Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal rabbits

EDGARDO D’ANGELO,1 MATTEO PECCHIARI,1 PAOLO BARAGGIA,1 MARINA SAETTA,2 ELISABETTA BALESTRO,2 AND JOSEPH MILIC-EMILI3

1Istituto di Fisiologia Umana I, Università di Milano, 20133 Milan; and 2Dipartimento di Medicina Clinica e Sperimentale, Università di Padova, 35128 Padua, Italy; and 3Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada H2X 2P2

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D’Angelo, Edgardo, Matteo Pecchiari, Paolo Baraggia, Marina Saetta, Elisabeta Balestro, and Joseph Milic-Emili. Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal rabbits. J Appl Physiol 92: 949–956, 2002. First published October 12, 2001; 10.1152/japplphysiol.00776.2001.—Lung mechanics and morphology of 10 normal open-chest rabbits (group A), mechanically ventilated (MV) with physiological tidal volumes (8–12 ml/kg), at zero end-expiratory pressure (ZEEP), for 3–4 h, were compared with those of five rabbits (group B) after 3–4 h of MV with a positive end-expiratory pressure (PEEP) of 2.3 cmH2O. Relative to initial MV on PEEP, MV on after 3–4 h of MV with a positive end-expiratory pressure resulted in a significant increase in quasi-static elastance (Ers; +71%) and airway (Rint; +22%), with no change in the viscoelastic time constant. After restoration of PEEP, quasi-static elastance and viscoelastic resistance returned to control levels, whereas Rint remained elevated (+22%). On PEEP, MV had no effect on lung mechanics. Gas exchange on PEEP was equally preserved in groups A and B, and the lung wet-to-dry ratios were normal. Both groups had normal alveolar morphology, whereas only group A had injured respiratory and membranous bronchioles. In conclusion, prolonged MV on ZEEP induces histological evidence of peripheral airway injury with a concurrent increase in Rint, which persists after restoration of normal end-expiratory volumes. This is probably due to cyclic opening and closing of peripheral airways on ZEEP.

METHODS

In 1984, Robertson (18) suggested that ventilation at low lung volumes may cause lung injury as a result of shear stresses caused by cyclic opening and closing of small airways. Using an ex vivo model of lavaged rat lung, Muscedere et al. (17) showed that ventilation with physiological tidal volumes (VT) from zero end-expiratory pressure (ZEEP) resulted in a significant increase in the histological injury scores in the respiratory (RIS) and membranous bronchioles (MIS) relative to ventilation from positive end-expiratory pressure (PEEP) above the lower inflection point on the static inflation volume-pressure (V-P) curve of the lung. In normal, closed-chest rabbits ventilated at low lung volumes for only 1 h, Taskar et al. (23) found no histological evidence of airway and parenchymal lung injury. In a subsequent study on normal, open-chest rabbits ventilated at low lung volumes for 3 h, Taskar et al. (22) again found no histological evidence of parenchymal lung injury, but they did not specifically assess peripheral airway injury with indexes such as RIS and MIS. Thus it is possible that ventilation at low lung volumes for >1 h may induce peripheral airway injury in the absence of preexistent parenchymal lung injury. In fact, to the extent that cyclic opening and closing of peripheral airways is responsible for lung damage, it is likely that the injury should be preferentially located in peripheral airways.

Accordingly, in the present study, we have assessed the effects of breathing at low lung volumes for 3–4 h in open-chest rabbits with normal lungs in terms of 1) histological indexes of peripheral airway and parenchymal injury and 2) lung mechanics. The latter was studied not only during the initial period of ventilation on PEEP and next on ZEEP, as in previous studies (17, 22), but also after restoration to PEEP from ZEEP to assess whether the changes in lung mechanics observed at ZEEP could be reversed.

Fifteen rabbits (weight range: 2.2–3.1 kg) were anesthetized with an intravenous injection of a mixture of pentobarbital sodium (20 mg/kg) and urethane (0.5 g/kg). A brass cannula and a polyethylene catheter were inserted into the trachea and carotid artery, respectively. The animals were paralyzed with pancuronium bromide (0.1 mg/kg) and mechanically ventilated (respirator 660; Harvard Apparatus, Holliston, MA) with a pattern similar to that during spontaneous breathing. Adequate levels of anesthesia and complete muscle relaxation were maintained with additional doses of the anesthetic mixture and pancuronium bromide. The chest was opened via a median sternotomy, and a coronal cut was made just above the costal arch. Application of PEEP (2–2.5 cmH2O) prevented lung collapse. During the measurements, the ribs on the two sides and the diaphragm were pulled...
widely apart, so that the lungs did not contact the chest wall, except in their lowermost parts.

