-C-based surfactant prepared by Byk Gulden (Konstanz, Germany). This material contains (wt/wt) 1.8% rSP-C, 63.4% 2-dipalmitoyl-sn-s-phosphatidylcholine (DPPC), 27.8% 1-palmitoyl-2-oleoyl-3-sn-phosphatidylglycerol, 4.5% palmitic acid, and 2.5% CaCl₂ and is available as a dry powder that may be resuspended in 0.9% NaCl in concentrations between 25 and 100 mg PL/ml. The surfactant is resuspended by shaking the vial containing powder and liquid by hand for 2 min; after standing for several minutes, it has the consistency of skim milk. The rSP-C in this product is based on the sequence of human surfactant apoprotein C (8, 28). Amino acid substitutions are introduced into the human sequence in two sites (C₁₈₅ to F₁₈₅ and M₁₂₅ to I₁₂₅) to prevent uncontrolled formation of intra- and intermolecular disulfide bonds and uncontrolled oxidation, respectively. Thus rSP-C lacks the palmitoylation of C₁₈₅ found in hSP-C, a modification that has not been shown to affect function (14).

Radiolabeled surfactant used to measure distribution in pulmonary tissue was prepared by first combining 9 mg DPPC and 10 µCi [³H]DPPC (89 Ci/mmol; New England Nuclear, Boston, MA) in 400 µl chloroform and then drying
the lipids under N₂. The mixture was resuspended in 0.9% NaCl by vigorous mixing and sonication. Ten microcuries of the lipid suspension were mixed thoroughly with an appropriate unlabeled surfactant dose just before administration.

Surfactant function was assessed by using a pulsating bubble surfactometer (6). Surfactant was suspended in 150 mM NaCl-5 mM CaCl₂, and determinations were made at 37°C, 20 pulsations/min, for 10 min. Use of plastic chambers that have been flushed for 30 min with flowing distilled water is critical for these determinations.

Control experiments were performed by using either no agent or a dried lipid preparation prepared by Byk-Gulden containing all components of rSP-C surfactant except rSP-C protein.

Animal preparation and injury induction. Pigs [20.2 ± 1.9 (SD) kg] were induced with ketamine (50 mg/kg im) and anesthetized with thiopental sodium (12.5 mg/kg iv). After a tracheostomy was performed and an 8-Fr endotracheal tube was tied in place, anesthesia was maintained with pentobarbital (300 mg/kg sc) and morphine sulfate (1.5 mg/kg sc). Additional pentobarbital and morphine sulfate were administered as necessary to maintain anesthesia. Catheters were placed in a jugular vein, a femoral artery, and the urinary bladder. The animal was ventilated with 100% oxygen by using a tidal volume (VT) of 15 ml/kg, 12 breaths/min, and a positive end-expiratory pressure (PEEP) of 5 cmH₂O. Blood hemoglobin oxygen saturation was monitored continuously by using a pulse oximeter. The ventilatory rate was adjusted to achieve an arterial PCO₂ (PA CO₂) of 40 ± 6 Torr, and baseline measurements of arterial blood gases and ventilatory parameters were taken when this value had been maintained for 20 min on unchanged ventilator settings.

Lung injury was induced by repetitive whole lung saline lavage. After the animal was disconnected from the ventilator, the table was tilted 60° with the head up, and 0.9% NaCl (37°C, 50 ml/kg) was administered via the endotracheal tube into the lung over ~10 s from a height of not more than 45 cm above the midthorax. The animal was gently jiggled for 45 s, and then the table was tilted 45° head down to allow passive drainage of the lavage fluid. Ventilation was reestablished for 1–2 min with the animal horizontal, and additional drainage was then performed for 10–20 s with the table tilted 45° head down. After the first lavage, the ventilation rate was maintained for the duration of the experiment at 1.5 times the baseline value. Lavages were performed every 10 min. Lavages 1–3 were performed with the animal supine. Thereafter, lavages were performed in the following sequence: prone, prone, supine. Arterial blood gases (always obtained with the animal in the prone position) were monitored after the third and each subsequent lavage. Time t = 0 was identified as the first time that the arterial PO₂ (Pa O₂) fell below 167 Torr and, without subsequent lavages, remained <167 Torr for 1 h. The volumes of saline administered and drained were recorded, and the difference between instilled and drained volumes was calculated.

