Partial liquid ventilation improves lung function in ventilation-induced lung injury


ABSTRACT: Disturbances in lung function and lung mechanics are present after ventilation with high peak inspiratory pressures (PIP) and low levels of positive end-expiratory pressure (PEEP). Therefore, the authors investigated whether partial liquid ventilation can re-establish lung function after ventilation-induced lung injury.

Adult rats were exposed to high PIP without PEEP for 20 min. Thereafter, the animals were randomly divided into five groups. The first group was killed immediately after randomization and used as an untreated control. The second group received only sham treatment and ventilation, and three groups received treatment with perfluorocarbon (10 mL·kg⁻¹, 20 mL·kg⁻¹, and 20 mL·kg⁻¹ plus an additional 5 mL·kg⁻¹ after 1 h). The four groups were maintained on mechanical ventilation for a further 2-h observation period. Blood gases, lung mechanics, total protein concentration, minimal surface tension, and small/large surfactant aggregates ratio were determined.

The results show that in ventilation-induced lung injury, partial liquid ventilation with different amounts of perfluorocarbon improves gas exchange and pulmonary function, when compared to a group of animals treated with standard respiratory care. These effects have been observed despite the presence of a high intra-alveolar protein concentration, especially in those groups treated with 10 and 20 mL of perfluorocarbon.

The data suggest that replacement of perfluorocarbon, lost over time, is crucial to maintain the constant effects of partial liquid ventilation.


It is known that modes of mechanical ventilation which allow end-expiratory alveolar collapse and/or end-inspiratory alveolar overstretching result in a decrease of lung compliance and gas exchange, and lead to atelectasis, pulmonary oedema, pneumonitis and fibrosis: for review see [1]. Development of intra-alveolar protein-rich oedema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by Webb and Tierney [2] and was later confirmed by Dreyfuss and coworkers [3, 4] who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury. Additionally, it is known that large changes in both volume and surface area result in surfactant depletion from the alveoli into the airways as well as to transformation from surface active large aggregates to inactive small aggregates [5–9]. Thus, loss of surfactant function will increase the surface tension at the air-liquid interface of the alveolar walls resulting in alveolar collapse and an increased suction force on the pulmonary interstitium which causes more alveolar oedema. The epithelial/endothelial damage results mainly from the shear forces which appear in a nonhomogeneous ventilated lung [4, 8].

It is known that perfluorocarbons (PFCs) have a surfactant-like activity due to their low surface tension (18 mN·m⁻¹), which in a surfactant-deficient lung decreases the high surface tension at the air-liquid interface [10–13]. Based on this low surface tension, the resulting peak inspiratory pressure (PIP) during volume-controlled ventilation are reduced [10, 11, 13]. Another property of PFCs is their high density which, mainly in the dependent part of the lung, recruits collapsed alveolar units [14, 15]; the combination of PFCs with gas ventilation, better known as partial liquid ventilation (PLV), also improves gas exchange in surfactant-deficient lungs [10, 11]. Additionally, because PFCs might not be affected by the presence of...
After tracheotomy, muscle relaxation was induced by Nembutal. Thereafter, a 5-min period of hypoventilation was maintained with pancuronium bromide 0.6 mL kg^-1 i.m. (Pavulon®). After tracheotomy, muscle relaxation was induced by pancuronium bromide 0.6 mL kg^-1, intramuscular (i.m.) (Nembutal®, Algin BV, Maassluis, the Netherlands). After tracheotomy, muscle relaxation was maintained with pancuronium bromide 0.6 mL kg^-1 i.m. Body temperature was kept within the normal range by means of a heating pad.

Experimental design

To produce ventilation VILI, PIP was increased to 45 cmH_2O and PEEP was decreased to zero for 20 min, whereas the other ventilator settings were not changed. Thereafter, a 5-min period of hypoventilation was introduced (i.e., PIP decreased to 26 cmH_2O and PEEP increased to 6 cmH_2O) to increase arterial carbon dioxide (CO_2). These ventilator settings were chosen based on a pilot study (unpublished data) which showed that animals ventilated at 45/0 cmH_2O (PIP/PEEP, respectively) for 20 min and then ventilated at 30/10 cmH_2O died from severe hypocapnia.