Airflow (V) was measured with a heated Fleisch pneumotachograph no. 00 (HS Electronics, March-Hugstetten, Germany) connected to the tracheal cannula and a differential pressure transducer (±2 cmH₂O; Validyne MP45, Northridge, CA). The response of the pneumotachograph was linear over the experimental range of V. Tracheal pressure (P₀) was measured with a pressure transducer (model 1290A; Hewlett-Packard, Palo Alto, CA) connected to the side arm of the tracheal cannula; there was no appreciable shift in the signal or alteration in amplitude up to 20 Hz. The signals from the transducers were amplified (model RS3800; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 14-bit analog-to-digital converter, and stored on a desk computer. Volume changes were computed by numerical integration of the digitized V signal. Arterial blood PO₂ (PaO₂), PCO₂ (PaCO₂), and pH (pHₐ) were measured by means of a blood-gas analyzer (IL 1620; Instrumentation Laboratory, Milan, Italy) on samples drawn at the beginning and at the end of the tests made on PEEP.

After completion of the surgical procedure, the rabbits were ventilated with a specially designed, computer-controlled ventilator, delivering water-saturated air from a high-pressure source (4 atm) at constant flows of the selected magnitudes and durations. The inspiratory and expiratory solenoid valves (model S50 and S80; Peter Paul, New Britain, CT) had a closing or opening time of 5 ms; they could also be operated so as to occlude the airways, either at end inspiration or end expiration, for 5 s. The inspiratory and expiratory valves were connected to the pneumotachograph attached to the animal's trachea by means of short, rigid tubings. A Fleisch pneumotachograph (no. 00) connected to the exhaust valve (model S50) of the inspiratory line and differential pressure transducer (±2 cmH₂O, MP45, Validyne) provided the feedback signal to the computer for the fine adjustment of the proportional valve (model FSV1; Aalborg, Orangeburg, NY) setting the inflation flow. A three-way stopcock allowed the connection of the expiratory valve, either to the ambient or to a drum in which the pressure was set at 2–2.5 cmH₂O by means of a flow-through system. The baseline ventilator setting consisted of fixed VT of 25 ml (8–12 ml/kg) and inspiratory (Tᵢ) and expiratory durations of 1 and 2.2 s, respectively. With this setting, no intrinsic PEEP was present under any experimental condition, as evidenced by an end-expiratory pause (zero flow) and absence of P₀ changes with airway occlusion at end expiration.

Procedure and Data Analysis

After the thorax was opened, 10 rabbits (group A) were subjected to the following sequence of PEEP and ZEEP while the baseline ventilatory settings remained constant in each rabbit: 1) 15 min of mechanical ventilation (MV) with PEEP (PEEPᵢ); 2) 3–4 h of MV at ZEEP; and 3) 15 min of MV with PEEP (PEEPᵢ). Lung mechanics were assessed with the rapid airway occlusion method (2, 5) during the PEEP₁ and ZEEP₁ periods and after 5–10 min (ZEEP₁) and at the end of the ZEEP period (ZEEP₂). In five rabbits (group B) that were subjected only to MV with PEEP for 3–4 h, assessment of lung mechanics was made 5–10 min after the onset of MV with PEEP (PEEPᵢ) and at the end of the PEEP period (PEEP₂). Before measurements during MV with PEEP, the lungs were inflated three to four times up to a P₀ of ~25 cmH₂O. Two types of experimental procedures were carried out: 1) while keeping VT at baseline values, test breaths were intermittently performed with different inspiratory flow (Vᵢ) and Tᵢ in the range of 0.25–3 s; and 2) while keeping VT at baseline values, test breaths were intermittently performed with different VT in the range of 8–61 ml to obtain quasi-static inflation V-P curves. End-inspiratory occlusions lasting 5 s were made in all test breaths, which were performed in random order and repeated four to five times in all experimental conditions. During ventilation at ZEEP, end-inspiratory occlusions were performed only for VT of 8 and 25 ml. During ventilation with PEEP, the expiratory valve was opened to the ambient for four to six expirations to measure the difference between the end-expiratory and the resting lung volume (ΔEELV); these breaths were followed by two inflations up to P₀ of 20–25 cmH₂O. The animals were from a single cohort, and the experiments were done in random order.