During and after injury induction, the inspired oxygen fraction and VT were held constant; allowance was made for the compliance of the ventilator circuit. Measurements of peak pressure were made after a 0.5-s inspiratory pause. Specific protocol-driven adjustments in ventilation were employed, based on preliminary experience, to ensure survival of animals during the experiments. For PaO₂ values ≤40 Torr, the PEEP was increased in increments of 2.5 mmHg to a maximum of 10 cmH₂O. For respiratory acidosis with pH values <7.24, the ventilatory rate was increased in increments of 5 breaths/min, as tolerated, to a maximum rate of 35 breaths/min. If the pH remained <7.24, PEEP was increased in increments of 2.5 cmH₂O. NaCl (0.9%) was administered intravenously at a rate of 1 ml·kg⁻¹·h⁻¹, and a bolus of 100 ml was administered if increments in PEEP resulted in arterial hypotension.

Treatment. After injury was established, the treatment to be administered was randomly assigned. The following groups were studied: (1) control, no treatment (Con); (2) PL control, 50 mg PL/kg in a volume of 2 ml/kg (Con-50/2); (3) PL control, 100 mg PL/kg in a volume of 2 ml/kg (Con-100/2); (4) 50 mg/kg rSP-C surfactant, in a volume of 1 ml/kg (Surf-50/1); (5) 50 mg/kg rSP-C surfactant in a volume of 2 ml/kg (Surf-50/2); (6) 50 mg/kg rSP-C surfactant in a volume of 4 ml/kg (Surf-50/4); (7) 50 mg/kg rSP-C surfactant in a volume of 6 ml/kg (Surf-50/6); (8) 100 mg/kg rSP-C surfactant in a volume of 1 ml/kg (Surf-100/1); (9) 100 mg/kg rSP-C surfactant in a volume of 2 ml/kg (Surf-100/2); and (10) 100 mg/kg rSP-C surfactant in a volume of 4 ml/kg (Surf-100/4). No significant differences existed among study groups after induction of lung injury and before treatment. Specifically, the pulmonary injury in different groups was equivalent, as measured by abnormalities in gas exchange and quasistatic compliance of the respiratory system (Crs) (Table 1). The number of lavages necessary to induce injury in each group averaged 7.3 ± 0.9 and was not significantly different among groups. The difference between instilled and drained saline volumes averaged 27 ± 1.2 ml/kg and was not significantly different among groups. The volume of saline administered intravenously did not differ among groups.

During treatment, the pig was first placed in the right lateral decubitus position with the head elevated 15°, and a catheter was advanced through an adapter on the Y endotracheal tube connector to a point ~2 cm proximal to the carina. The ventilator was paused (while maintaining PEEP), one-half of the surfactant dose to be administered was instilled, and ventilation was resumed. After 1–2 min, this procedure was repeated with the pig in the left lateral decubitus position. The total time required for treatment was ~4 min. The pig was then placed prone for the remainder of the experiment. At specified intervals, samples were obtained for measurement of arterial blood gases, and ventilatory parameters were recorded.

Tissuesampling and analysis. Four hours after treatment, the animal was killed with an overdose of barbiturate. The

Table 1. Group characteristics just before treatment (at t = 1 h)

