Perfluorochemical rescue after surfactant treatment: effect of perflubron dose and ventilatory frequency

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Wolfson, Marla R., Nancy E. Kechner, Robert F. Roache, Jean-Pierre DeChadarevian, Helena E. Friss, S. David Rubenstein, and Thomas H. Shaffer. Perfluorochemical rescue after surfactant treatment: effect of perflubron dose and ventilatory frequency. J. Appl. Physiol. 84(2): 624–640, 1998.—To test the hypotheses that perfluorochemical (PFC) liquid rescue after natural surfactant (SF) treatment would improve pulmonary function and histology and that this profile would be influenced by PFC dose or ventilator strategy, anesthetized preterm lambs (n = 31) with respiratory distress were studied using nonpreoxygenated perflubron. All animals received SF at 1 h and were randomized at 2 h as follows and studied to 4 h postnatal age: 1) conventional mechanical gas ventilation (n = 8), 2) 30 ml/kg perflubron with gas ventilation (partial liquid ventilation [PLV]) at 60 breaths/min (n = 8), 3) 10 ml/kg perflubron with PLV at 60 breaths/min (n = 7), and 4) 10 ml/kg perflubron with PLV at 30 breaths/min (n = 8). All animals tolerated instillation without additional cardiopulmonary instability. All perflubron-rescued groups demonstrated sustained improvement in gas exchange, respiratory compliance, and reduction in pressure requirements relative to animals receiving SF alone. Improvement was directly related to perflubron dose and breathing frequency; peak inspiratory pressure required to achieve physiological gas exchange was lower in the higher-dose and -frequency groups, and mean airway pressure was lower in the lower-frequency group. Lung expansion was greater and evidence of barotrauma was less in the higher-dose and -frequency group; regional differences in expansion were not different as a function of dose but were greater in the lower-frequency group. Regional differences in lung perflubron content were reduced in the higher-dose and -frequency groups and greatest in the lower-dose and -frequency group. The results suggest that, whereas PLV of the SF-treated lung improves gas exchange and lung mechanics, the protective benefits of perflubron in the lung may depend on dose and ventilator strategy to optimize PFC distribution and minimize exposure of the alveolar-capillary membrane to a gas-liquid interface.

Lung histomorphology; pulmonary stability; ventilation strategy

LIQUID-ASSISTED VENTILATION can be defined as pulmonary gas exchange supported by tracheal instillation of perfluorochemical (PFC) liquid. The mechanisms that support pulmonary gas exchange are associated with the physicochemical properties of the PFC liquid and biophysical effects of the liquid on lung mechanics. In this regard, as a class, PFC liquids dissolve large volumes of respiratory gases and have relatively low surface tension, which supports spreading through the lung (21, 26, 35, 40). On PFC instillation, a liquid-liquid interface replaces the gas-liquid interface at the lung surface; surface tension from gas is eliminated, and interfacial tension is reduced (34). In the completely PFC liquid-filled lung, interfacial tension exists at the PFC-lung interface, whereas in the partially liquid-filled lung, interfacial tension exists at the gas-lung and PFC-lung interface. As such, the degree of reduction in collapsing tensions is dependent on the distribution of the PFC liquid and gas. In this way, lung volume is recruited, compliance is increased, and inflation pressures and pulmonary barotrauma are reduced (31, 42, 45).

In the purest form, liquid breathing involves the transport of respiratory gases in dissolved form through tidal volume (VT) exchange of PFC to and from the lung. Mechanically assisted liquid breathing, tidal liquid ventilation, has been shown to effectively support pulmonary gas exchange and improve lung mechanics in adult, neonatal, and preterm animals with respiratory distress (25, 42, 45). Rufer and Spitzer (27) were first to suggest that long-term liquid ventilation may not be necessary per se and that simply adding low-surface-tension PFC liquid to the lungs of minipigs in respiratory distress may be sufficient to improve pulmonary compliance and gas exchange. It was demonstrated subsequently that improvements in cardiopulmonary function seen during tidal liquid ventilation remained on return to gas breathing, during which time the remaining volume of PFC liquid was gradually volatilized out of the lung into the expired gas; improvement in pulmonary mechanics and gas exchange was thought to be related to the possible effects of residual low-surface-tension PFC in the lung (3, 25, 30, 31). Similar results were seen during gas ventilation after administration of low-dose PFC liquid, which reduced surface tension in the immature lamb lung (10). The method of gas ventilation in the presence of PFC liquid was described later as partial liquid ventilation (PLV) (37) or PFC-associated gas exchange (9). Mechanistically, it has been suggested that instilled or residual PFC liquid could serve to improve lung function and gas exchange through a "surfactant-like" effect and/or alveolar recruitment (32).

On the basis that animals were successfully ventilated by moving PFC fluid in the lung, recovery to mechanical gas ventilation with remaining PFC in the lung, and ultimately achieved effective ventilation by spontaneous gas breathing, the first clinical trial of PFC ventilation was performed in 1989 (11). Subsequent clinical trials in human neonates with respiratory distress syndrome were delayed until a medical-
grade PFC liquid became available, and the trials were resumed in 1993 (15). In the interim, several studies demonstrated efficacy of gas ventilation after single or multiple tracheal instillations of PFC liquid in normal, immature, or lung-injured animals (16, 24, 36–38). These studies used various ventilatory strategies to maintain gas exchange and demonstrated improvement in respiratory compliance (Crs) of the immature and injured lung. One study, performed in the adult saline-lavaged rabbit, demonstrated that the initial and maintained improvement in oxygenation was dependent on the dose of PFC liquid, whereas the reduction in ventilatory pressure was not dependent on PFC dose (38). Whereas gas exchange was also found to improve in surfactant-deficient or synthetic surfactant-treated preterm animals, PFC liquid instillation methodology varied within the studies (14, 16). In this regard, initial and subsequent PFC dosing and positive end-expiratory pressure (PEEP) were not well quantitated and were were, depending on the appearance of PFC liquid in the endotracheal tube or pressure spikes during the early phase on inspiration. Ventilation was supported by prospectively limiting breathing frequencies to ≤30 breaths/min and inspiratory times to ≤0.75 s in deference to minimizing Vr and peak inspiratory pressure (PIP). To the degree that this ventilatory scheme would tend to require higher Vr and PIP than a higher-frequency strategy, this strategy may expose the immature lung to higher peak pressure over a longer duration than would a strategy involving higher frequency, lower Vr, and shorter inspiratory time.

Uncertainty remains regarding the appropriate initial PFC liquid volumes and the need to preoxygenate the PFC fluid or to reduce ventilatory frequencies and increase inspiratory time during and after PFC instillation, beyond preinstillation settings required in the immature lung. Furthermore, the effect of this treatment on lung structure and PFC deposition has not been quantitated. In this study we hypothesized that perflubron rescue after surfactant treatment would significantly improve pulmonary mechanics, gas exchange, and lung histology and that perflubron dose or breathing frequency would influence the pulmonary outcome. Cardiopulmonary tolerance to intratracheal instillation of room air-equilibrated perflubron during conventional mechanical gas ventilation (CMV) was assessed, perflubron treatment after surfactant treatment was compared with surfactant treatment alone, and effects of perflubron dose or breathing frequency strategy on cardiopulmonary function, lung histology, and perflubron deposition were evaluated.