The end-inspiratory airway occlusions were followed by a rapid, initial drop in P₀ (ΔP₁) and by a slow decay (ΔP₂) to an apparent plateau value (quasi-static pressure (Pₛₚ)). This pressure, computed as the mean pressure recorded during the interval between 4.5 and 5 s after the occlusion, was taken to represent the quasi-static lung recoil pressure, whereas ΔP₁ and ΔP₂ divided by VT yielded the lung interrupter (Rᵢnt) and additional resistances (ΔR), respectively. Viscoelastic parameters, viscoelastic resistance (Rvisc) and Tᵢvisc = Rvisc/viscoelastic elastance (Evisc), were computed by fitting the values of ΔR and durations of inflation (Tᵢ) with the function (5)

$$\Delta R = Rvisc \times (1 - e^{-Tᵢ/Tᵢvisc}) \tag{1}$$

whereas lung quasi-static elastance (Est) was obtained as (Pₛₚ – EEP)/VT, where EEP is end-expiratory pressure. After completion of the mechanics measurements, the left or right lung was processed for histological analysis, whereas the other one was weighed immediately after removal, left overnight in an oven at 120°C, and weighed again to compute the wet-to-dry ratio.

Histological Analysis

After excision and isolation, the lungs were fixed by intrabronchial infusion of 10% formalin with the pressure maintained at 20 cmH₂O for 24 h. Technically adequate fixation was achieved in seven lungs from rabbits of group A and five from rabbits of group B. Five blocks, 1 cm thick, involving both subpleural and parahilar regions, were obtained in each animal: two, one, and two blocks from the upper, middle, and lower lobe, respectively, for the right lung; and two and three blocks from the upper and lower lobe, respectively, for the left lung. Each block was processed through a graded series of alcohols and embedded in paraffin. From each block, sections of 5 μm thickness were cut and stained with hematoxylin-eosin for light microscopic analysis. Histological evaluation was done without knowledge of the mechanical data. The following measurements were performed: 1) mean linear intercept (Lₘ), which is a measure of air space enlargement, as described by Thurlbeck (24); 2) indexes of parenchymal injury, as described by Taskar et al. (22); and 3) presence of bronchiolar epithelial necrosis and sloughing, which is a measure of bronchiolar injury, as described by Muscedere et al. (17).

For Lₘ measurements, one section from each block was examined at a magnification of ×125, and 40 non-overlapping fields were analyzed on each section, giving a total of 200 fields/animal. The value of Lₘ was obtained as the ratio between the length in micrometers of a line passing transversely through each field and the number of alveolar walls intercepting the line, with the final result for a given animal.
being the average $L_m$ of the 200 fields examined. Additional histological evidence of parenchymal injury was assessed according to the following five parameters: namely, focal alveolar collapse, intra-alveolar edema, hemorrhage, epithelial desquamation in alveoli, and presence of granulocytes in the air spaces (22). Each parameter was evaluated semiquantitatively in a single, blind manner, using a four-grade scale (absent, mild, moderate, and prominent).

Bronchiolar injury was assessed from the presence of epithelial necrosis and sloughing (i.e., separation of necrotic tissue) in the respiratory bronchioles, i.e., airways with alveolar outpouchings in their walls, and in the membranous bronchioles, i.e., airways without cartilage, including terminal bronchioles and the parent generation to respiratory bronchioles. At least 50 bronchioles were examined per animal. Three indexes were obtained for each lung: 1) the RIS computed as the percent ratio of injured to total respiratory bronchioles examined; 2) the MIS computed as the percent ratio of injured to total membranous bronchioles examined; and 3) the total injury score computed as the percent ratio of injured respiratory and membranous bronchioles to total respiratory and membranous bronchioles (17).

Statistics

Results from mechanical studies are presented as means ± SE. The least squares regression method was used to assess the parameters in Eq. 1 and of the P-V relationship of the lungs. Comparisons among experimental conditions were performed using one-way ANOVA; when significant differences were found, the Bonferroni test was performed to determine significant differences between different experimental conditions. Results from histological studies are expressed as median and range, and the statistical analysis was performed using the Mann Whitney U-test. The level for statistical significance was taken at $P \leq 0.05$.

RESULTS

Ventilation on PEEP

In each animal, the values of $P_{aO_2}$, $P_{aCO_2}$, and $pH_a$ obtained at the beginning and at the end of the sessions on PEEP did not differ significantly and were thus averaged. The mean values of $P_{aO_2}$, $P_{aCO_2}$, and $pH_a$ during PEEP1 and PEEP2 were similar for both group A and B rabbits (Table 1). Also, the mean values of the wet-to-dry ratio assessed at the end of the experiments in the two groups of rabbits did not differ significantly (Table 1) and were virtually the same as those obtained in 29 lungs (4.61 ± 0.07) removed from rabbits 30–40 min after the induction of anesthesia, in which the only other intervention was the excision of part of the pericardium (3).