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Con-50/2</th>
<th>Con-100/2</th>
<th>Surf-50/1</th>
<th>Surf-50/2</th>
<th>Surf-50/4</th>
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<th>Surf-100/1</th>
<th>Surf-100/2</th>
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<td>Te (h)</td>
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<td>4</td>
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<tr>
<td>PaO₂ (Torr)</td>
<td>80 ± 7</td>
<td>96 ± 11</td>
<td>73 ± 14</td>
<td>105 ± 8</td>
<td>75 ± 6</td>
<td>88 ± 7</td>
<td>72 ± 7</td>
<td>95 ± 7</td>
<td>97 ± 8</td>
<td>85 ± 11</td>
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<tr>
<td>PaCO₂ (Torr)</td>
<td>54 ± 5</td>
<td>54 ± 2</td>
<td>55 ± 2</td>
<td>51 ± 1</td>
<td>47 ± 1</td>
<td>50 ± 2</td>
<td>42 ± 3</td>
<td>52 ± 1</td>
<td>48 ± 2</td>
<td>49 ± 0</td>
</tr>
<tr>
<td>Crs (ml/cmH₂O)</td>
<td>10.4 ± 0.7</td>
<td>10.5 ± 0.1</td>
<td>10.0 ± 1.2</td>
<td>11.4 ± 0.3</td>
<td>10.8 ± 0.9</td>
<td>9.4 ± 0.7</td>
<td>9.5 ± 0.6</td>
<td>11.0 ± 0.4</td>
<td>11.1 ± 0.4</td>
<td>10.1 ± 0.2</td>
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Values are means ± SE of n pigs. Con, control; Con-50/2 and Con-100/2: phospholipid control with 50 and 100 mg phospholipid/kg in 2 ml/kg volume, respectively; Surf-50/1, -50/2, -50/4, and -50/6: 50 mg/kg surfactant in 1, 2, 4, and 6 ml/kg volume, respectively; Surf-100/1, -100/2, and -100/4: 100 mg/kg surfactant in 1, 2, and 4 ml/kg volume, respectively; PaO₂ and PaCO₂: arterial PO₂ and PCO₂, respectively; Crs, respiratory system compliance.
lungs were removed and weighed, and samples from six predetermined sites were obtained for histological examination. Histopathological procedures were as described previously (12). The slides were coded and evaluated by a pathologist blinded to the treatment history, and an overall semiquantitative score (0–4) was given to each slide for the extent and degree of hyaline membrane formation, neutrophil influx, alveolar edema, interstitial edema, alveolar hemorrhage, interstitial hemorrhage, alveolar distension, atelectasis, interstitial emphysema, mucus accumulation, and bronchiolitis. A mean grade for each variable for each animal was calculated after the slides were decoded.

Determination of distribution of instilled surfactant. Three of the six animals in each of the four groups receiving 50 mg/kg rSP-C surfactant were administered radiolabeled surfactant. At the termination of the experiment, the lung lobes were separated, weighed, and frozen. Subsequently, the portions were thawed, mixed with 4 ml water/g tissue, and homogenized by using a Polytron tissue homogenizer (Brinkman, Westhaven, NY). Samples were mixed with Protosol (DuPont NEN, Boston, MA) in a proportion of 0.8:1.0 (vol/vol) and digested for 18 h at 50°C. After cooling, the digested tissue was mixed 1.8:10 (vol/vol) with EcoScint (National Diagnostics, Atlanta, GA) and counted in a well scintillation counter. The counts per minute per gram of lung tissue for each lung lobe were then calculated.

Data analysis. Animals in the groups receiving 50 mg/kg in various dose volumes were studied as a group, with random allocation of animals to receive surfactant or control treatment (Con group). Subsequently, animals in the groups receiving 100 mg/kg and additional control animals (Con group) were studied. Therefore, results are compared within dose (50 or 100 mg/kg) groups and between dose groups and the Con group.

Quasistatic Crs was calculated by dividing the Vr by the difference between the plateau pressure and the PEEP value. To obtain information on increases in PaO2 that occurred during the entire posttreatment period, the area under the curve (AUC) was calculated for the ∆PaO2 vs. time plot. Here, \( \Delta \text{PaO}_2 = (\text{PaO}_2)_t - (\text{PaO}_2)_0 \), where \((\text{PaO}_2)_0\) is the value of PaO2 at each observations time point, \( t \), during the posttreatment period, and \((\text{PaO}_2)_t\) is the value at the time of treatment. Information about sustained effects of treatment were obtained by analysis of values at the final (\( t = 5 \) h) time point. Unless otherwise noted, data are presented as mean values ± SE. Error bars are omitted from most figures for purposes of presentation.

Statistical analysis was performed by using SigmaStat (Jandel Scientific, San Rafael, CA). Differences among groups were determined by using a one-way ANOVA or, when assumptions for parametric testing were not met, a Kruskal-Wallis one-way ANOVA on ranks. Pairwise post hoc testing was performed by using the Student-Newman-Keuls method. Differences within groups were detected by one-way repeated-measures ANOVA or, when assumptions for parametric testing were not met, the Friedman repeated-measures ANOVA on ranks. Post hoc comparison to pretreatment values was performed by using Bonferroni t-test or, for data failing nonparametric testing, the method of Dunn. Significant differences were assumed if the probability of the null hypothesis was <5%.