Experimental groups

The animals were randomized to one of five groups (n = 6 per group). In the first group (untreated controls), the animals were killed after the 5-min ventilation period of 26/6 (PIP/PEEP) with an overdose of pentobarbital. In each animal the thorax and diaphragm were opened, the tracheotomy catheter was connected to a pressure transducer and a pressure-volume curve (P/V curve) was immediately recorded (see later); thereafter a bronchoalveolar lavage (BAL) was performed five times with saline-CaCl_2 (see later). This group was used as an untreated control group. The second group (sham-treated controls) received a sham bolus of air 28 mL kg^-1 intratracheally and was mechanically ventilated at a PIP of 30 cmH_2O, PEEP of 10 cmH_2O. I:E ratio of 1:2, FIO_2 1.0, and respiratory rate of 40 bpm for 2 h. These ventilator settings were chosen based on a preliminary study which showed that pressures of 26/6 cmH_2O (PIP/PEEP, respectively) and 28/10 cmH_2O were too low to keep animals alive for a 2-h observation period. Three groups received PFC at a dose of 10 mL kg^-1 (PFC10), 20 mL kg^-1 (PFC20), or 20 mL kg^-1 plus after 60 min, an extra dose of 5 mL kg^-1 (PFC20+R); this extra dose was based on a previous experience with this model (unpublished data) and aimed to maintain oxygen tension in arterial blood (PaO_2) as stable as possible during the remainder of the study period.

Gas exchange and haemodynamics

Arterial blood gas samples were taken in all groups before, after VILI, and at 5 min after the 26/6 period, and in the four ventilated groups at 5 min after the 30/10 period, and at 30, 60, 90 and 120 min after PFC instillation. The samples were analysed for PaO_2 and arterial carbon dioxide tension (PaCO_2) by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the same time points, arterial pressure was recorded. Haemodynamic support was provided by infusion of 1 mL of saline (to a maximum of 2 mL h^-1) when mean arterial pressure (MAP) decreased <60 mmHg.

Pressure/volume curves

At 120 min after administration of PFC all animals were killed with an overdose of pentobarbital sodium.
injected through the penile vein. Then static P/V curves were recorded. After the thorax and diaphragm were opened (to eliminate the influence of chest wall compliance and intra-abdominal pressure), the tracheotomy catheter was connected to a pressure transducer (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument Co., Quincy, MA, USA). Using a syringe filled with nitrogen the lungs were first inflated (within 10 s) to an airway pressure of 35 cmH2O, which was maintained for 5 s, followed by deflation to an airway pressure of 0 cmH2O. Then the lungs were re-inflated in steps of 0.5 mL until an airway pressure of 35 cmH2O was reached. Each inflation step took 1–2 s followed by a 5-s pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cmH2O was reached. The volume of nitrogen left in the syringe was recorded. The lower inflection point (LIP) was determined from the intersection of the lines representing the minimum slope of the compliance curve and the maximum slope of the compliance curve. Maximal compliance (Cmax) was calculated from the steepest part of the deflation limb [18]. Total lung capacity (TLC35) was defined as lung volume at inflation with a distending pressure of 35 cmH2O [19].

Gruenwald index

The Gruenwald index which characterizes the surfactant system in situ [20], was calculated from the P/V curve, defined as (2V5 + V10)/2Vmax, where V5, V10 and Vmax are the lung volumes at airway pressures of 5, 10 and 35 cmH2O from the deflation limb, respectively.

Bronchoalveolar lavage

After the P/V curve recordings a BAL (30 mL·kg⁻¹) was performed five times with saline-CaCl2 1.5 mmol·L⁻¹. In the resultant BAL (crude lavage) there were two visible layers, the upper layer was composed of the saline-CaCl2, oedema fluid and cell debris, and the bottom layer comprised only PFC. Thereafter, cell debris and PFC were removed from BAL by centrifugation at 400 × g for 10 min. The active surfactant component in the BAL fluid was separated from the nonactive surfactant component by differential centrifugation, followed by phosphorus analysis, and the ratio of nonactive to active (small to large aggregate) surfactant was calculated [21]. Finally, the protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [22].