METHODS

Animal preparation. Thirty-one premature lambs [age = 125–127 days gestation, full term = 147 ± 3 (SE) days] were delivered by cesarean section. The dated, pregnant ewe was sedated (500 mg of ketamine HCl), epidural anesthesia was induced (1 mg/kg of 0.75% bupivacaine HCl), the ewe was restrained in the side-lying position, and a cesarean section was performed. After the uterus was exposed and opened sufficiently for the head of the fetal lamb to emerge, a rubber glove containing warmed saline solution was placed over its snout. The skin and soft tissues were anesthetized (4 mg/kg of 0.5% lidocaine HCl), catheters (5- to 8-Fr) were placed in a jugular vein and carotid artery, and a 3.5-mm-ID tracheostomy cannula (HiLo Jet Tube, Mallinckrodt) was placed with the tip positioned proximal to the carina. Pancuronium bromide (0.1 mg/kg) and sodium bicarbonate (2.5 meq/kg) were administered intravenously, the rubber glove was removed, and the lamb was delivered, wiped dry, weighed, covered with a plastic blanket, and warmed by a radiant heat source. After the cord was clamped, pressure-limited CMV was initiated with the animal in the supine position, the catheters were connected to appropriate transducers, and a constant infusion of nutrient substance (glucose at 0.50 g·kg⁻¹·h⁻¹) and pancuronium bromide (0.1 mg·kg⁻¹·h⁻¹) was begun. In this mode of ventilation, at a constant flow and inspiratory time, Vr will increase on the basis of lung mechanics until the pressure limit is reached. Initial ventilator settings consisted of a ventilator rate of ≤60 breaths/min, 4 cmH₂O PEEP, 30 cmH₂O maximum inspiratory pressures, and fraction of inspired O₂ of 1.0. Evaluation of the mechanics of breathing (see below) was used to maximize the ventilation schema by altering inspiratory pressures (≤35 cmH₂O), ventilator rate (≤60 breaths/min), or PEEP (3–5 cmH₂O). These parameters were adjusted while pressure-volume (P-V) loops and pulmonary mechanics data were monitored to achieve the highest compliance and lowest resistance while preventing overdistension, as assessed by flattening of the P-V loop. Inspired Po₂ (Pio₂) was kept constant at 100%. On-line pulmonary P-V relationships were monitored to prevent overdistension and minimize the risk of lung rupture. Electrocardiogram electrodes and a rectal temperature probe were inserted for monitoring. The animal’s temperature was maintained within 37–39°C. In light of potential cardiopulmonary instability and the risk of hypotension associated with transition and prematurity, supplemental anesthesia was used judiciously; pentobarbital sodium (≤10 mg·kg⁻¹·h⁻¹) was administered if a tachycardic response to soft tissue pinch was observed. Animals were managed according to the Guiding Principles in the Care and Use of Animals of the National Institutes of Health. All procedures in this protocol were approved by the Institutional Animal Care Committee of Temple University School of Medicine.

Experimental protocol. All animals received CMV (InfantStar, Infrasonics, San Diego CA) with PIP and peak expiratory pressures of ≤35 and 5 cmH₂O, respectively, frequency of ≤60 breaths/min, and inspiratory time of ≤0.50 s, and inspired O₂ was maintained constant at 100%. The choice of ventilator was based on that used in clinical trials in premature infants (15). At 1 h of life all animals were briefly disconnected from the ventilator and treated with exogenous bovine surfactant extract (4 ml/kg, Survanta, Ross Laboratories, Columbus, OH). The surfactant was delivered in four equal aliquots as the animal was rotated from supine, right- and left-side lying, and head down. Between each aliquot the animal was reconnected to the ventilator at preinstillation settings. At 2 h of life the animals were randomized into four groups: 1) continuous CMV (n = 8), 2) instillation of 30 ml/kg PFC liquid with sustained gas ventilation (PLV) and ventilator frequency of 60 breaths/min (n = 8), 3) instillation of 10 ml/kg PFC liquid with sustained gas ventilation (PLV) and ventilator frequency of 60 breaths/min (n = 7), and 4) instillation of 10 ml/kg PFC liquid with sustained gas ventilation (PLV) and ventilator frequency of 30 breaths/min (n = 8). This test paradigm was developed to examine the cardiopulmonary and histological profile of surfactant-treated preterm...
To the extent that an alveolar PFC layer produces diffusion infusion to minimize opening pressure and prevent overdistension of liquid. The P-V loop was monitored to guide the rate of PFC dose was instilled in the supine, left-side lying, right-side lying, or prone positions to promote distribution of the PFC liquid. The P-V loop was adjusted to prevent PFC reflux into the ventilator lines or development of a visible fluid column in the endotracheal tube, which would increase resistance and decrease VT.

Experimental management and measurements. All lambs were managed utilizing practices standard in the care of critically ill human neonates and previous experience with gas- and liquid-ventilated lambs (31, 42). For the purposes of the protocol, this management also included serial arterial blood samples, which were drawn every 15 min for hematocrit (Clay-Adams autocrit centrifuge), hemoglobin, arterial Po2 (Pao2), arterial Pco2 (Paco2), pH, HCO3-, and base excess (models ABL 330 and OSM 3, Radiometer, Copenhagen, Denmark). Arterial and central venous pressure (transducers, Statham, Los Angeles, CA) and heart rate were continuously recorded (model 7, Grass, Quincy, MA) and monitored (Air-Shields, Athens, Hatboro, PA); arterial O2 saturation (model 100, Nellcor, Pleasanton, CA) was continuously monitored. Bicarbonate solution was administered intravenously (intermittently in ≤2 meq/kg boluses) if pH was <7.25 and Paco2 was ≤50 Torr to manage metabolic acidosis. The amount of supplemental bicarbonate required was calculated as follows: meq base added = base deficit (meq/l) × body wt × 0.3. Nonbicarbonate buffering (tris(hydroxymethyl)aminomethane (THAM), 0.3 M; ml added over 15 min = body wt × base deficit (meq/l)) was utilized to correct predominant respiratory acidosis. The lamb was transfused with 10 ml/kg of fresh whole blood collected from the placenta if the hematocrit was <35%. Mean arterial blood pressure was calculated from systolic and diastolic pressure measurement. The alveolar-arterial O2 difference [(A-a)D02] was calculated from measurements of PaO2 and P1O2, where PaO2 = P1O2 - PaCO2/R (where PaO2 is alveolar Po2 and R is respiratory exchange ratio), with the assumption that PaCO2 = alveolar Pco2 (Paco2) and R = 1. To the extent that an alveolar PFC layer produces diffusion limitation, the assumption of equilibrium between PaCO2 and Paco2 may not hold true during PLV. Data of Mates et al. (17) indicate that substitution of PaCO2 for Paco2 may overestimate PaO2, imposing a mean error of ~6 and 12 Torr at doses of 10 and 30 ml/kg, respectively. Therefore, the (A-a)D02 as represented in the present study is a maximum estimate of this parameter during PLV. Ventilation was evaluated further from the calculated ventilator efficiency index [VEI = 3,800/(PIP - expiratory pressure) × ventilator frequency × PaCO2] (22).

Functional residual capacity (FRC) was measured before perflubron instillation using the closed-circuit helium-dilution technique (PANDA, MAS, Hatfield, PA) (29). Mechanics of breathing were determined at least every 30 min from measurements of tracheal pressure, flow, and volume. Airflow was measured with a pneumotachometer (no. 00, Fleish, Epalinges, Switzerland), and VT was calculated from digital integration of the flow signal. P-V loops, constructed from digitized data, were referenced to the measured gas FRC (before and after surfactant) and subsequently to the volume of PFC instilled. Crs and respiratory resistance were calculated by the least-mean-square analysis of the tracheal pressure, flow, and volume data (PeDLS-LAB, MAS) (2). Minute ventilation (V), respiratory resistance, and time constants were also calculated from these signals. All animals were rotated on the quarter-hour and sequentially positioned supine, left-side lying, right-side lying, or prone to support distribution of respiratory media and pulmonary blood flow. Ventilator pressures and/or frequency (group 1) or pressures alone (groups 2–4) were adjusted to optimize pulmonary mechanics (highest compliance and lowest resistance, while overdistension, as assessed by flattening of the P-V loop, was prevented) and gas exchange and to minimize ventilatory pressures, with the goal of eucarbia and prevention of hypoxia. All animals were transilluminated on the half-hour and immediately before and after surfactant or PFC treatment. Pneumothorax or fluorothorax was assessed by transillumination and deterioration of vital signs and was treated with drains. Fluorothorax was confirmed postmortem by evidence of PFC in the thorax.