The end-expiratory pressure applied to rabbits of both groups A and B was almost the same during PEEP1 and PEEP2; its average value was $2.3 \pm 0.1$ cmH$_2$O. Similarly, the mean values of $\Delta$EELV did not differ significantly among the various conditions in both groups of rabbits (Table 2).

Static V-P relationships. In each animal, both before and after the prolonged ventilation on ZEEP or PEEP, the inflation V-P curve on PEEP could be closely fitted ($r > 0.95$) by a function in the form $V_o\cdot(1 - e^{-k\cdotPst})$, where $V_o$ is maximum volume above the resting volume of the lung and $k = 1/P$ is a shape factor (4, 19).

The group mean values of these constants during PEEP1 and PEEP2 are reported in Table 2. Because, in all animals, the values of $V_o$ and $k$ did not change after prolonged ventilation on ZEEP (group A) or PEEP (group B), a unique relationship could be used to describe the quasi-static lung V-P curve above the end-expiratory lung volume with PEEP, as shown in Fig. 1.

Elastance. On the basis of the $V_o$ and $\Delta$EELV values in Table 2, tidal ventilation with PEEP occurred in the range of 30–65% $V_o$. The average values of $E_{st}$ obtained under the various conditions in the two groups of animals are given in Table 3. During ventilation with PEEP, $E_{st}$ was almost the same before and after the prolonged period of ventilation on ZEEP (group A), as well as with PEEP (group B).

$R_{int}$. In all animals and conditions, $R_{int}$ was independent of flow; hence, the values of $R_{int}$ obtained in each animal and condition were averaged (Table 3). With PEEP1, $R_{int}$ did not differ significantly between groups A and B ($P = 0.45$). In group A rabbits, with PEEP2, $R_{int}$ increased significantly relative to PEEP1 in seven animals, decreased significantly in one, and was unchanged in two animals. On average, $R_{int}$ was, however, significantly increased [change ($\Delta$) in $R_{int} = 3.5 \pm 1.2$ cmH$_2$O·s·L$^{-1}$; $P < 0.02$] after the prolonged ventilation on ZEEP. On the other hand, in group B

| Table 1. $P_{aO_2}$, $P_{aCO_2}$, $pH_a$, and wet-to-dry ratio of the lung of group A and B rabbits during PEEP1 and PEEP2 |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | $P_{aO_2}$, Torr | $P_{aCO_2}$, Torr | $pH_a$ | Wet-to-Dry Ratio |
| **Group A**                    |                 |                 |       |                  |
| PEEP1                          | 85 ± 7          | 38.5 ± 3.5      | 7.34 ± 0.04 |                  |
| PEEP2                          | 94 ± 11         | 37.8 ± 4.5      | 7.30 ± 0.07 | 4.59 ± 0.07      |
| **Group B**                    |                 |                 |       |                  |
| PEEP1                          | 95 ± 7          | 35.5 ± 4.1      | 7.33 ± 0.09 |                  |
| PEEP2                          | 96 ± 15         | 34.0 ± 5.5      | 7.30 ± 0.07 | 4.72 ± 0.10      |

Values are means ± SE. $P_{aO_2}$, $P_{aCO_2}$, and $pH_a$: arterial PO$_2$, PCO$_2$, and pH, respectively. PEEP$_1$, ventilation with positive end-expiratory pressure (PEEP) of 2.3 cmH$_2$O before ventilation on zero end-expiratory pressure (ZEEP) (group A) or on PEEP (group B); PEEP$_2$, ventilation with PEEP of 2.3 cmH$_2$O after 3–4 h of ventilation on ZEEP (group A) or on PEEP (group B).

| Table 2. Mean values of constants in equation $V_o\cdot(1 - e^{-k\cdotPst})$ used to fit the lung inflation volume-pressure curve and $\Delta$EELV during PEEP1 and PEEP2 in groups A and B |
|---------------------------------|-----------------|-----------------|
|                                | $V_o$, ml       | $k$, cmH$_2$O$^{-1}$ | $\Delta$EELV, ml |
| **Group A**                    |                 |                 |                  |
| PEEP1                          | 75.7 ± 2.4      | 0.180 ± 0.005   | 24.2 ± 1.0       |
| PEEP2                          | 75.7 ± 2.4      | 0.176 ± 0.004   | 24.3 ± 1.2       |
| **Group B**                    |                 |                 |                  |
| PEEP1                          | 78.3 ± 4.0      | 0.187 ± 0.012   | 25.0 ± 1.4       |
| PEEP2                          | 80.5 ± 5.3      | 0.184 ± 0.011   | 25.5 ± 1.3       |

Values are means ± SE. $V_o$, maximum volume above resting volume; $k$, shape factor; $\Delta$EELV, difference between end-expiratory and resting lung volume.