RESULTS

Gas exchange. The injury induced in this model produced a pulmonary shunt that resulted in a mean PaO2 value of 80 ± 7 Torr 1 h after the final lavage (\( t = 1 \) h). None of the untreated Con animals showed evidence of improvement in gas exchange over the subsequent 4 h. The highest PaO2 observed in the Con group was 113 Torr, and mean PaO2 5 h after the final lavage (\( t = 5 \) h) was 65 ± 8 Torr. In contrast, oxygenation did improve in all treated groups (Fig. 1). Values for Surf-50/1 and Surf-50/2 at \( t = 1.5 \) h and later times were significantly different both from the \( t = 1 \) h value for each group and from values for the Con group at each time point (Fig. 1A). At \( t = 5 \) h, the Surf-50/2 group value was significantly greater than the values for Surf-50/4 or Surf-50/6. Values for the Surf-50/4 and Surf-50/6 groups were significantly greater than Con values at \( t = 4 \) and 4.5 h. Values for Surf-100/1 and
Surf-100/2 at $t = 1.5$ h and later times were significantly greater than the $t = 1$ h value for each group (Fig. 1B). From $t = 1.5$ to 4.5 h, values were significantly greater than corresponding values for the Con group. From $t = 1.5$ to 2.5 h, values for these two groups were also significantly greater than those for the Surf-100/4 group. Surf-100/4 values were significantly greater than those of the Con group at $t = 1.5, 4$, and 4.5 h.

There were no significant differences among the three control groups (Con, Con-50/2, and Con-100/2) in mean values for gas exchange or quasistatic Crs at any time point. However, several animals receiving PL had transient increases in PaO2 values. Two of the four animals in the Con-50/2 group had PaO2 values of 185 and 289 Torr at 1.5 and 2.0 h after treatment, respectively. These values fell to $<167$ Torr (the prospectively defined threshold value that defined response to treatment) within 0.5 and 2.5 h, respectively. One of three animals in the Con-100/2 group had a PaO2 value of 341 Torr at 0.5 h after treatment that fell to $<167$ Torr within 2 h. Comparisons of mean values between groups treated with rSP-C surfactant and the Control group yield results comparable to comparisons made between treated groups and the aggregate of all control animals (Con, Con-50/2, and Con-100/2); the former comparisons are presented in this study.

We also compared the oxygenation response among groups by calculating the AUC response to treatment for all groups. Results are shown in Fig. 2. AUC values significantly different from those of the Con group were detected for the Surf-50/1 (50 mg PL/ml) and Surf-50/2 (25 mg PL/ml) groups and for the Surf-100/1 (100 mg PL/ml) and Surf-100/2 (50 mg PL/ml) groups.

All treated groups tended to have lower PaCO2 values than those observed in the Control group (Fig. 3); however, the variability in response precluded statistical significance at most time points. PaCO2 in the Surf-50/2 group was significantly less than the Con value at $t = 2.5$ h. PaCO2 in the Surf-100/2 group decreased significantly, relative to the pretreatment value, between $t = 1.5$ and 4 h (Fig. 3B), and was significantly less that the Con value at $t = 2.5$ h.

Ventilation. Protocol-driven changes resulted in an increase in PEEP in the Control group over the course of the experiment from 5.0 ± 0.0 to 7.5 ± 0.9 cmH2O at $t = 5$ h. In contrast, no PEEP increase was required for Surf-50/2 and Surf-100/2 animals, and lesser changes were required for other groups (Fig. 4).

Measured quasistatic Crs decreased markedly (from ~23 to 10 ml/cmH2O) with induction of pulmonary injury (Fig. 5). Subsequent changes in either Con or treated groups were small, although statistically significant. Quasistatic Crs values for the Control group and for all treated groups except the Surf-50/1 group continued to fall significantly during the 5 h after injury induction.