The animals were killed with an overdose of pentobarbital sodium and KCl at 4 h postnatal age. The chest was opened, and the lungs and thorax were inspected with and without the ventilator cycling to assess gross morphology, air/PFC leak, and evidence of PFC in the thorax. Within 5 min of death, the ventilator was stopped and continuous positive airway pressure, equivalent to the final PEEP, was applied. The trachea was then clamped, and random samples of the lung within the dependent and nondependent regions of the lung were obtained and identified with respect to an anatomic matrix. No attempt was made to remove perfluorbron from the lung. The lung samples (0.50- to 1.0-cm3 blocks) were immediately placed in 10% Formalin. Additional lung tissue samples, obtained from the dependent and nondependent regions of the lung as described previously, were placed in gastight containers for analysis by X-ray fluorescence spectroscopy (XRF) technique (Tara Fields, Epalinges, Switzerland). Perflubron content of the lung tissue homogenate samples was analyzed by Alliance Pharmaceutical using a proprietary X-ray fluorescence spectroscopy (XRF) technique (Tara Fields, Epalinges, Switzerland).
personal communication). XRF is an element-specific technique used in the present application to measure the bromine signal in perflubron (4). Tissue samples were homogenized, and XRF was performed using an energy-dispersive spectrometer (model 770, Kevek Instruments, San Carlos, CA). Aqueous potassium bromide solutions were used to create a calibration standard curve. The mass of perflubron in the tissue was calculated stoichiometrically from the mass of bromine.

Two-factor analysis of variance for repeated measurements and post hoc testing with Student-Newman-Keuls correction for multiple comparison were performed to evaluate statistical differences in gas exchange, acid-base, and cardiopulmonary function as a function of time (all groups), perflubron rescue compared with surfactant treatment and CMV alone, perflubron dose (10 vs. 30 ml/kg), and ventilator frequency (30 vs. 60 breaths/min). One-factor analysis of variance and Tukey’s post hoc test were used to test for significant difference in morphometric indexes and perflubron content. Statistical significance was accepted at $P < 0.05$.

RESULTS

Survival. Twenty-seven animals (88%) survived the full 4-h protocol. Four animals experienced pneumothorax after surfactant treatment. Two of these animals, randomized to CMV and surfactant treatment alone, experienced progressive hypotension and died at 3 and 3.5 h. Of the remaining two animals, randomized to PLV 30 ml/kg and 60 breaths/min, one animal experienced acute arrhythmia at 3.5 h and died and the other animal survived the entire protocol. One animal randomized to PLV 30 ml/kg and 60 breaths/min demonstrated a progressive decrease in $P_{aO_2}$ with evidence of pneumothorax or fluorothorax by the end of PFC instillation and died at 2.25 h. The weight $[2.53 \pm 0.01 \text{ (SE)} \text{ kg}]$ and age $[125.6 \pm 0.16 \text{ days gestation}]$ of the animals were not different as a function of group, nor were they correlated with survival.

During PFC instillation. Trends in gas exchange, arterial blood pressure, and P-V relationships during PFC instillation are shown in Fig. 1. There was little difference in $P_{aO_2}$, $P_{aCO_2}$, and mean arterial pressure within the first 5 min of instillation. Thereafter, oxygenation improved, while CO2 elimination and arterial blood pressure responses were variable. There was a biphasic $P_{aCO_2}$ response characterized by a decrease to below-preinstillation values followed by an increase back to preinstillation values toward the end of instillation, which then resolved to below-preinstillation values within 5 min after instillation. Marked changes in the P-V relationship were noted during instillation. With progressive filling (Fig. 1B, loops C–E), opening pressures decreased (i.e., arrows, pressure point on inflation limb where VT increased) and Crs and VT increased. After PFC instillation, each P-V loop (loops C–E) demonstrated that hysteresis was greater at low lung volume and decreased with increased lung volume toward the end of inspiration. In addition, hysteresis of the individual loops (loops C–E) decreased with increasing perflubron lung volume. The presence of fluid in the endotracheal tube at zero end-expiratory pressure (i.e., meniscus) did not consistently correlate with the volume of perflubron instilled or the predetermined gas FRC and was sensitive to body position.

Cardiopulmonary profile. The effect of perflubron liquid dose and ventilatory frequency on gas exchange over time is depicted in Fig. 2. $P_{aO_2}$ increased significantly ($P < 0.05$) in all groups after surfactant treatment. Within 5 min after PFC instillation, there was a significant ($P < 0.01$) further increase in $P_{aO_2}$ indepen-
dent of perflubron dose. This initial increase was significantly greater ($P < 0.05$) in animals ventilated at the higher rate (60 breaths/min) than in those ventilated at the lower rate (30 breaths/min). By 90 min after instillation, there was no difference in PaO$_2$ among the perflubron-treated animals. In all PFC-treated animals, PaO$_2$ gradually decreased but remained significantly ($P < 0.001$) higher than in animals treated with surfactant alone. There was a variable response in PaCO$_2$ to surfactant treatment, with residual hypercarbia noted in all groups. After perflubron instillation, there was a significant ($P < 0.001$) and sustained reduction in PaCO$_2$ to surfactant treatment, with residual hypercarbia noted in all groups. After perflubron instillation, there was a significant ($P < 0.001$) and sustained reduction in PaCO$_2$ to within physiological range in all perflubron-treated animals compared with those receiving surfactant treatment alone. After perflubron instillation, PaCO$_2$ was not statistically different between the groups treated with 30 and 10 ml/kg, except at 195 min. PaCO$_2$ initially decreased to a significantly ($P < 0.05$) greater degree in animals ventilated at the higher rate than in animals ventilated at the lower rate. According to protocol, this guided a reduction in ventilatory pressures and VT (Figs. 3 and 4) in animals ventilated at the higher rate, such that by 4 h PaCO$_2$ was equivalent in all perflubron-treated animals and significantly less than PaCO$_2$ in animals treated with surfactant alone. As reflected by the pH (Table 1) and PaCO$_2$ (Fig. 2), a persistent respiratory acidosis was noted before and after surfactant treatment in all animals; the respiratory acidosis resolved in the perflubron-treated animals. During the first 2 h of the protocol, all groups required exogenous buffer: 80% required THAM and 20% received bicarbonate. There was no significant difference in the amount of buffer given across groups during this time period: THAM at 4.8 ± 0.75 and 5.62 ± 0.5 (SE) ml/kg and bicarbonate at 2.6 ± 0.41 and 2.9 ± 0.4 (SE) meq/kg at 1 h before and 2 h after surfactant treatment, respectively. Over the remaining 2 h of the protocol, the animals treated with surfactant alone required more buffer (predominantly THAM on the basis of PaCO$_2$) than did animals treated with PLV; there was no significant difference in buffer requirements between the PLV groups: for surfactant alone, THAM at 4.2 ± 0.9 and 5.4 ± 0.4 (SE) ml/kg and bicarbonate at 2.2 ± 0.17 and 2.1 ± 0.8 meq/kg at 3 and 4 h, respectively; for PLV, THAM at 2.2 ml/kg in one animal at 3 h only and bicarbonate at 2.1 ± 0.4 and 2.2 ± 0.2 (SE) meq/kg at 3 and 4 h, respectively. As shown in Table 1, there was a gradual decrease in mean arterial pressure in all animals throughout the protocol, with no significant differences noted among groups.