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rabbits, the prolonged ventilation with PEEP did not change Rint significantly ($\Delta R_{\text{int}} = -0.6 \pm 0.5 \text{ cmH}_2\text{O} \cdot \text{s}^{-1}$; $P > 0.2$).

Viscoelastic properties. In all animals and conditions, a unique function in the form of Eq. 1 adequately described the experimental $\Delta R_{\text{TI}}$ relations ($r = 0.97$), allowing computation of $R_{\text{visc}}$ and $\tau_{\text{visc}}$. Figure 2A depicts the relationship of $\Delta R$ to TI obtained in one animal during ventilation with PEEP, before and after prolonged ventilation on ZEEP (left), and the average results obtained from the 10 lungs (right). Also shown in Fig. 2B are an individual (left) and the group mean relationship (right) obtained before and after prolonged ventilation on PEEP. No significant changes in $R_{\text{visc}}$ and $\tau_{\text{visc}}$ occurred before and after prolonged ventilation on ZEEP or on PEEP (Table 4).

Elastance. According to the $V_o$ values in Table 2, baseline tidal ventilation ($V_T = 25 \text{ ml}$) on ZEEP occurred in the range of 0–35% $V_o$. There was both an immediate and a progressive increase in Est with vent-

Table 3. Mean values of $E_{\text{st}}$ and $R_{\text{int}}$ of group A rabbits during PEEP$_1$, PEEP$_2$, ZEEP$_1$, and ZEEP$_2$, and of group B rabbits during PEEP$_1$ and PEEP$_2$

<table>
<thead>
<tr>
<th>Group</th>
<th>Est, cmH$_2$O/l</th>
<th>Rint, cmH$_2$O·s$^{-1}$</th>
<th>Est, cmH$_2$O/l</th>
<th>Rint, cmH$_2$O·s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEEP$_1$</td>
<td>178 ± 9</td>
<td>16.2 ± 1.1</td>
<td>ZEEP$_1$</td>
<td>219 ± 9†</td>
</tr>
<tr>
<td>PEEP$_2$</td>
<td>182 ± 10</td>
<td>19.7 ± 1.5*</td>
<td>ZEEP$_2$</td>
<td>242 ± 9*†</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEEP$_1$</td>
<td>166 ± 10</td>
<td>14.8 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEEP$_2$</td>
<td>166 ± 10</td>
<td>14.2 ± 0.7</td>
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</table>

Values are means ± SE. Est, quasi-static pulmonary elastance; Rint, interrupter resistance. *Significantly different from values on PEEP$_1$, $P < 0.01$; †significantly different from corresponding values on PEEP, $P < 0.01$.
titation on ZEEP (Table 3). With VT = 8 ml, Est was significantly larger than that for VT = 25 ml (ΔEst = 6.4 ± 1.8 cmH2O/l; P < 0.001); this was related to the pronounced “knee” in the lowest part of the dynamic inspiratory V-P curve that was practically absent during ventilation with PEEP, as shown in Fig. 3. Indeed, under the latter condition, Est at VT = 8 ml was significantly smaller than that at VT = 25 ml (ΔEst = −6.6 ± 1.2 cmH2O/l; P < 0.001).

Rint. As during ventilation with PEEP, Rint was independent of flow in all animals. The mean values of Rint obtained with ZEEP1 and ZEEP2 are shown in Table 3. Although larger, Rint with ZEEP1 was not significantly different from that with PEEP1 (ΔRint = 4.1 ± 2.5 cmH2O·s−1·l−1; P > 0.05), suggesting that, in the volume range of 35–65% Vo, there is little or no change in Rint. However, Rint increased with ZEEP2, becoming significantly larger than that with both PEEP1 (ΔRint = 11.4 ± 3.6 cmH2O·s−1·l−1; P < 0.01) and PEEP2 (ΔRint = 8.9 ± 3.1 cmH2O·s−1·l−1; P < 0.02).