Pathology. The lungs of lavaged animals weighed 24.2 ± 0.6 g/kg body wt, and lung weight was not significantly different among experimental groups. We find in normal pigs ($n = 4$) of similar size that are not lavaged a lung weight of 11.3 ± 0.7 g/kg body wt. The control animals (with acute lung injury and no surfactant treatment) demonstrated moderate formation of hyaline membranes (severity grades for all controls of
Hyaline membrane formation was significantly less in treated animals (severity grade for all treated animals of 1.90, 25th–75th percentile range of 1.33–2.33; \( P < 0.05 \); Fig. 6). No influence of surfactant dose or dose volume on hyaline membrane formation was detected. All animals demonstrated moderate infiltration of polymorphonuclear neutrophils, moderate interstitial and alveolar edema, mild alveolar and interstitial hemorrhage, and moderate bronchiolitis.

Distribution of instilled surfactant. Normalized distribution within the lungs of instilled surfactant was relatively homogenous, independent of the volume instilled (Fig. 7). Counts within a specified lobe (or lobe portion) are expressed relative to the size of that lobe (as estimated by weight) and normalized to the overall ratio of counts per minute per gram for the entire lung. Thus a value of 1 represents homogeneous distribution.

In vitro surface tension measurements. The results of in vitro surface tension measurements of rSP-C surfactant dilutions ranging from 0.125 to 2 mg/ml are shown in Fig. 8. Concentrations <1 mg/ml demonstrated significant loss of surface activity.

DISCUSSION

We evaluated a surfactant containing only rSP-C, PL, and palmitic acid to determine its physiological effect in an animal model of acute lung injury. The influence of dose volume on two doses of this surfactant was determined. The lavage model that we used has been of unique value in the study of surfactant treatment of acute lung injury. It exhibits certain characteristics of ARDS, including profound hypoxemia due to pulmonary shunt, diminished pulmonary quasistatic Crs, high-permeability pulmonary edema, loss of surfactant function, hyaline membrane formation, and the presence of intense inflammation (3). The lavage model is highly reproducible, as indicated in part by the minimal variation in gas-exchange impairment and quasistatic Crs among animals (Table 1). This reproducibility is critical for studies such as this that evaluate...
the influence on gas exchange and lung mechanics of various surfactant components or of elements of complex treatment strategies; unfortunately, other models of acute lung injury do not share this reproducibility.

The most significant findings of this study are 1) an rSP-C-based surfactant was effective in improving gas exchange in the porcine lung lavage model, 2) this improvement was not accompanied by a significant improvement in quasistatic Crs, 3) the greatest improvement in gas exchange was associated with administration of relatively lower surfactant dose volumes, and 4) treatment with surfactant resulted in a decrease in hyaline membrane formation.

A variety of observations, both in vitro and in vivo, suggest that the hydrophobic surfactant apoproteins B and C enhance the surface activity of PL (4, 21, 26, 30). Recent studies in preterm rabbits and lavaged adult rats demonstrate the in vivo surface activity of PL combined with rSP-C in which cysteines at positions 4 and 5 are replaced by either serines or phenylalanines (5, 11, 14). In the present study, we have extended these observations and demonstrated the efficacy of rSP-C surfactant in a large animal model.

The improvement in oxygenation after treatment with rSP-C surfactant was rapid and significant. Improvement in blood oxygenation, as indicated by improvement in blood hemoglobin saturation, occurred within 2–3 min and was documented on the first blood-gas analysis taken after treatment. Frequently, the increase in PaO₂ seen after the single dose of surfactant peaked after treatment and then declined, suggesting the possible need for repeated doses. Also, there was substantial variability in the timing and magnitude of the response seen within each group. For example, the individual peak PaO₂ values for animals in the Surf-50/2 group were 392, 384, 401, 581, 518, and 241 Torr. These values were reached at t = 5, 2, 3.5, 2.5, 4, and 2 h, respectively. The mean value for this group at t = 5 h was 332 ± 67 Torr. The infrequent, modest, and transient increases in PaO₂ values seen in control animals that received only PL and palmitic acid (without apoprotein) underscore the importance of administering apoprotein in association with PL.

The mechanisms responsible for the improvement in oxygenation in these animals are not fully defined. In
Distribution of 4 ml/kg instilled surfactant was contrast to experience in other large animals in which appeared rather homogeneous over the range of dose (7). Surprisingly, the distribution of instilled surfactant result in more homogeneous delivery of lung surfactant in 4 ml/kg. These results were counterintuitive. We and in a dose volume of 1 or 2 ml/kg had improved blood Similarly, animals treated with 100 mg/kg surfactant the same amount of surfactant in either 4 or 6 ml/kg. Animals treated with 50 mg/kg surfactant be required.