Figures 3 and 4 depict the effect of PFC liquid dose and ventilatory frequency on Crs, VT, PIP, and mean...
airway pressure (Paw) requirements. As shown in Fig. 3, there was no significant difference in Crs after surfactant treatment in all groups. There was a significant and sustained increase in Crs after perflubron instillation compared with animals treated with surfactant alone. This increase was initially significantly greater (P < 0.01) in animals treated with the higher dose (30 ml/kg at 60 breaths/min) than in other perflubron-treated groups; this difference resolved by 15 min after instillation. Crs was not significantly different as a function of ventilator frequency. Figure 3 also demonstrates that there was no significant difference in VT after surfactant treatment in any group; animals treated with surfactant alone demonstrated a trend (P = 0.07) toward increasing VT over the final hour of the protocol. After perflubron instillation, there was a significant (P < 0.001) and sustained increase in VT in all perflubron-treated animals compared with animals treated with surfactant alone. On the basis of the reduction in Paco2 described above, PIP was reduced such that VT was decreased in the animals treated at the higher frequency compared with animals ventilated at the lower frequency. The reduction in VT was greater (P < 0.05) at the higher than at the lower dose. By 1 h after perflubron instillation, VT requirements were greater (P < 0.05) in the animals ventilated at the lower frequency-dose combination than in all other groups and remained higher. By 90 min after PFC instillation, VT requirements to achieve physiological Paco2 in animals ventilated at the higher frequency, independent of dose, were not statistically different from those of the animals treated with surfactant alone, which demonstrated persistent hypercapnia. As shown in Fig. 4, there was no significant reduction in PIP requirements after surfactant treatment in any group. After perflubron instillation, there was a significant (P < 0.01) and sustained reduction in PIP in all PFC-treated animals. PIP could be initially decreased to a significantly (P < 0.001) greater degree in the animals ventilated at the higher dose and frequency (30 ml/kg and 60 breaths/min) than in all other animals; these differences resolved by 1 h after PFC instillation. PIP could be maintained significantly lower (P < 0.001) in perflubron-treated animals ventilated at the higher frequency than in those ventilated at the lower frequency. As also shown in Fig. 4, Paw was not significantly different over time in animals treated with surfactant alone. Paw in all perflubron-rescued animals was significantly different as a function of time (P < 0.001) and significantly lower (P < 0.001) than in the control group. The decrease in Paw was not significantly different as a function of perflubron dose. The reduction in Paw was significantly different as a function of
frequency and time. Whereas Paw was lower (P < 0.001) in animals ventilated at the lower rate, the frequency-dependent difference decreased as a function of time (120–180 vs. 180–240 min, P < 0.001). PEEP (Table 1) was not statistically different as a function of time or treatment group.

There was a small increase in FRC after surfactant treatment [12.8 ± 1.1 and 14 ± 3 (SE) ml/kg before and after surfactant, respectively]. Additional cardiopulmonary indexes are shown in Table 1. There were no significant differences in the (A-a)DO2, VEI, V˙, expiratory resistance, or expiratory time constant (tE) after surfactant treatment. After perflubron instillation, there was a significant initial decrease (P < 0.05) in (A-a)DO2 and an increase (P < 0.01) in VEI in all PFC-treated groups. The initial reduction in (A-a)DO2 was significantly less (P < 0.05) in animals receiving 10 ml/kg and 30 breaths/min than in the other perflubron-treated animals. Although the improvement in (A-a)DO2 diminished over time, (A-a)DO2 remained lower in all perflubron-treated groups than in animals treated with surfactant alone. The VEI was significantly greater (P < 0.05) in animals treated with 10 ml/kg and 30 breaths/min and sustained in all perflubron-treated groups. After PFC instillation, there was a significant initial increase (P < 0.005) in V in animals ventilated at the higher frequency; V then returned to near-preperflubron treatment values according to the protocol-directed decrease in PIP and resultant decrease in V˙ needed to maintain physiological PaCO2. There were no significant differences in expiratory resistance after surfactant or perflubron instillation. The tE was not different after surfactant treatment in any group. After perflubron instillation there was a significant and sustained increase (P < 0.01) in the tE in all perflubron-treated groups; the increase was not statistically different across groups.

Histology and morphometry. Macroscopically, lungs treated with surfactant alone demonstrated marked atelectasis in the dependent regions and appeared less well expanded and more red in color than those treated with perflubron. On inspection with the ventilator cycling, the nondependent regions appeared to inflate before the dependent regions in all lungs. Expiration appeared more homogenous. The perflubron-treated lungs demonstrated regional differences in color during inflation ranging from a pink "snowflake-like" appearance in the nondependent region to a deeper, consistent red color in the dependent region. At end expiration, these lungs demonstrated a consistent red color and appeared larger than lungs treated with surfactant alone. Small amounts of perflubron were observed (≤3
Table 1. Summarized cardiopulmonary indexes

<table>
<thead>
<tr>
<th>Post-natal Time, min</th>
<th>pH</th>
<th>HCO₃⁻, meq/l</th>
<th>MAP, mmHg</th>
<th>PEEP, cmH₂O</th>
<th>(A-a)ĐO₂</th>
<th>VEI, %</th>
<th>Vₑ, ml·min⁻¹·kg⁻¹</th>
<th>Rₑ, cmH₂O·l⁻¹·s</th>
<th>τₑ, s</th>
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<td>CMV</td>
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<tr>
<td>Pre-SF</td>
<td>60</td>
<td>7.03 ± 0.07</td>
<td>20.9 ± 0.84</td>
<td>53 ± 5</td>
<td>4.8 ± 0.3</td>
<td>545 ± 12</td>
<td>3.5 ± 0.3</td>
<td>360 ± 51</td>
<td>79 ± 6</td>
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<tr>
<td>30 min post-SF</td>
<td>90</td>
<td>7.02 ± 0.07</td>
<td>20.8 ± 1</td>
<td>48 ± 4</td>
<td>4.3 ± 0.3</td>
<td>457 ± 35</td>
<td>3 ± 0.29</td>
<td>377 ± 46</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>90 min post-SF</td>
<td>150</td>
<td>7.05 ± 0.08</td>
<td>20.7 ± 1.9</td>
<td>40 ± 4</td>
<td>4.2 ± 0.4</td>
<td>444 ± 39</td>
<td>3.2 ± 0.38</td>
<td>419 ± 87</td>
<td>68 ± 3</td>
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<tr>
<td>3 h post-SF</td>
<td>240</td>
<td>7.12 ± 0.07</td>
<td>20.8 ± 1.6</td>
<td>38 ± 2</td>
<td>5 ± 0.3</td>
<td>579 ± 22</td>
<td>2.82 ± 0.37</td>
<td>486 ± 60</td>
<td>79 ± 26</td>
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<tr>
<td>PFC 30 ml/kg-60 breaths/min</td>
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<tr>
<td>Pre-SF</td>
<td>60</td>
<td>7.08 ± 0.03</td>
<td>23.2 ± 0.9</td>
<td>59 ± 3</td>
<td>4.9 ± 0.1</td>
<td>591 ± 8</td>
<td>2.6 ± 0.19</td>
<td>312 ± 24</td>
<td>83 ± 5</td>
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<tr>
<td>30 min post-SF</td>
<td>90</td>
<td>7.11 ± 0.05</td>
<td>23 ± 1.2</td>
<td>45 ± 2</td>
<td>4.45 ± 0.2</td>
<td>564 ± 15</td>
<td>3.21 ± 0.19</td>
<td>369 ± 50</td>
<td>81 ± 16</td>
</tr>
<tr>
<td>30 min post-PFC</td>
<td>150</td>
<td>7.36 ± 0.04*</td>
<td>20.8 ± 2</td>
<td>40 ± 4</td>
<td>4.2 ± 0.3</td>
<td>392 ± 52*</td>
<td>8.13 ± 0.7*</td>
<td>449 ± 68*</td>
<td>71 ± 12</td>
</tr>
<tr>
<td>2 h post-PFC</td>
<td>30</td>
<td>7.34 ± 0.02*</td>
<td>23.1 ± 1.3</td>
<td>38 ± 3</td>
<td>4 ± 0.3</td>
<td>428 ± 46</td>
<td>8.77 ± 0.72*</td>
<td>380 ± 36</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>PFC 10 ml/kg-60 breaths/min</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre-SF</td>
<td>60</td>
<td>7.09 ± 0.08</td>
<td>22.8 ± 2</td>
<td>48 ± 2</td>
<td>4.6 ± 0.3</td>
<td>606 ± 18</td>
<td>3.66 ± 0.22</td>
<td>265 ± 37</td>
<td>87 ± 17</td>
</tr>
<tr>
<td>30 min post-SF</td>
<td>90</td>
<td>7.14 ± 0.05</td>
<td>23.1 ± 1.4</td>
<td>47 ± 2</td>
<td>4.6 ± 0.3</td>
<td>595 ± 30</td>
<td>2.95 ± 0.17</td>
<td>304 ± 52</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>30 min post-PFC</td>
<td>150</td>
<td>7.46 ± 0.03‡</td>
<td>22.5 ± 2.3</td>
<td>35 ± 3</td>
<td>4.2 ± 0.3</td>
<td>396 ± 29*</td>
<td>9.09 ± 0.59*</td>
<td>542 ± 63*</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>2 h post-PFC</td>
<td>240</td>
<td>7.35 ± 0.02*</td>
<td>23.9 ± 1.4</td>
<td>31 ± 3</td>
<td>4.2 ± 0.3</td>
<td>464 ± 53</td>
<td>8.48 ± 0.64*</td>
<td>332 ± 36</td>
<td>91 ± 10</td>
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<tr>
<td>PFC 10 ml/kg-30 breaths/min</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-SF</td>
<td>60</td>
<td>7.19 ± 0.08</td>
<td>21.1 ± 0.3</td>
<td>60 ± 6</td>
<td>4.8 ± 0.2</td>
<td>573 ± 16</td>
<td>3.63 ± 0.28</td>
<td>361 ± 38</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>30 min post-SF</td>
<td>90</td>
<td>7.19 ± 0.08</td>
<td>20.1 ± 1.4</td>
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<td>4.6 ± 0.2</td>
<td>554 ± 25</td>
<td>3.95 ± 0.3</td>
<td>327 ± 41</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>30 min post-PFC</td>
<td>150</td>
<td>7.37 ± 0.05*</td>
<td>23.7 ± 2</td>
<td>42 ± 2</td>
<td>4.6 ± 0.2</td>
<td>452 ± 43*</td>
<td>11.3 ± 0.56*</td>
<td>322 ± 24</td>
<td>93 ± 14</td>
</tr>
<tr>
<td>2 h post-PFC</td>
<td>240</td>
<td>7.31 ± 0.03*</td>
<td>24.9 ± 2.2</td>
<td>42 ± 9</td>
<td>4.6 ± 0.2</td>
<td>502 ± 48</td>
<td>11.2 ± 0.79*</td>
<td>282 ± 22</td>
<td>83 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SE. CMV, conventional mechanical gas ventilation; SF, surfactant; PFC, perflubron perfluorochemical; MAP, mean arterial pressure; PEEP, positive end-expiratory pressure; (A-a)ĐO₂, alveolar-arterial O₂ difference; VEI, ventilatory efficiency index; Vₑ, minute ventilation; Rₑ, expiratory resistance; τₑ, expiratory time constant. *P < 0.05 vs. 30 min post-SF within group; †P < 0.05 vs. 30 min post-PFC; ‡P < 0.05 vs. other groups at same time point.
Fig. 5. Photomicrographs (×180) of lung sections from nondependent (A, C, E, and G) and dependent (B, D, F, and H) regions from animals treated with surfactant and conventional mechanical ventilation (A and B), perfluorobutane at 30 ml/kg and 60 breaths/min (C and D), perfluorobutane at 10 ml/kg and 60 breaths/min (E and F), and perfluorobutane at 10 ml/kg and 30 breaths/min (G and H).
PFC uptake. Perflubron content in the lung tissue is displayed in Fig. 7 as the content in the total lung across all regions as well as regional distribution (nondependent vs. dependent lung). Perflubron content of the total lung was significantly greater ($P < 0.05$, +80%) for a dose of 30 ml/kg than for 10 ml/kg and not statistically different between frequencies. Regional differences in lung perflubron content were statistically different as a function of dose and frequency. Regional differences were noted only in animals treated with the lower dose (10 ml/kg; dependent vs. nondependent, $P < 0.05$). The regional difference was significantly greater ($P < 0.001$) in animals ventilated at 30 breaths/min (+68%) than in those ventilated at 60 breaths/min (+33%).

