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Abstract

The involvement of pulmonary circulation in the mechanical properties was studied in isolated rat lungs. Pulmonary input impedance (ZL) was measured at a mean transpulmonary pressure (Ptpmean) of 2 cmH2O before and after physiological perfusion with either blood or albumin. In these lungs and in a group of unperfused lungs, ZL was also measured at Ptpmean values between 1 and 8 cmH2O. Airway resistance (Raw) and parenchymal damping (G) and elastance (H) were estimated from ZL. End-expiratory lung volume (EELV) was measured by immersion before and after blood perfusion. The orientation of the elastin fibers relative to the basal membrane was assessed in additional unperfused and blood-perfused lungs. Pressurization of the pulmonary capillaries significantly decreased H by 31.5 +/- 3.7% and 18.7 +/- 2.7% for blood and albumin, respectively. Perfusion had no effect on Raw but markedly altered the Ptpmean dependences of G and H < 4 cmH2O, with significantly lower values than in the unperfused lungs. At a Ptpmean of 2 cmH2O, EELV increased by 31 +/- 11% (P = 0.01) following pressurization of the capillaries, and the elastin [...]

Reference


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Impact of microvascular circulation on peripheral lung stability

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Impact of microvascular circulation on peripheral lung stability. Am J Physiol Lung Cell Mol Physiol 287: L879–L889, 2004. First published June 18, 2004; 10.1152/ajplung.00263.2003.—The involvement of pulmonary circulation in the mechanical properties was studied in isolated rat lungs. Pulmonary input impedance (ZL) was measured at a mean transpulmonary pressure (Ptpmean) of 2 cmH2O before and after physiological perfusion with either blood or albumin. In these lungs and in a group of unperfused lungs, ZL was also measured at Ptpmean values between 1 and 8 cmH2O. Airway resistance (Raw) and parenchymal damping (G) and elastance (H) were estimated from ZL. End-expiratory lung volume (EELV) was measured at Ptpmean values between 1 and 8 cmH2O, with significantly lower values than in the unperfused lungs. At a Ptpmean of 2 cmH2O, EELV increased by 31 ± 11% (P = 0.01) following pressurization of the capillaries, and the elastin fibers became more parallel to the basal membrane. Because the organization of elastin fibers results in smaller H values of the individual alveoli, the higher H in the unperfused lungs is probably due to a partial alveolar collapse leading to a loss in lung volume. We conclude that the physiological pressure in the pulmonary capillaries is an important mechanical factor in the maintenance of the stability of the alveolar architecture.

Forced oscillations; alveolar wall; elastin; end-expiratory lung volume

The mechanical properties of the lungs are significantly influenced by changes in the pulmonary hemodynamic conditions (3, 7, 9, 11, 20, 21, 27, 29, 35–37, 40). Numerous clinical (3, 7, 9, 11, 20, 27) and experimental studies (29, 36, 37) have demonstrated that elevation of the pulmonary blood pressure (Paw) and/or pressure (3, 11, 29, 36, 37) leads to a deterioration of the lung function via a decrease in functional residual capacity (FRC) (7) and/or stiffening of the alveolar wall (40). Although the qualitative examinations performed by von Basch (38a) in 1889 suggested that not only congestion, but also pulmonary hypoperfusion, can alter the lung configuration, few data are available concerning the changes in the mechanical conditions of the lungs during hypoperfusion or the complete absence of pulmonary perfusion (21, 25, 29, 35). Mitzner et al. (25) observed a transient increase in the respiratory resistance and a decrease in the compliance after occluding a pulmonary artery in mice, but the explanation of this finding remained unclarified. Furthermore, we recently demonstrated that low pulmonary venous pressures cause an impairment in lung mechanics, manifested in increases in the parenchymal resistive and elastic parameters, while the airway properties remain unaffected (29). However, the mechanisms responsible for the compromised lung mechanics at low vascular pressure or when there is no perfusion are still unclear. In the present study, we hypothesize that the pressurized pulmonary capillary network exerts a mechanical tethering effect that contributes to the maintenance of a normal lung function. In an attempt to test this hypothesis, we set out to investigate how the absence or presence of physiological perfusion affects the airway and parenchymal mechanical parameters and lung volume at different transpulmonary pressures in isolated rat lungs. We also examined whether or not the changes in lung function after the onset of pulmonary perfusion are similar for blood and albumin perfusates.