Minimal surface tension

Minimal surface tension of the crude lavage was determined by means of a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In this method, a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100–20% at a cycle speed of 0.33 min⁻¹. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1 × 1 cm) is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface compression and expressed as milli Newton·metre⁻¹ (mN·m⁻¹). Surface tension characteristics of a BAL sample are measured after application on the surface of the saline-filled trough. In this study 300 μL of BAL fluid was applied to the surface of the trough; surface tension was measured after 3 cycles [23].

Statistical analysis

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Intragroup comparisons were analysed with repeated measures using analysis of variance (ANOVA); intergroup comparisons were analysed with ANOVA. If a difference was found, a post hoc test was performed (Tukey-Kramer). Statistical significance was accepted at p-values <0.05. All data are expressed as mean ± standard deviation.

Results

Figure 1 shows the $P_{a,O_2}$ levels during the whole study period. After VILI and the ventilator settings were set at 26/6 cmH2O for 5 min the $P_{a,O_2}$ decreased $<13.3$ kPa in all groups. After PFC instillation and

![Fig. 1. – Arterial oxygen tension (mean ± SD) during the whole study period. B: baseline; VILI: ventilation with peak inspiratory pressure (PIP) of 45 cmH2O without peak end-expiratory pressure (PEEP) after 20 min; 26/6: after 5 min at PIP 26 cmH2O PEEP; arterial carbon dioxide tension ($P_{a,CO_2}$) at 60 min in the group PFC20, R was measured before instillation of the additional 5 mL perfluorobron dose; PFC: perfluorocarbons. PFC10: PFC at dose of 10 mg·kg⁻¹; PFC20: PFC at dose of 20 mg·kg⁻¹; PFC20, R: PFC at dose of 20 mg·kg⁻¹ plus 5 mg·kg⁻¹ after 60 min of ventilation. #: untreated; ●: sham-treated; ■: PFC10; ■: PFC20; ◊: PFC20, R. *: significant difference with sham-treated control group; #*: significant difference with PFC10; **: significant difference with PFC20.
The MAP values were comparable in all groups during the whole study period.

Table 2.—Amount of recovered bronchoalveolar lavage fluid (BAL) fluid, protein concentration, lung volume above functional residual capacity at pressure 35 cmH₂O (TLC₃5), maximum compliance (Cₘₐₓ), Gruenwald Index, lower inflection point of the pressure volume curve (LIP), minimal surface tension (min surf) of crude BAL fluid, total phosphorous concentration, and small aggregates (SA)/large aggregates (LA) ratio

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Untreated</th>
<th>Sham-Treated</th>
<th>PFC₁₀</th>
<th>PFC₂₀</th>
<th>PFC₂₀+R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery BAL fluid %</td>
<td>90±1</td>
<td>90±1</td>
<td>90±1</td>
<td>90±1</td>
<td>90±1</td>
</tr>
<tr>
<td>Protein concentration BAL mg·mL⁻¹</td>
<td>1.3±0.3*</td>
<td>1.4±0.4</td>
<td>1.9±0.2</td>
<td>1.8±0.3</td>
<td>1.4±0.1*</td>
</tr>
<tr>
<td>TLC₃₅ cm³·kg⁻¹</td>
<td>35±2**</td>
<td>31±3*+**</td>
<td>38±4</td>
<td>39±4</td>
<td>42±5</td>
</tr>
<tr>
<td>Cₘₐₓ mL·kg⁻¹</td>
<td>1.5±0.5*+<em>+</em></td>
<td>1.4±0.2*+<em>+</em></td>
<td>2.4±0.2</td>
<td>2.6±0.2</td>
<td>2.8±0.6</td>
</tr>
<tr>
<td>Gruenwald index</td>
<td>0.30±0.09</td>
<td>0.40±0.17</td>
<td>0.40±0.07</td>
<td>0.4±0.07</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>LIP cmH₂O</td>
<td>15.3±1.4*+<em>+</em></td>
<td>18.2±2*+<em>+</em></td>
<td>10.8±1.2**</td>
<td>10.6±3.3**</td>
<td>6.7±1.9*</td>
</tr>
<tr>
<td>Min surf mN·m⁻¹</td>
<td>33±3.1</td>
<td>31±1.6</td>
<td>35±2.1</td>
<td>35±0.5</td>
<td>32±3.9</td>
</tr>
<tr>
<td>Total phosphorus mmol</td>
<td>2.0±0.6</td>
<td>1.4±0.5</td>
<td>1.7±0.4</td>
<td>1.5±0.3</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>SA/LA ratio</td>
<td>1.7±1.1*+<em>+</em></td>
<td>4.6±2.7</td>
<td>4.6±2.3</td>
<td>5.6±3.5</td>
<td>4.2±2.7</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD. Untreated: nonventilated control; Sham-treated: Sham-treated, ventilated control; PFC₁₀: PFC 10 mg·kg⁻¹; PFC₂₀: PFC 20 mg·kg⁻¹; PFC₂₀+R: PFC 20 mg·kg⁻¹ plus 5 mg·kg⁻¹ after 60 min of ventilation; TLC₃₅: ventilation induced lung injury; BAL: bronchoalveolar lavage fluid; PFC: perfluorocarbons. *: versus PFC₁₀, p<0.05; **: versus PFC₂₀+R, p<0.05.