**DISCUSSION**

This study demonstrates improvement in gas exchange and Crs during perflubron instillation in the presence of acute respiratory failure in the surfactant-treated preterm lamb. During the initial period after instillation, improvement in this profile with reduction in ventilatory pressure requirements was directly related to the perflubron dose and breathing frequency. Whereas dose- and frequency-related differences in gas exchange and Crs resolved within 90 min after perflubron instillation treatment, PIP requirements were lower in the higher-dose and -frequency groups for most of the protocol. All perflubron-rescued groups demonstrated sustained improvement in gas exchange and Crs and reduction in ventilator pressure requirements relative to animals receiving surfactant treatment alone. The histological profile of the lungs of animals treated with perflubron at 30 ml/kg showed improved expansion and reduced evidence of barotrauma. With respect to perflubron treatment, lung expansion was greater and evidence of barotrauma was less in the higher-dose and -frequency group; regional differences in expansion were not different as a function of dose but were greater in the lower-frequency group. Regional differences in lung perflubron content were reduced in the higher-dose and -frequency groups and greatest in the lower-dose and -frequency group.

Although previous studies have demonstrated improvement in the cardiopulmonary profile during gas ventilation after instillation of preoxygenated PFC liquid (9, 14, 16, 24, 36–38), this profile has not been characterized quantitatively during the instillation process. In general, liquids have lower diffusion coefficients for gases than equal volumes of gas within a gas reservoir; as such, gases diffuse more slowly in liquids than in a gaseous medium (23, 35). Whereas preoxygenation of the PFC fluid may offset the diffusional limitations by increasing the partial pressure driving force, the additional step to precondition the PFC fluid may not be necessary or desirable. If the PFC fluid is completely saturated with O2, the PO2 in the PFC fluid would be higher than the PO2 in the lung before instillation of PFC. On the basis that diffusion of gases requires a partial pressure difference, we reasoned that presaturation of the PFC with O2 would delay the absorption of the gas FRC into the PFC fluid. Although it is possible that residual gas may “bubble out,” this process could create gas locks. Delayed gas absorption

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**Table 2. Summarized qualitative histological assessment**

<table>
<thead>
<tr>
<th></th>
<th>Hyaline Membrane</th>
<th>Hemorrhage</th>
<th>Patchy Expansion</th>
<th>Uniform Expansion</th>
<th>Edema</th>
<th>Lymphatic Dilation</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>1.43 ± 0.39</td>
<td>2.4 ± 0.51</td>
<td>2 ± 0.1</td>
<td>-2.6 ± 0.18</td>
<td>0.67 ± 0.26</td>
<td>1.6 ± 0.34</td>
<td>1.75 ± 0.33</td>
</tr>
<tr>
<td>PFC 30 ml/kg-60 breaths/min</td>
<td>1.11 ± 0.42</td>
<td>0.66 ± 0.32</td>
<td>1.75 ± 0.43</td>
<td>-2.24 ± 1.24</td>
<td>0.2 ± 0.2</td>
<td>0.75 ± 0.18</td>
<td>1.05 ± 0.04</td>
</tr>
<tr>
<td>PFC 10 ml/kg-60 breaths/min</td>
<td>0.6 ± 0.31</td>
<td>0.7 ± 0.34</td>
<td>2.3 ± 0.48</td>
<td>-3.13 ± 0.18</td>
<td>0.4 ± 0.24</td>
<td>1.5 ± 0.41</td>
<td>1.31 ± 0.27</td>
</tr>
<tr>
<td>PFC 10 ml/kg-30 breaths/min</td>
<td>1.46 ± 0.23</td>
<td>1.2 ± 0.3</td>
<td>2.75 ± 0.59</td>
<td>-1.98 ± 0.17</td>
<td>0.25 ± 0.22</td>
<td>0.75 ± 0.19</td>
<td>1.32 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE.
and gas locks would, in turn, impede the migration of the PFC into the distal lung, present an additional source of interfacial tension, contribute to ventilation heterogeneity, and delay establishment of an effective liquid FRC. In addition, underlying heterogeneity of ventilation before PFC instillation does not ensure that the instilled PFC liquid will be ventilated equally; all regions of the lung will not necessarily have the same PAO₂ or PACO₂. Mates et al. (17–19) demonstrated ventilation heterogeneity as well as heterogeneously distributed diffusion limitation during PLV. For these reasons, it was unclear whether the temporary increase in diffusional limitation associated with nonpreoxygenated PFC could be tolerated in the presence of extreme hypoxemia and hypercarbia, particularly in the immature animal with limited cardiopulmonary reserve.