Methods

Preparation of lungs. The procedure for preparation of the isolated lungs was identical to that described in detail previously (29). After approval by the Animal Care Committee of the Canton of Geneva and by the Institutional Ethics Committee, 21 adult male Sprague-Dawley rats weighing 360–390 g were anesthetized with isoflurane (3% induction, 1.4% maintenance dose), tracheotomized with a polyethylene cannula (14-gauge; Braun, Melsungen, Germany), and mechanically ventilated (model 683; Harvard Apparatus, South Natick, MA) with a tidal volume of 7 ml/kg and a respiratory rate of 70–80 breaths/min while a positive end-expiratory pressure (PEEP) of 2.5 cmH2O was maintained. Airway opening pressure was monitored continuously (DP 45 transducer and 2D15 carrier demodulator; Validyne, Northridge, CA). The femoral artery was cannulated with a 28-gauge catheter (Portex, Hythe, UK) for monitoring the systemic blood pressure (model 156 PCE 06-G2; Honeywell, Zurich, Switzerland). The femoral vein was also cannulated for drug delivery. Heparin (1.5 IU/g) was then administered intravenously for complete anticoagulation of the blood. Next, 35 ml of arterial blood was gently withdrawn while the collected blood was continuously replaced by the intravenous infusion of colloid solution (hydroxyethyl starch 6%). This maneuver maintained a constant intravascular volume and a mean systemic blood pressure >50 mmHg and thus minimized the risk of ischemic lesions in the lungs. The collected diluted blood was centrifuged (4,000 rpm for 10 min), and 17 ml of plasma was extracted. The resulting concentrated blood with a hematocrit level of ~35% served as priming perfusate.

The chest was widely retracted after a midline sternotomy, and a polyethylene catheter (14-gauge, Braun) was placed into the main
pulmonary artery via the right ventricular outflow track, advanced until it was immediately proximal to the bifurcation, and was next connected to medical-grade silicone tubing [1.47-mm inner diameter (ID); Ulrich, St. Gallen, Switzerland]. The animals were then completely exsanguinated by widely opening the left ventricle and the left atrium. To minimize the warm ischemic time period until reperfusion, the lungs were immediately flushed via the pulmonary artery cannula with 30 ml of cold (10°C) hydroxyethyl starch 6% solution from a height of 30 cm. Through the left ventriculotomy, another catheter was placed into the left ventricle, into which a Combifix-7-Adapter (Bard, Orchard Park, NY) was tightly fixed and connected to medical-grade silicone tubing. Finally, a third catheter (polyethylene tubing, ID 0.88 mm, Portex) was introduced directly into the left atrium for measurement of the left atrial pressure (P la). The lungs and the heart were excised in a single block, dissected free of adjacent tissue, and weighed. The heart-lung block was suspended from an isometric force displacement transducer (Grass FT03; Quincy, MA) in a thermostabilized, humidified Plexiglas chamber. The lungs were ventilated with air mixed with 5% CO₂, and a respiratory rate of 50 breaths/min, a tidal volume of 7 ml/kg, and a PEEP of 2.5 cmH₂O were maintained. A series of hyperinflations (peak pressure of 25–30 cmH₂O) was applied by occluding the expiratory port of the ventilator until the atelectatic areas were completely abolished. Pulmonary vascular pressures were recorded continuously at end expiration. The pressure in the loudspeaker were measured successively at Ptp mean levels of 1, 2, 4, 6, and 8 cmH₂O. These pressure levels were set by adjusting the PEEP during ventilation to the corresponding Ptp mean level, and ZL was recorded at end expiration. The pressure in the loudspeaker chambers was also adjusted to the pressure level at which the oscillatory measurements were made. A 2-min period was kept between the changes in PEEP and the oscillatory measurements.

Study protocol. Ten to fifteen minutes after the onset of ventilation, a sigh was given to standardize the volume history. The PEEP level was then decreased to 2 cmH₂O, and four to six ZL recordings were collected in all lungs at end expiration while a mean transpulmonary pressure (Ptp mean) of 2 cmH₂O was maintained. After the start of perfusion in the lungs receiving either blood or 8% albumin, a period of ~3–5 min was allowed for stabilization of the physiological pulmonary hemodynamics. A sigh was given next, and another set of ZL measurements started 1 min later at the same Ptp mean level. These measurements allowed an estimation of the effects of the onset of perfusion in the normal range of breathing. To investigate how the perfusion affects the pressure dependence of the pulmonary mechanics, the unperfused lungs were ventilated until a steady-state condition had been established. The tracheal tube and the perfusion cannula were then clamped at a Ptp mean level of 2 cmH₂O, and the preparation was immersed in a glass cylinder containing 37°C saline to read its total volume. The heart-lung preparation was returned to the Plexiglas box, steady-state perfusion with blood was established, and the immersion procedure was repeated. The values of EELV were calculated by subtracting the volumes of the connecting tubing and of the heart-lung tissue from the total saline displacement. The EELV of the perfused lungs was corrected for the elevations in vascular volume; the weight of the heart-lung blocks was measured immediately after the immersions, and the increase in vascular volume due to the filling of the pulmonary capillaries with blood was calculated by dividing the increases in weight by the density of the blood.