Table 1.—Data on arterial carbon dioxide tension (Pₐ,CO₂) and mean arterial pressure (MAP) over time in both the treated and untreated groups

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Untreated</th>
<th>Sham-Treated</th>
<th>PFC₁₀</th>
<th>PFC₂₀</th>
<th>PFC₂₀+R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pₐ,CO₂ kPa</td>
<td>Baseline</td>
<td>4.7±0.7</td>
<td>5.3±1.6</td>
<td>4.8±0.97</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td>VILI</td>
<td>2.5±0.3</td>
<td>2.4±0.6</td>
<td>2.5±0.35</td>
<td>2.7±0.4</td>
<td>2.9±0.5</td>
</tr>
<tr>
<td>5 26/6</td>
<td>4.7±0.7</td>
<td>5.3±1.0</td>
<td>5.0±2.1</td>
<td>5.2±0.89</td>
<td>6.7±1.7</td>
</tr>
<tr>
<td>5 30/10</td>
<td>30</td>
<td>5.6±1.3</td>
<td>3.9±0.4</td>
<td>4.4±0.9</td>
<td>5.4±0.9</td>
</tr>
<tr>
<td>60</td>
<td>6.3±1.6</td>
<td>4.4±1.3</td>
<td>4.4±0.7</td>
<td>4.7±1</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>6.5±1.3</td>
<td>4.8±1.2</td>
<td>4.7±0.5</td>
<td>5.6±0.8</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>6.9±1.8</td>
<td>4.8±2.1</td>
<td>4.9±0.3</td>
<td>4.4±0.9</td>
<td></td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>Baseline</td>
<td>135±16</td>
<td>134±15</td>
<td>144±6</td>
<td>140±24</td>
</tr>
<tr>
<td>VILI</td>
<td>75±25</td>
<td>84±32</td>
<td>72±25</td>
<td>85±22</td>
<td>71±21</td>
</tr>
<tr>
<td>5 26/6</td>
<td>74±30</td>
<td>63±42</td>
<td>47±24</td>
<td>67±36</td>
<td>89±32</td>
</tr>
<tr>
<td>5 30/10</td>
<td>111±12</td>
<td>88±24</td>
<td>106±25</td>
<td>97±14</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>91±24</td>
<td>80±20</td>
<td>89±21</td>
<td>90±9</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>89±19</td>
<td>78±17</td>
<td>95±15</td>
<td>95±37</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>86±29</td>
<td>76±17</td>
<td>97±15</td>
<td>97±12</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>89±27</td>
<td>85±9</td>
<td>73±24</td>
<td>80±13</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Untreated: nonventilated control. Sham-treated: Sham-treated, ventilated control; PFC₁₀: partial liquid ventilation (PLV) with perfluorocarbons (PFC) at dose 10 mg·kg⁻¹; PFC₂₀: PLV with PFC at dose 20 mg·kg⁻¹; PFC₂₀+R: PLV with PFC at dose 20 mg·kg⁻¹ plus 5 mg·kg⁻¹ after 60 min of ventilation; VILI: ventilation induced lung injury; BAL: bronchoalveolar lavage fluid from all ventilated groups were not significantly different from the untreated control group. For data on TLC₃₅, Cₘₐₓ, and LIP see table 2 and figures 2a and b. The total phosphorous concentration in the BAL fluid was not different between groups. The ratio of small to large aggregates in BAL fluid was significantly higher in the four ventilated groups compared with the untreated control group, and there was no significant difference between the sham-treated control group and all the PFC-treated groups.