The results demonstrate that the process of instilling nonpreoxygenated perflubron improved gas exchange without compromise in blood pressure. Several interesting patterns in gas exchange were noted during instillation. As shown in Fig. 1, improvement in oxygenation and CO₂ elimination was noted 5 min after initiation of perflubron instillation. As the perflubron volume increased, oxygenation continued to improve, whereas there was a biphasic response in CO₂ elimination. The continual improvement in oxygenation is ostensibly related to the high solubility of O₂ in perflubron (53 ml/dl at 37°C), lung volume recruitment, and improved ventilation-to-perfusion matching and Crs. Gradual fluid migration to the distal lung surface would serve to 1) reduce the alveolar interfacial tension by replacing the air-lung surface interface with a perflubron-lung surface interface in the regions of fluid migration and 2) establish a liquid end-expiratory volume that would prevent alveolar collapse and increase the effective lung volume throughout the respiratory cycle. Oxygenation of the perflubron liquid by the gas ventilator during instillation may serve to increase the partial pressure difference for O₂, reduce the diffusional limitations coupled to a liquid medium, reverse alveolar hypoxia, and improve pulmonary blood flow. As such, the combination of increased surface area for exchange and high solubility of O₂ in the perflubron liquid would explain early maintenance of oxygenation followed by continual improvement during instillation.

In contrast to the oxygenation response pattern, the biphasic PAO₂ profile during instillation may be explained by the low arterial–alveolar difference for CO₂ ([a–A]DCO₂) and high solubility of CO₂ (210 ml/dl at 37°C) in perflubron compared with O₂, changes in respiratory mechanics, and dose-dependent distribution of perflubron in the lung. During the early phase of instillation (i.e., 0–5 min; 10 ml/kg), lung recruitment would support improved compliance, matching of ventilation and perfusion, oxygenation, and CO₂ elimination. In addition, CO₂ diffusing from the blood readily...
dissolves in the perflubron alveolar reservoir, thus decreasing PaCO₂. Although PFC fluids of high CO₂ solubility may provide a greater carrying capacity for CO₂, additional ventilation is required to deplete CO₂ from the PFC reservoir in the lungs. Increasing perflubron lung volumes could increase the diffusional limitation for CO₂ and transiently increase alveolar dead space, thus requiring additional ventilation to eliminate CO₂. As such, PaCO₂ could increase during gas ventilation with instillation of larger volumes of PFC if a proportional increase in ventilation is not achieved. As shown in Fig. 1, by 10 min, 20 ml/kg of perflubron accumulated in the lung and resulted in a smaller increment in Crs. This further increase in perflubron lung volume without a substantial increase in Crs and Vt could increase the diffusional limitation for CO₂, which coupled with the low (a-A)DCO₂ would explain the observed transient increase in PaCO₂. As also shown in Fig. 1, by 15 min, maximal recruitment of lung volume and distribution of perflubron occurred with instillation of 30 ml/kg of perflubron, resulting in a further and substantial increase in Crs, Vt, and V. As such, CO₂ was more effectively removed from the alveolar reservoir and PaCO₂ continued to improve. Utilizing perflubron treatment in saline-lavaged adult rabbits, Tutuncu et al. (36) demonstrated a perfubron dose-dependent (3–9 ml/kg) decrease in the elevated ratio of alveolar dead space to VT. Whereas higher doses of perflubron failed to reduce this ratio, physiological levels of PaCO₂ could be supported with continual ventilation (36). In addition, Mates et al. (17, 18) demonstrated that O₂ shunt and (a-A)D CO₂ increased linearly with the volume of perflubron in the gas-ventilated normal lung. These studies indicated that impairment in gas exchange could be associated with mass transport limitations imposed by the fluid due to creation of anatomic shunt in poorly ventilated fluid-filled regions, diffusional equilibrium time for O₂ and CO₂ exceeding pulmonary capillary transit time, and a “sump” effect of perflubron related to the high solubility for respiratory gases. As such, these studies suggest that the improvement in oxygenation and biphasic response in PaCO₂ with incremental filling in the immature animals of the present study may be attributed to alveolar recruitment and improvement in compliance and ventilation-perfusion matching, all of which would serve to offset potential deleterious effects of intrinsic diffusional limitations associated with a fluid medium of high respiratory gas solubility. Finally, although the dynamic response in gas exchange during fluid instillation was measured only in the animals treated with 30 ml/kg and ventilated at 60 breaths/min, we expect that the variation in CO₂ elimination would occur to a lesser degree in the animals treated with 10 ml/kg and ventilated at 30 breaths/min and possibly not at all in the animals treated with 10 ml/kg and ventilated at 60 breaths/min. We speculate that the increase in effective lung volume associated with instillation of 10 ml/kg perflubron would result in a substantial increase in Crs and reduction of PaCO₂ (Fig. 1) without significant physiological manifestations due to diffusional limitations and that this effect would be sustained throughout the study.

Inspection of the P-V relationship during PFC instillation revealed a perfluorobron dose-dependent decrease in opening pressures and hysteresis and an increase in Crs. Before perflubron instillation, the gas FRC [14 ± 3 (SE) ml/kg] was indicative of substantial lung instability with primarily elastic collapsing forces as reflected by opening pressures approximating peak pressures. With the initial phase of perflubron instillation there was a substantial increase in Crs ostensibly due to the combined effect of lung volume recruitment and reduction in surface tension, which resulted in a decrease in opening pressures relative to surfactant treatment alone. With progressive filling up to 30 ml/kg, Crs continued to increase and opening pressures continued to decrease. The reduction in opening pressures and improvement in Crs concomitant with the increase in liquid lung volume reflect alveolar recruitment without overdistension and reduction of air-liquid interfacial tension at the alveolar-capillary membrane. The presence of an incompressible fluid in a surfactant-treated lung would prevent alveolar collapse at end expiration, promote lung stability, and reduce pressure required to initiate volume expansion (i.e., opening pressures).

As shown in Fig. 1, at each perflubron dose the difference in volume at each given pressure during inflation and deflation (i.e., hysteresis) was less toward the peak of the Vt. In addition, hysteresis throughout the entire gas Vt was reduced at higher perfluorobron lung volumes. Bachofen et al. (1) demonstrated a similar relationship between lung volume and hysteresis in a study of a hexadecane-rinsed and gas-inflated lung. Micrographs of the hexadecane-rinsed and gas-inflated lungs demonstrated empty spaces on the lung surface surrounded by a bovine lipid extract film; the empty spaces were presumed to be filled with (nonfixable) hexadecane. The authors proposed that, since hexadecane is a short hydrocarbon compound that may act like a solvent for the lipid tails of dipalmitylphosphatidylcholine (DPPC), it may interdigitate with the lipid tails of DPPC and nearly eliminate interfacial forces. Using a less lipophilic PFC, Schurch et al. (28) reported that the interfacial tension between a surface and PFC droplet is decreased as the concentration of DPPC is increased. In vitro measurements of interfacial tension using the captive bubble methodology demonstrated an interaction between the surfactant film and hexadecane. On expansion, hexadecane forms a layer on top of the surfactant lining layer, the surfactant film is less compressible, and interfacial tension is increased. On deflation, the hexadecane layer contracts and becomes sequestered into surfactant-covered droplets that are molded into the lung tissue (i.e., empty spaces in the micrograph), the surfactant film between the air and hexadecane ostensibly is changed from a monolayer to a multilayer and is compressible, and surface tension is reduced, resulting in greater volume at any given pressure. The results of the present study may be related in part to this mechanism.
Although differences exist between the hydrocarbon hexadecane and the brominated fluorocarbon perfluoron, several similarities suggest that interaction between perfluoron and bovine-based exogenous surfactant used in the present study may influence the mechanical properties of the lung. The terminal bromine on the perfluoron molecule confers relative lipophilicity, which would favor interdigitation with the lipophilic tails of the bovine surfactant, not unlike the interaction of hexadecane and DPPC. This would markedly reduce, if not eliminate, interfacial tension between perfluoron and the surfactant film. Utilizing excised lungs from preterm lambs, Tarczy-Hornoch et al. (34) reported that exogenous bovine surfactant reduces the interfacial tension at the air-lung as well as the perfluoron-lung interface. With repeated inflation and deflation, as occurs during gas ventilation, surfactant-coated perfluoron micelles could form and present as the noncellular vacuoles noted on microscopy in the present study, which were similar to the surfactant-covered empty spaces seen in the hexadecane study. Although the surfactant film around these “micelles” may be compressible, fostering reduced surface tension, the relatively incompressible perfluoron core may confer added stability to the lung during deflation. As such, during gas ventilation at low doses of perfluoron, although inflation pressures are greater than at high doses because of stratification with large regions of the nondependent lung remaining gas filled, greater volume is maintained at any given pressure during expiration. At higher doses, distribution of the perfluoron is enhanced, the gas-surfactant-lung interface is replaced with a gas-surfactant-PFC-lung interface in more regions of the lung, and lung volume is recruited. As such, inflation pressures for the same Vt would be reduced, and less volume would be maintained at any given pressure during expiration (i.e., reduced hysteresis). Higher end-expiratory volumes of the incompressible PFC would prevent alveolar collapse.