Histological preparations. To assess the potential structural changes responsible for the altered lung mechanics, histological studies were performed on eight additional isolated lungs, which were prepared in the same manner as described above. Four of them remained unperfused; the others were perfused with blood while physiological perfusion pressures were maintained (Ppa = 17.5 mmHg, Pao = 7.5 mmHg). In each group, two lungs were ventilated during maintenance of a PEEP of 2 cmH₂O, whereas a PEEP of 8 cmH₂O was kept in the other two lungs. The lungs were then immersed in buffered 4% formaldehyde solution for 12 h while a Ptp mean corresponding to the level of PEEP was maintained. The perfused lung was kept under normal hemodynamic conditions for 1 h after immersion to ensure adequate lung fixation while the pulmonary capillary network was filled with blood. Lung specimens (1 from each side) were embedded in paraffin. Two 5-μm sections were prepared in each lung specimen and were stained with the Miller elastic van Gieson stain to label elastin fiber in the alveolar walls. The orientation of the elastin fibers was characterized by using an image analysis system (Q550-iw Quantimet, Leica) connected to a DMRBE microscope (Leica) via a video camera (Sony DYC 930t pr ccd). Image
acquisition was performed by using a ×63 dry objective (NPL-Fluothar 63/0.9) and a ×10 lens. Four to six gray images were randomly selected from each histological preparation, and the acute angles (<90°) of all elastin fibers relative to the local orientation of the basal membrane were recorded by using the interactive measurements facilities of QWin software. A total of 5–15 angles were obtained on each image, resulting in 250–350 angle readings in each condition (PEEP 2 and 8 cmH₂O in unperfused and blood-perfused lungs). We occasionally noticed folds and buckles in the basement membrane, particularly in the unperfused lungs. Because these folds were generally smaller than the length of the elastin fibers, we were able to estimate the angle between the basement membrane and the elastin fibers even in this condition.

Statistical analysis. Scatters in the parameters are expressed in SE values. The paired t-test was utilized to estimate the effects of the onset of perfusion on the mechanical parameters. Two-way repeated-measures ANOVA was used with the perfusate as the first variable and the Ptp mean level as the second variable to establish the effects of perfusion on the pulmonary mechanical parameters at different values of Ptp mean. One-way ANOVA was used to compare the protocol groups involved in the histological studies as concerns the angle of the elastin fibers. The Student-Newman-Keuls multiple comparison procedure was employed to compare the lung mechanical parameters under different conditions. In each test, a significance level of P < 0.05 was applied.

RESULTS

The animals involved in the three protocol groups were comparable with regard to body weight and baseline lung mechanical parameters (Table 1).

Lung mechanical measurements. Figure 1 shows the airway and tissue parameters before and after perfusion with blood or albumin. The changes in Raw and Iaw that occurred after the onset of perfusion with blood (7.5 ± 9.1% and 6.6 ± 4.3%, respectively) or 8% albumin (6.1 ± 4.9% and 10.1 ± 7.4%, respectively) were not significant. However, perfusion with blood and with 8% albumin each induced significant decreases in G (−31.8 ± 5.0% and −29.2 ± 2.1%) and H (−31.5 ± 3.7% and −18.7 ± 2.7%). A mild, but statistically significant, decrease in η was observed in the lungs perfused with 8% albumin (−12.7 ± 3.0%), whereas η fell only slightly on blood perfusion (−0.8 ± 3.8%).

Figure 2 illustrates the Raw values in the unperfused and the perfused lungs at different Ptp mean levels. In the unperfused lungs, an increase of Ptp mean from 1 to 8 cmH₂O induced significant stepwise decreases in Raw, whereas the return of Ptp mean to 1 cmH₂O resulted in similar increases in Raw. The dependence of Raw on Ptp mean was comparable in the perfused lungs, and there was no significant difference between the values of Raw in the three groups at any Ptp mean level. The invariance values were not affected by either Ptp mean or the perfusion (data not shown).