Figure 2a shows the inflation limbs from the P/V curves. The three PFC-treated groups had a significantly lower opening pressure than both the untreated and the sham-treated control groups. The PFC₂₀+R group had a significantly higher TLC₃₅ than both the untreated and sham-treated control groups. Figure 2b shows the deflation limbs from the P/V curves. The three PFC-treated groups had a significantly lower residual capacity at pressure 35 cmH₂O (TLC₃₅), maximum compliance (Cₘₐₓ), lower inflection point of the pressure volume curve (LIP), minimal surface tension (min surf) of crude BAL fluid, total phosphorous concentration, and small aggregates (SA)/large aggregates (LA) ratio.
has been demonstrated that loss of surface active molecules due to mechanical ventilation with high inspiratory lung volumes without PEEP is produced by displacement of surfactant from the alveolar air-liquid interface into the small airways [2, 5]. Moreover, the surface area changes produced by the high inspiratory lung volumes lead to an increased rate of conversion of active into nonactive surfactant subtractions [7–9]. All these mechanisms will lead to alveolar collapse and protein infiltration [2–4, 8, 9] in which the latter leads to further inactivation of surfactant [24, 25]. In the current study PLV was used to improve the disturbed lung function caused by VILI. The results showed that after VILI, PLV produced an immediate dose and time-dependent improvement in $P_{aO_2}$. In the group treated with 10 mL·kg$^{-1}$ PFC the pre-VILI values of $P_{aO_2}$ were never reached, whereas in both groups treated with 20 mL·kg$^{-1}$ PFC, within 5 min there was a significant increase in $P_{aO_2}$ values compared with values after VILI, and these improved values were comparable with baseline values. However, $P_{aO_2}$ decreased over time in both groups in which PFC was not replaced. It has been shown that in surfactant-deficient animal lungs PLV provides adequate gas exchange as long as a sufficient amount of PFC is present in the lungs [10–13]. The present group has demonstrated that higher doses of PFC lead to higher levels of oxygenation in animals suffering from acute respiratory failure as a result of dose-dependent recruitment of collapsed atelectatic alveoli by PFC [10, 11]. It is also known that oxygenation deteriorates over time if no additional doses of PFC are instilled; this is attributed to evaporation of PFC which will cause affected alveoli to collapse [10, 11].

In the present study, the inflation limbs of the P/V curves showed on the one hand, a significantly lower opening pressure in the three PFC-treated groups and, on the other, a significantly higher maximal compliance compared with both control groups. It may be speculated that one of the reasons for this is that in the surfactant-deficient lungs, the decrease of surface tension at the air-liquid interface by PFC (vapour) improves the mechanical properties of the lung [10, 11, 26–29]. These findings have previously been demonstrated by Kirmse et al. [14] showing that with increasing amounts of perfluorobron the LIP decreases to a point where additional doses of perfluoron have no further influence on the detectable LIP.

A side-effect of PFC may be the constant surface tension which does not change with the changes in surface area (which is a property of natural surfactant) so that the end-expiratory stability in the PFC-treated animals (characterized by the Gruenwald Index) was not statistically different from the sham-treated control animals [13].