Ventilation of the patient treated with surfactant may distending pressures, and that novel strategies for gas-exchanging areas recruited after administra-

namic compliance focuses attention on the possibility that gas-exchanging areas recruited after administration of surfactant, results are variable. Increase in blood oxygenation dissociated from a change in dynamic compliance has been noted in guinea pigs (3) and rabbits (when no volume recruitment maneuver was used; Ref. 16). On the other hand, in studies of rats (1) and sheep (18), an apparent improvement in dynamic compliance (i.e., fall in peak inspiratory pressure during constant-volume ventilation) that correlates with improvement in gas exchange after administration of surfactant was seen. Clinical observations suggest that gas exchange in patients with ARDS may improve without a concomitant increase in quasiastic Crs (10). Certainly these comparisons, as well as those in this report, may be influenced by measurements made at different points on the pressure-volume curve. Overall, however, discordance between gas exchange and dynammic compliance focuses attention on the possibility that gas-exchanging areas recruited after administration of surfactant are subjected to potentially injurious distending pressures, and that novel strategies for ventilation of the patient treated with surfactant may be required.

We did detect significant differences in gas exchange among groups treated with different dose volumes of surfactant. Animals treated with 50 mg/kg surfactant in a dose volume of either 1 or 2 ml/kg had improved blood oxygenation relative to animals receiving the same amount of surfactant in either 4 or 6 ml/kg. Similarly, animals treated with 100 mg/kg surfactant in a dose volume of 1 or 2 ml/kg had improved blood oxygenation relative to animals receiving that amount in 4 ml/kg. These results were counterintuitive. We and others had believed that larger dose volumes would result in more homogeneous delivery of lung surfactant and, therefore, in greater improvement in gas exchange. Surprisingly, the distribution of instilled surfactant appeared rather homogeneous over the range of dose volumes delivered (Fig. 7). These observations are in contrast to experience in other large animals in which the distribution of 4 ml/kg instilled surfactant was rather heterogeneous (18). However, use of different instillation techniques and different species may explain these differences.

Thus explanations other than variability in distribution of instilled surfactant are needed to explain the influence of dose volume on gas exchange. Although careful histological examination disclosed no evidence of airway obstruction, it is possible that the larger volumes of surfactant filled more of the airways than did the smaller volumes and thereby interfered with gas exchange. Similarly, although we did not detect differences in interstitial or alveolar edema among treatment groups, it is possible that impaired clearance by the damaged epithelium of water and solutes in the more dilute surfactant preparations contributed to their relative lack of effect. Also, it is possible that, as the rSP-C surfactant is diluted, its ability to withstand inhibition by proteins present in the alveolar space becomes limited. Alternatively, surface activity might be diminished in less concentrated suspensions.

To explore the last possibility, dilutions of rSP-C surfactant were tested in a bubble surfactometer. Improvement in in vitro surface activity was detectable at concentrations of \( \leq 0.5 \) mg/ml (Fig. 8). This value is significantly greater than the value of 0.01 mg PL/ml reported recently (5), a remarkably low value that was obtained by using a Wilhelmy balance. Thus dilution of administered surfactant in the edematous lung may contribute to loss of surface-tension-lowering activity.

Control animals, whether untreated or treated with PL alone, had similar degrees of hyaline membrane formation (data not shown). In contrast, animals treated with surfactant had significantly reduced hyaline membrane formation (Fig. 6). This observation suggests modification by surfactant treatment either of the flux of fibrin into alveoli or of thrombotic and/or thrombo-
lytic processes within alveoli.

In conclusion, surfactant composed of PL and rSP-C appears effective in a porcine lavage model of acute lung injury. The beneficial effect on gas exchange is not fully sustained, suggesting the possible utility of multiple doses. Surfactant delivered in two large boluses, each administered during a pause of mechanical ventila-
tion, was distributed homogeneously within the lungs. These studies provide rationale and guidance for clinical investiga-
tions using rSP-C-based surfactants and suggest that the volume in which exogenous surfactant is delivered may be of critical importance.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-23584 and a grant from Byk-Gulden, Konstanz, Germany.

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Received 23 November 1998; accepted in final form 22 October 1999.

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