Viscoelastic properties. Figure 2A, bottom, depicts the relationship of ΔR to Ti pertaining to one animal (left) and to the entire group (right) obtained with ZEEP1 and ZEEP2. In all animals and conditions, a unique function in the form of Eq. 1 adequately described the data points, with the mean values of Rvisc and τvisc being reported in Table 4. With ZEEP1, Rvisc increased significantly relative to that with PEEP1 (ΔRvisc = 13.8 ± 4.1 cmH2O·s−1·l−1; P < 0.02), and a further significant increase occurred between ZEEP1 and ZEEP2 (ΔRvisc = 8.7 ± 3.6 cmH2O·s−1·l−1; P < 0.05). In contrast, τvisc remained essentially the same under all conditions.

Histology

The results of Lm for the animals that underwent the prolonged period of ventilation on ZEEP (group A) and on PEEP (group B) are shown in Table 5. The Lm did not differ significantly between groups A and B, whereas the MIS, RIS, and total injury score were significantly greater in group A (P < 0.05). There was no histological evidence of lung edema on specimens from both groups A and B, in line with the normal values of the wet-to-dry ratio of the lung (Table 1), nor of focal alveolar collapse, hemorrhage, or epithelial desquamation in alveoli. Signs of mild inflammation, as judged from the presence of granulocytes in the air spaces, were found only in two out of seven animals of group A and one animal of group B.

DISCUSSION

Using an ex vivo model of lavaged rat lungs ventilated with physiological VT from ZEEP or PEEP above the lower inflection point on the static inflation V-P relationship of the lung, Muscedere et al. (17) showed that, on ZEEP, there was a significant increase in RIS and MIS. In line with the latter results, we found that the values of RIS and MIS were significantly higher in group A than B (Table 5). In group A rabbits, however, the MIS were substantially lower than those obtained in the lavaged rat lungs (17). Because lavaged lungs axiomatically exhibit greater regional structural inhomogeneity, such a discrepancy is predictable based on the concept of parenchymal interdependence postulated by Mead et al. (15). Thus marked regional structural inhomogeneity should enhance the shear stresses and related injury due to cyclic opening and closing of peripheral airways. This has been recently discussed in detail by Marini (14). In the present study, we have also measured Lm, which did not differ significantly between groups A and B, indicating that MV on ZEEP does not cause enlargement of air spaces compared with MV on PEEP.

Contrary to Taskar et al. (23), we have found that, in normal open-chest rabbits, ventilation at low lung vol-

![Fig. 3. Average relationships (solid lines) between ΔV and transpulmonary pressure (Ptp) obtained in 10 open-chest rabbits during PEEP1 and at ZEEP2. Symbols and dotted lines, corresponding static end-expiratory and end-inspiratory conditions for tidal volumes of 25 and 8 ml, respectively.](http://www.jap.org)
peripheral airways. This discrepancy is probably due to the fact that these authors ventilated their rabbits at low volume for only 1 h, compared with 3–4 h in the present study. In a subsequent study, Taskar et al. (22) found no evidence of lung injury in normal open-chest rabbits ventilated at low lung volume for 3 h. This was based on the following six parameters: namely, focal alveolar collapse, intra-alveolar edema, hema
line membranes, hemorrhage, epithelial desquamation in airways and alveoli, and presence of granulocytes in the air spaces. In both group A and B rabbits, there were no histological signs of alveolar injury, like hemorrhage, focal alveolar collapse, alveolar epithelial desquamation, or intra-alveolar edema, as also evidenced by normal values of lung wet-to-dry ratio (Table 1), whereas, at variance with the results of Taskar et al. (22), there was evidence of epithelial desquamation in the respiratory and membranous bronchioles (Table 5). It should be noted, however, that Taskar et al. (22, 23) did not use specific, quantitative indexes of peripheral airway injury like those used in the present study. Air space enlargements, emphysema-like lesions, bronchiectasis, and pseudocysts are characteristic feature of baro- and volotrauma in patients with severe respiratory distress syndrome (7). Such changes, which have also been found in pigs with multifocal pneumonia ventilated at high lung volumes (10), were absent in the present model of low-volume injury.

Lung injury during ventilation at low lung volumes is generally attributed to cyclic opening and closing of relatively small airways with concomitant generation of shear stresses that may be responsible for some of the lung damage (17, 18). With PEEP, there was no evidence of airway closure because, as shown in Fig. 1, the static inflation V-P curve of the lung was concave to the pressure axis (8). Accordingly, at PEEP of 2 cmH2O, the static compliance with VT = 8 ml was higher than that with VT = 25 ml (Fig. 3). In contrast, at ZEEP, the initial part of the static inflation V-P curve was convex to the pressure axis, and, accordingly, the compliance with VT = 8 ml was lower than that with VT = 25 ml (Fig. 3). This change in shape of the static V-P curve at low lung volumes has been attributed to airway closure (8). The site of closure, as determined by serial sections of quick-frozen dog lungs, is in small (≤1 mm in diameter) airways (11). Thus, based on the above-mentioned considerations, it appears that, during MV on ZEEP, the present rabbits exhibited cyclic airway opening and closing, which should be responsible for the changes in RIS and MIS, as well as the increase in Rint on PEEP2 relative to PEEP1.