In the current study, the protein level from the crude BAL fluid in all ventilated groups was as high as in the untreated ventilated control group. Moreover, in both PFC-treated groups without replacement, an increase in the total protein concentration was observed. Additionally, the data show that with a larger amount of PFC (PFC20–R) in the lung, the total amount of protein in the BAL fluid is less. How

Discussion

This study shows that partial liquid ventilation improves $P_{aO_2}$ and lung mechanics in a model of ventilation-induced lung injury, despite the presence of a high intra-alveolar protein concentration. The authors have previously shown that this model of VILI is characterized by a decrease in lung mechanics and Gruenwald Index, and a high minimal surface tension in BAL fluid compared with healthy rat lungs [8]. The exact mechanism by which the lung damage is produced by artificial ventilation is not entirely clear, but the role of surfactant changes is being stressed [8, 9, 16]. The present group have shown that modes of ventilation with large tidal volume without PEEP disturb the surfactant system in a VILI model [8].

![Image](image_url)

Fig. 2. - a) Inflation limbs from the pressure/volume curves (mean ± SD). Untreated: untreated, nonventilated control group. Lower inflection point. b) Deflation limbs from the pressure/volume curves (mean ± SD). PFC10: PFC at dose of 10 mg·kg$^{-1}$ PFC20: PFC dose of 20 mg·kg$^{-1}$; PFC20–R: PFC dose at 20 mg·kg$^{-1}$ plus 5 mg·kg$^{-1}$ ventilation after 60 min of ventilation. ●: untreated; ▼: sham-treated; □: PFC10; △: PFC20; ○: PFC20–R. *: significant differences between PFC20–R versus sham untreated; **: significant differences between PFC20–R versus sham untreated; #: PFC10 versus sham-treated; §: PFC10 versus untreated. *: significant difference between PFC10 versus sham-treated; ¶: significant difference between PFC20 versus sham-treated.

significantly higher $C_{max}$ than both untreated and the sham-treated control groups.

Fig. 2. - a) Inflation limbs from the pressure/volume curves (mean ± SD). Untreated: untreated, nonventilated control group. Lower inflection point. b) Deflation limbs from the pressure/volume curves (mean ± SD). PFC10: PFC at dose of 10 mg·kg$^{-1}$ PFC20: PFC dose of 20 mg·kg$^{-1}$; PFC20–R: PFC dose at 20 mg·kg$^{-1}$ plus 5 mg·kg$^{-1}$ ventilation after 60 min of ventilation. ●: untreated; ▼: sham-treated; □: PFC10; △: PFC20; ○: PFC20–R. *: significant differences between PFC20–R versus sham untreated; **: significant differences between PFC20–R versus sham untreated; #: PFC10 versus sham-treated; §: PFC10 versus untreated. *: significant difference between PFC10 versus sham-treated; ¶: significant difference between PFC20 versus sham-treated.
PFC prevents protein infiltration and alveolar flooding is not entirely clear. Dreyfuss et al. [26] showed in rats that PFC partially reversed the effects of alveolar flooding (the so-called tamponade effect), but in some rats, did not reduce the permeability changes on the alveolo-capillary membrane measured by 125I-labelled serum albumin. It has been suggested that as a result of the PFC-filled alveoli, the suction forces on the interstitium more or less disappear thus preventing protein influx into the PFC-filled alveoli. However, alveoli which have only a PFC film at the air-liquid interface and which may collapse during expiration will promote alveolar flooding due to their high surface tension at end-expiration; this could be one reason why the amount of proteins in the PFC group receiving only 10 mL·kg⁻¹ PFC is significantly higher than in the PFC20 + R group.

In conclusion, the results of the present animal study show that in ventilator-induced lung injury, partial liquid ventilation with different amounts of perflubron improves gas exchange and pulmonary function, when compared to a group of animals treated with standard respiratory care. These effects have been observed despite the presence of a high intra-alveolar protein concentration, especially in those groups treated with 10 and 20 mL of perflubron. The data suggest that replacement of perfluorocarbon lost over time is crucial to maintain constant effects of partial liquid ventilation.

Acknowledgements. The authors thank S. Majoor and E. Hendrik for expert technical assistance, and the Depts of Cardiorespirochemistry and Paediatrics at Erasmus University Rotterdam for assisting with phospholipid measurements. They would also like to thank L. Visser-Iles for the English-language editing.

References

22. Bradford MM. A rapid and sensitive method for the