Tutuncu et al. (36, 38) suggested that the perfluoron-related improvement in oxygenation and Crs might occur by different mechanisms. After saline lavage, adult rabbits demonstrated a dose-dependent increase in PaO₂ with incremental doses of perfluoron from 3 to 15 ml/kg (38), with a marked difference in the PaO₂ response between 3 and 6 ml/kg and little difference at 9–15 ml/kg (36); improvement in Crs was not dose dependent (36, 38). These authors speculated that increasing doses of perfluoron would serve to progressively recruit lung volume and prevent alveolar collapse in more regions of the lung, thereby enabling more regions of the lung to participate in gas exchange. They hypothesized that, unlike the effect of dose on oxygenation, instillation of very small doses of perfluoron was sufficient to reduce surface tension to that of the perfluoron liquid. In the present study, our finding of little dose-dependent differences in the PaO₂ response was similar to that of the adult rabbits treated with 9–15 ml/kg perfluoron. In contrast to the previous study, the preterm lambs demonstrated an initial dose-dependent increase in Crs that resolved within 15 min after perfluoron instillation. Differences between the studies might be related to the animal preparations and perfluoron dose range. In contrast to the relatively low initial gas lung volume of the preterm lambs in the present study, gas lung volume was not reported and residual saline may have maintained a certain degree of alveolar recruitment in the adult rabbits. Whereas incremental perfluoron doses would provide an increasing reservoir to support gas exchange, it is possible that additional volume may have placed these lungs toward the top and relatively curvilinear portion of the P-V curve. Within this context, it is possible that incremental doses could have offset the effect of reducing surface tension, thereby limiting further improvement in compliance. In addition, the preterm lambs in the present study were pretreated with bovine surfactant before perfluoron instillation.

As previously discussed, interaction between the perfluoron and surfactant film synergistically would reduce interfacial tension at the air-lung as well as perfluoron-lung interface (33). Although it is less complete to characterize Crs without reference to an exact lung volume, it is difficult to determine the exact contribution of gas relative to perfluoron liquid in the composite FRC. This point is particularly difficult with respect to a low dose if the amount of instilled PFC is less than the measured gas FRC. With respect to progressive filling, we can speculate with reasonable certainty on the basis of radiographic and PFC elimination data that the effective FRC immediately after instillation approximates the PFC volume (20, 44). Because the P-V loops shown in Fig. 1B were obtained immediately after the perfluoron was instilled, predating a substantial artifact due to evaporative loss, the liquid lung volume represents a reasonable assessment of end-expiratory volume. When compliance (Fig. 1B) is normalized to the gas FRC measured before (point A = 0.013 I/cmH₂O) and after surfactant (point B = 0.016 I/cmH₂O) and the PFC liquid FRC immediately after PFC administration (i.e., point D = 0.028 I/cmH₂O; point E = 0.023 I/cmH₂O), the data demonstrate that, whereas compliance increased by a factor of 2.6–3.2, there was a smaller change in specific compliance (1.4–1.7). As such, whereas Crs initially increased to a greater degree at the higher dose, this study indicates that specific Crs remained relatively unchanged. This finding suggests that Crs increased in proportion to lung volume recruitment. To the degree that improvement in compliance may reflect the balance of reduction in surface tension and increase in lung volume, it is likely that the greater initial increase in compliance in the higher-dose group is due to the greater PFC lung volume, which achieved greater lung recruitment and distribution of the PFC liquid and replaced the gas-liquid interface of more of the lung than did the low-dose group. Because lung volume is recruited more homogeneously with the higher dose of perfluoron, this may also improve the distribution of the exogenous surfactant and result in a further increase in Crs. The attenuation of differences in compliance over time between the dose groups may be related to PFC elimina-
tion or redistribution of the PFC liquid to the dependent region of the lung (20, 44).

All perflubron-treated animals demonstrated an increase in $P_aO_2$ and reduction in the intrapulmonary shunt as evidenced by the decrease in $(A-a)D_O_2$. The increase in $P_aO_2$ and reduction in intrapulmonary shunt were greater within the 1st h after instillation. A similar time-dependent attenuation in the $P_aO_2$ response has been demonstrated in saline-lavaged perflubron-treated adult rabbits (36). There may be several explanations for this biphasic response in oxygenation indexes during the 2 h after fluid instillation. Before perflubron instillation, alveolar volume, oxygenation, and pulmonary blood flow are presumably low throughout the lung. As perflubron is instilled, lung volume is recruited, surface tension is reduced, alveolar hypoxia is decreased, and pulmonary blood flow would increase. This would serve to improve lung stability, ventilation-perfusion matching, oxygenation, and $CO_2$ removal. As such, whereas ventilatory pressures could be reduced to maintain eucarbia, $P_aCO_2$ would also decrease. Over time, as the perflubron is volatilized from the lung, fewer alveoli would remain expanded at the end of expiration. The pattern of stratification reflected by the micrographs and perflubron lung content in this study, as well as previous radiographic (43) and histological evidence from other studies (12, 33), indicates that the perflubron is distributed primarily to the dependent lung, whereas the nondependent regions of the lung are primarily gas filled. This pattern is ostensibly related to the inherent difference in density and kinematic viscosity between the gas and perflubron respiratory media. Because this pattern of stratification appears to be a function of perflubron dose, it is reasonable to assert that at any given time more of the lung would be fluid filled at the higher dose than at the lower dose. In addition, the rate of PFC elimination depends on $V_t$, the duration of contact between the inspired gas and PFC, and the PFC surface area in contact with the gas, which in turn depends on the distribution of the inspired gas and PFC volume. In this regard, one could hypothesize that the rate of perflubron elimination could, in fact, be greater at a higher perflubron lung volume or $V_t$. This could explain why there was little difference in $P_aO_2$ and $(A-a)D_O_2$ over time as a function of dose, whereas the biphasic response in $P_aO_2$ and $(A-a)D_O_2$ after perflubron instillation appeared accentuated at 60 breaths/min. It is reasonable to speculate that supplemental perflubron dosing or ventilation with perflubron-saturated inspired gas may serve to offset perflubron volatilization; however, the marked difference between the physicochemical properties of the gas and perflubron media suggests that these approaches could not completely eliminate redistribution during gas ventilation. In addition, existing dosing guidelines are qualitative at best. Studies including supplemental dosing and ventilation with perflubron-enriched inspired gas are in progress to address these issues.