The parenchymal resistive and elastic parameters are plotted against Ptp mean in Figs. 3 and 4, respectively. In the unperfused lungs, the increase of Ptp mean induced gradual decreases in G and H until Ptp mean reached 4 cmH₂O, which was followed by marked and statistically significant increases in both parameters between 6 and 8 cmH₂O. The decrease of Ptp mean from 8 to 2 cmH₂O resulted in profound drops in G and H, the values at 4 and 2 cmH₂O being significantly lower than those obtained at the corresponding pressure levels during the increasing phase of Ptp mean. A further decrease of Ptp mean from 2 to 1 cmH₂O caused substantial increases in G and H, which approached their corresponding initial values. In the lungs perfused with either blood or 8% albumin, the changes in G and H were much less in the Ptp mean range between 1 and 4 cmH₂O. The differences between the G and H values in the unperfused and the perfused lungs were statistically significant at the Ptp mean levels of 1, 2, and 4 cmH₂O, but no differences were observed at all at higher pressures. The nature of the fluid perfused had no effect on G and H at any stage of the experiment. In all groups, the parallel changes in G and H resulted in fairly constant η values when Ptp mean was <6 cmH₂O. Slight, but statistically significant, decreases in η were observed at higher Ptp mean levels in both the unperfused lungs (−26.7 ± 3.1% between the Ptp mean levels of 2 and 8 cmH₂O) and those perfused with either blood (−24.0 ± 1.9%) or 8% albumin (−11.2 ± 4.1%). The η values in the protocol groups were not different at any level of Ptp mean.

Changes in lung volume. Perfusion with blood led to an increase in EELV from 3.5 ± 0.6 ml to 5.0 ± 0.2 ml (P < 0.05) at the Ptp mean of 2 cmH₂O. The changes in EELV (1.53 ± 0.50 ml) correlated well with decreases in G (r² = 0.76) and H (r² = 0.64). Furthermore, the relative increase in FRC after the onset of perfusion (31% ± 11%, P = 0.01) was similar in magnitude to the decreases in G (32% ± 6%) and H (29% ± 4%).

Lung histology. Figure 5 illustrates the lung histology in an unperfused isolated lung and in a lung perfused with autologous blood. In the unperfused lung, the shape of the alveolar wall is distorted and convoluted, whereas in the perfused lung, the alveolar structure appears smooth and regular. There is a near radial arrangement of the elastin fibers across the alveolar wall in the unperfused lung, which is in contrast with the linear distribution of the fibers around the alveolar septa, including perfused capillaries. Figure 6 summarizes the angles of the elastin fibers in the unperfused and perfused lungs fixed at normal and high Ptp mean levels. The angle of the elastin fibers relative to the local orientation of the basal membrane was significantly greater in the unperfused lungs when a Ptp mean of 2 cmH₂O was maintained, suggesting a radial distribution of the fibers in the alveolar septa. The significantly smaller angles in the other three groups of lungs demonstrated that the elastin fibers were distributed more tangentially to the basal membrane around the alveolar septa.

Table 1. Airway resistance (Raw) and inertance (Iaw), tissue damping (G), elastance (H), hysteresivity (η), and body weight (BW) under baseline conditions before onset of perfusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>Raw, cmH₂O·s/l</th>
<th>Iaw, cmH₂O·s²/l</th>
<th>G, cmH₂O/I</th>
<th>H, cmH₂O/I</th>
<th>η</th>
<th>BW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unperfused</td>
<td>11.3 ± 0.87</td>
<td>0.055 ± 0.0052</td>
<td>215 ± 13</td>
<td>1594 ± 87</td>
<td>0.135 ± 0.0061</td>
<td>331 ± 19</td>
</tr>
<tr>
<td>Blood perfusion</td>
<td>12.3 ± 0.68</td>
<td>0.057 ± 0.0033</td>
<td>272 ± 30</td>
<td>1855 ± 164</td>
<td>0.145 ± 0.0044</td>
<td>347 ± 12</td>
</tr>
<tr>
<td>Albumin perfusion</td>
<td>10.8 ± 0.53</td>
<td>0.065 ± 0.0038</td>
<td>226 ± 15</td>
<td>1534 ± 108</td>
<td>0.148 ± 0.0024</td>
<td>373 ± 16</td>
</tr>
<tr>
<td>P value</td>
<td>0.33</td>
<td>0.23</td>
<td>0.15</td>
<td>0.18</td>
<td>0.14</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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Perfusion parameters. Figure 7 depicts the changes in weight, $R_v$, and $Q_p$ with altered $P_{tp\text{mean}}$ in the perfused lungs. The stability of lung weight throughout the study protocol indicates the absence of edema in our experiments. $R_v$ was generally lower and $Q_p$ was systematically higher in the lungs perfused with albumin. Increases of $P_{tp\text{mean}}$ did not have statistically significant effects on the weight, $R_v$, or $Q_p$ in the lungs perfused with either blood or albumin.