On ZEEP, Ptr increased more markedly and rapidly at the onset of inflation than on PEEP, as illustrated in Fig. 4, which shows the time course of Ptr, V, and volume changes in a rabbit at PEEP1 and ZEEP2. During the initial 90 ms of inflation, the average rate of rise of Ptr [ΔPtr/Δtime (t)] on PEEP1 and ZEEP2 was 6.8 and 32.9 cmH2O/s, respectively. The corresponding average values for all rabbits were 6.1 ± 0.8 and 33.7 ± 2.9 cmH2O/s, respectively. The high values of ΔPtr/Δt on ZEEP probably contributed to the histological damage of the peripheral airways observed after ventilation on PEEP in group A. In contrast, in group B, the values of ΔPtr/Δt were low and almost constant throughout the ventilation period. The increase in the initial ΔPtr/Δt on ZEEP was due to increased impedance caused by atelectasis and/or airway closure.

Table 5. Lm, RIS, MIS, and TIS from lungs subjected to 3–4 h of ventilation on ZEEP (group A) or PEEP (group B)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Lm, μm</th>
<th>RIS, %</th>
<th>MIS, %</th>
<th>TIS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>(286–403)</td>
<td>(0–33)</td>
<td>(9–22)</td>
<td>(6–16)</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>(294–414)</td>
<td>0</td>
<td>2.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Values are medians with range in parentheses. n, No. of animals. Lm, mean linear intercept; RIS, respiratory injury score; MIS, membranous injury score; TIS, total bronchiole injury score. *P < 0.05, group A vs. B.

Fig. 4. Ensemble average of records of flow (V), ΔV, and tracheal pressure (Ptr) from 10 consecutive breath cycles in an open-chest rabbit during baseline ventilation with PEEP1 and after ZEEP2.
Two mechanisms can account for the increase of $Est$ that occurs on ZEEP: namely, an increase in stiffness of lung tissue due to larger surface forces, and a decrease in the amount of ventilated tissue caused by airway closure and/or alveolar collapse. Both mechanisms could also be responsible for the progressive increase in $Est$ with time. An increase in surface forces with time at low end-expiratory transpulmonary pressure and lung volume has been advocated to explain the changes in lung compliance in the absence of detectable airway closure (25, 26). However, changes in surface forces alone cannot account for lung behavior at very low lung volumes (20). Airway closure and atelectasis represent, therefore, conditions that may contribute to the progressive increase of $Est$ on ZEEP.

In fact, a theoretical study of Stamenovic and Wilson (21) indicates that regional mechanical inhomogeneities should lead to diffuse alveolar collapse at low transpulmonary pressures. Presence of focal atelectasis was found, however, only in one out of three additional rabbit lungs fixed after 4 h on ZEEP, with a transpulmonary pressure similar to the peak $Ptr$ during MV (~8 cmH$_2$O) to avoid reexpansion of collapsed areas, whereas, for essentially the same end-inspiratory pressure, the lung volume was ~25 ml larger on PEEP than on ZEEP (Fig. 3). Hence, atelectasis alone cannot account for such a volume reduction (~30% $V_e$). Accordingly, it is likely that small airway closure is the main mechanism leading to increased $Est$ during ventilation on ZEEP.

The present study shows, for the first time, that, on ZEEP, there is a significant time-dependent increase in $Rvisc$, whereas $\tau_{visc}$ does not change (Table 4). In principle, the same two mechanisms that have been invoked to explain the increase in $Est$ with ventilation on ZEEP could account for the concurrent increase of $Rvisc$ (Table 4). In line with previous observations in dog lungs (6, 12), most of the resistive properties of the rabbit lung arise from tissue, as $Rvisc$ was markedly larger than $Rint$ under all experimental conditions (Tables 3 and 4), and these mainly reside in the air-liquid interface (1). Changes in the properties of the surface film during ventilation at ZEEP could, therefore, have contributed to the increase in $Rvisc$. Increased inhomogeneity within the lung due to peripheral airway closure is another mechanism that could have contributed to the increase in $Rvisc$ at ZEEP. A decrease in the amount of ventilated tissue that occurs with airway closure and/or atelectasis could also cause an increase per se in $Rvisc$ without affecting $\tau_{visc}$. The fact that this was the case (Table 4) suggests that airway closure and/or atelectasis may have been the main cause of the changes in $Rvisc$ on ZEEP. Moreover, a reduction in ventilated tissue should have a proportional effect on both $Est$ and $Rvisc$. In fact, there was a highly significant correlation between changes in $Est$ and $Rvisc$ (Fig. 5).