It is particularly noteworthy that the higher dose and frequency resulted in a greater decrease in the PIP required to maintain physiological $CO_2$ elimination than did the lower dose and frequency. Although $P_aCO_2$ decreased markedly in all perflubron-treated animals, the reduction in $P_aCO_2$ occurred earlier in animals ventilated at the higher rate. As shown in Figs. 2–4, the decrease in $P_aCO_2$ in animals receiving the high or the low dose and ventilated at the higher rate, in turn, resulted in reduced $V_t$ and, thus, PIP requirements, whereas $V_r$ and, therefore, PIP requirements remained higher in animals ventilated at the lower dose and frequency. Although the VEI and Paw might suggest reduced ventilatory requirements in the low-dose and -frequency group, it is important to recognize that these indexes reflect the twofold decrease in rate to a greater degree than the higher $V_t$ and peak pressure requirements to support physiological $P_aCO_2$ in these animals. In addition, as would be expected, the lower Paw values in animals ventilated at the lower dose and frequency combination also yielded lower $P_aO_2$. After the transient dose-dependent differences in Crs, Crs, respiratory resistance, and $τ_e$ were not different as a function of dose or frequency. Histological, morphometric, and perflubron content analyses demonstrated improved expansion and distribution of perflubron at the higher dose, whereas regional differences were noted at the lower dose. Markedly increased evidence of pulmonary trauma was noted in the nondependent region of the lungs ventilated at the lower frequency. These findings indicate that the higher rate or perflubron dose supports gas exchange and lung mechanics with a more favorable histological outcome.

A similar result was demonstrated previously with high-frequency oscillatory ventilation, in which higher Paw and end distending pressure were shown to increase and maintain lung volume more homogeneously, improve oxygenation, and prevent lung injury in the treatment of respiratory distress syndrome (5). Venegas and Fredberg (39) reported that because lung volume is low in the lung with respiratory distress syndrome and relatively few alveoli are available to accommodate $V_t$, peak alveolar distension approaches total lung capacity at low ventilatory frequencies and PEEP. This suggests the importance of using higher frequencies and higher PEEP to support adequate ventilation with safe distension of recruited alveoli. Froese and Bryan (8) implemented this approach in high-frequency oscillatory ventilation, in which volume-recruitment maneuvers and small-volume oscillations are used to maintain alveolar recruitment without exposing the parenchyma and small airways to excessive peak pressures. With this in mind, the results of the present study suggest that, during PLV, the reduction of PIP using the higher-dose and -frequency combination may be a relatively more important determinant than Paw in preserving the immature lung. In this regard, it is possible that a higher Paw to support alveolarization of the PFC liquid in conjunction with lower PIP may provide greater global protection from barotrauma, as shown in this study. These findings have particular clinical relevance, in that potential candidates for this type of treatment are generally characterized by a restrictive pulmonary disorder, are
at risk for lung rupture, and are supported with maximal ventilator settings. To the degree that the larger part of the pressure requirements in these patients is required to overcome elastic rather than resistive forces, a common strategy is to optimize ventilation by reducing PIP rather than frequency. A previous study indicated that longer inspiratory times (≥0.75 s), variable levels of PEEP, and lower ventilatory rates (≤30 breaths/min) are required for effective gas ventilation in the presence of PFC liquid (16). In light of the preexisting cardiopulmonary instability in candidates for this treatment, we reasoned that reduction in ventilatory settings to these levels for the purposes of initiating PFC treatment may not be necessary or desirable. Our findings indicate that, because peak pressure and volume requirements are reduced and PFC distribution is fostered at higher volumes, pulmonary trauma may be reduced by ventilating at the higher rate at doses titrated up to 30 ml/kg.

Although the distribution of ventilation and perfusion was not quantitated directly in this study, resultant differences in the histological and perflubron content profiles of the lung at the time the animals were killed suggest that dosing and ventilation strategies may impact the matching of ventilation and perfusion over time during PLV. On initial instillation, lung volume is recruited, alveolar hypoxia is decreased, and pulmonary blood flow would ostensibly increase to the ventilated regions of the lung. In addition, previous studies have demonstrated that pulmonary blood flow is more homogeneous in the fluid-filled than in the gas-filled lung (41). These factors would serve to improve ventilation-to-perfusion matching. Over time, the perflubron stratifies to the dependent region of the lung, as demonstrated by the regional differences in perflubron lung content in the present study and previously reported radiographic evidence (44). Our results indicate improved lung expansion and distribution of perflubron at the higher dose, whereas regional differences in these indexes were seen at the lower dose. In addition, increased evidence of pulmonary trauma was noted in the nondependent region of the lungs ventilated at lower frequency. These findings indicate that redistribution of perflubron away from the nondependent region of the lung removes the protective effect of the perflubron and places the nondependent regions of the lung at higher risk for trauma than the dependent region. Preliminary studies in a subgroup of animals in the present study indicate that total pulmonary blood flow is maintained (43); other preliminary studies of animals receiving PLV indicate redistribution of pulmonary blood flow (7). On these bases, it is possible that over time, as the nondependent region of the lung becomes injured but still perfused due to the effect of blood flow redistribution in the fluid-filled lung, the ventilation-to-perfusion ratio in nondependent regions of the lung gradually decreases. This mechanism may explain the gradual decrease in PaO₂ over time in the present study and highlights the importance of determining the optimal dosing and ventilation strategy to maintain homogeneous distribution of perflubron at the alveolar-capillary membrane.

The presence of active air leak (i.e., pneumothorax) or interstitial air leak due to preexisting barotrauma warrants consideration with PLV. In the case of a pneumothorax, leakage of the incompressible PFC liquid into the pleural space may cause pulmonary or cardiac tamponade. On the basis of the physical properties of PFCs, it is likely that tracheally instilled PFCs will migrate to any plane within or between tissues of the damaged lung because of the positive spreading attributes of many PFCs. As such, preexisting air leak (even if undetectable by standard means) and, particularly, diffuse pulmonary interstitial air leak will influence the determination of optimal PFC volumes and elimination kinetics. In addition, the presence of non-communicating interstitial PFC may present a significant diffusion barrier, lead to marked complications in gas exchange, and impede volatilization of PFC from the lung. In the case of the present study, we have three experiences with perflubron instillation under these conditions. As noted previously, on the basis of the histology of the surfactant-treated control group, we believe that animals in all perflubron treatment groups had some degree of barotrauma before perflubron instillation. Evidence suggestive of perflubron outside gas exchange spaces was noted on histological sections. In both lambs in which clinically determined nonaccumulating pneumothorax was identified, perflubron instillation had positive compliance and PaO₂ and PaCO₂ responses. The PFC instillation procedures were not appreciably different, and the cardiovascular responses during the instillation in the animals with positive responses were comparable to those in animals without preexisting pneumothorax. In a third animal in which pneumothorax or fluorothorax was associated with perflubron administration, chest excursion and VR were impeded during instillation; PaO₂ deteriorated while compliance and PaCO₂ improved. Although we believe the available data are too limited to support the general application of intratracheal PFC in the presence or suspicion of air leak at this time, the possibility of lung healing due to the protective effect of PLV to reduce ventilator pressures should not be discounted; as the lung heals, fewer planes would be exposed.

In summary, we conclude that up to 30 ml/kg of room air-equilibrated perflubron liquid can be instilled during CMV up to a frequency of 60 breaths/min in the surfactant-treated preterm lamb with acute respiratory failure without imposing additional cardiopulmonary instability. After perflubron instillation, gas exchange and Crs increased, ventilatory pressures were reduced, and the lung demonstrated less evidence of barotrauma than in animals receiving surfactant treatment alone. The improvement in this profile was directly related to the perflubron dose and breathing frequency, in that PIP requirements were lower in the higher-dose and -frequency groups. Whereas a heterogenous pattern of expansion was noted in all groups, regional differences in expansion, evidence of barotrauma, and lung perflubron content were reduced in
the higher-dose and -frequency groups. The results of this study suggest that PLV may support improvement in gas exchange and lung mechanics. However, the results also indicate that the protective benefits of PFC liquids as therapeutic respiratory media may depend on maintaining the distribution of the fluid throughout the lung to minimize exposure of the alveolar surface to a gas-liquid interface similar to the effect of tidal liquid ventilation. During PLV, as the PFC liquid volatilizes from the lung over time, the relative gas-liquid composition of the FRC and interface at the alveolar surface are dynamically changing. Whereas supplemental dosing may foster improved distribution, quantitative guidelines to optimize dosing schedules are unavailable. For PLV to impact on long-term pulmonary morbidity, further study is warranted to guide dosing strategies and weaning procedures.

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