Airway resistance has been found to increase with acute reductions in lung volume, and this is ascribed to the concomitant decrease in lung recoil (13). Indeed, $Rint$ increased with ZEEP$_1$, although not significantly (Table 3). It should be noted, however, that, as a result of the reduced lung compliance on ZEEP$_1$, the recoil pressure at end inflation was only slightly smaller than that on PEEP$_1$. At ZEEP$_2$, $Rint$ became significantly larger than on PEEP$_1$ (Table 3). The increase in $Rint$ between ZEEP$_1$ and ZEEP$_2$ cannot be related to loss of lung recoil, as $Est$ became even larger on ZEEP$_2$ than on ZEEP$_1$ (Table 3) and the transpulmonary pressure at end inflation was essentially the same as that with PEEP$_1$ (Fig. 3). Because the increase of $Rint$ on ZEEP occurred despite an increased lung recoil, these changes in $Rint$ should be due to damage of peripheral airways, as evidenced by the RIS and MIS (Table 5), and/or to increased bronchomotor tone.

After the return of group A rabbits to PEEP (PEEP$_2$), $Rvisc$ as well as $Est$ reversed to the initial (PEEP$_1$) values, whereas $Rint$ remained significantly ($P < 0.01$) larger (Table 3). The increase in $Rint$ on PEEP$_2$ could not be related to changes in arterial blood gases or $pHa$, as the latter were not significant (Table 1). Similarly, in group B animals, $PaO_2$, $PaCO_2$, and $pHa$ were essentially the same on PEEP$_1$ and PEEP$_2$, indicating that, on PEEP, gas exchange was stable during the entire experimental period. Because the elastic recoil of the lung was also the same on PEEP$_1$ and PEEP$_2$ (Fig. 1), the increase in $Rint$ was probably due to changes in mechanical properties of the peripheral airways, as reflected by the significant increase in RIS and MIS (Table 5), and/or increased bronchomotor tone caused by the release of inflammatory mediators on ZEEP. Although signs of inflammation, as evaluated by the presence of granulocytes in the air spaces, were very modest in both groups A and B, this does not exclude the possibility of a different release of inflammatory mediators in the two groups, because the number of inflammatory cells does not necessarily reflect their state of activation. In group A, the increase in $Rint$ between PEEP$_1$ and PEEP$_2$ averaged 3.5 cmH$_2$O·s$^{-1}$ (Table 3). Assuming that, under normal conditions, peripheral airway resistance ($Rpaw$) contributes 20% of $Rint$ (13), $Rpaw$ with PEEP$_1$ should have amounted...
to 3.2 cmH₂O·s⁻¹·l⁻¹. Thus, in the absence of changes in bronchomotor tone, Rₚₚₚₚ should have doubled from PEEP₁ to PEEP₂ (i.e., from 3.2 to 6.7 cmH₂O·s⁻¹·l⁻¹).

In the present open-chest rabbits, peripheral airway lesions were found throughout the lungs because pleural pressure was essentially uniform. In closed-chest, normal lung models, however, the lesions should be confined to the lower lung zones as a result of the vertical gradient in pleural surface pressure (9). Indeed, with a closed chest, peripheral airway closure at low volumes occurs preferentially in the dependent lung zones, which are subjected to lower transpulmonary pressure (16).

In conclusion, the present study shows for the first time that, in normal lungs of open-chest rabbits, 3- to 4-h MV with physiological Vₚ at ZEEP induces histological evidence of peripheral airway injury, with a concurrent increase in airway resistance (Rₚₚₚₚ), which persists after the return of MV to a PEEP value that restores normal end-expiratory volumes. In contrast, when shifting from ZEEP to PEEP, both Eₚₚ and Rᵥ return to the initial values observed with PEEP. That MV at low lung volume may cause airway damage in normal lungs is of substantial interest. In fact, our open-chest rabbit model may serve to study the effects of acute low-volume injury on release of pulmonary inflammatory mediators in the absence of preexisting alterations or lesions.

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