DISCUSSION

The present study was designed to investigate the combined effects of pulmonary perfusion and inflation pressure on the mechanical properties of the pulmonary system in isolated rat lungs. We demonstrated that 1) the reestablishment of pulmonary perfusion significantly decreased the viscous resistance and the elastance of the parenchyma, whereas it had negligible effects on airway mechanics, 2) the decreases in the parenchymal mechanical parameters were associated with increases in lung volume, 3) the filling of the pulmonary vasculature markedly affected the pressure dependence of the pulmonary mechanical properties, 4) the effects of perfusion did not depend on the hemoglobin content of the perfusate, and 5) the orientation of the elastin fibers in the alveolar septa was more radial in the unperfused lungs at a $P_{tp\text{mean}}$ level of 2 cmH$_2$O than for those with an intact pulmonary microcirculation, and a higher $P_{tp\text{mean}}$ led to a significant reorganization of the elastin fiber network with near circumferential orientation.

Methodological considerations. The isolated rat lung model offers ideal conditions for comparison of the mechanical behavior of unperfused lungs with that of lungs perfused under the physiological hemodynamic conditions encountered in vivo. In this experimental setting, the interactions between pulmonary perfusion and lung mechanics can be determined in the absence of confounding systemic hormonal and neurogenic influences (39). Furthermore, the in vitro model applied in the present investigation allows control of the intra-alveolar CO$_2$ level through maintenance of a constant inspired and expired fraction of CO$_2$. Thus the potential biasing effects of hypocapnia on the lung mechanics (28, 33) were also avoided.

To decide whether the physiological transport of metabolites maintained by the blood plays a part in the distinct difference in parenchymal mechanics between unperfused and perfused lungs, we further applied 8% albumin as a perfusate. This solution does not contain hemoglobin, which plays an important role in the transport abilities of the perfusate and additionally modulates the pulmonary vascular tone (32).
identical mechanical behavior of the lungs was observed with these two perfusates, it can be suggested that the mechanical effects of the perfusates on the alveoli played the major role in our findings.

Another feature of the blood and albumin perfusions is that they resulted in significantly different $Q_p$ and $R_v$ levels while the same arterial-venous pressure gradient was maintained in the lungs. Although the lower $R_v$ and the higher $Q_p$ for albumin can be attributed to its lower viscosity, the differences in $R_v$ and $Q_p$ (~1.5 times) are smaller than the difference in the viscosities of blood with 35% hematocrit and plasma in the pulmonary circulation (~1.9 times) (1). This discrepancy could be explained by the lack of hemoglobin in the albumin perfusate, which may have led to a moderate vasoconstriction by inhibiting the release of nitric oxide from the pulmonary epithelium due to a decreased shear stress (32). Nevertheless, neither the different $Q_p$ nor the possible vascular contraction during albumin perfusion was reflected in the lung mechanical parameters, which indicates the primary importance of the intravascular pressure in the pulmonary circulation in our findings.

The pulmonary mechanical parameters obtained in the present study are in good agreement with earlier data on isolated perfused rat lungs (29). Although the values of EELV in the perfused lungs are ~30% higher than those estimated in vivo by the nitrogen closed-circuit method (16) and body plethysmography (18), this can be explained by the different lung configuration and transpulmonary pressure prevailing in the closed-chest supine animals.

**Effects of perfusion on lung mechanics.** We found that the onset of blood or 8% albumin perfusion caused an immediate and significant improvement in the lung parenchymal function, characterized by simultaneous decreases in the magnitudes of the tissue elastic ($H$) and viscous ($G$) properties (Fig. 1). Interestingly, the airway properties were not affected by perfusion. Two distinct mechanisms can produce decreases in $G$ and $H$. The improvement can be attributed either to changes in the intrinsic mechanical properties of the parenchyma (40) or to an increase in the lung volume available for gas exchange as a result of the reopening of air spaces (3).

The intrinsic viscoelastic properties of the parenchyma are determined by a number of important structural components, such as the connective tissue network, the interstitial cells, and the surface lining layer (41). Indirect effects of the altered airway properties on the parenchymal mechanics (24) can be excluded, as no difference in airway properties was observed between the protocol groups. Changes in the surface-active forces were also unlikely to contribute to our findings for the following reasons. Surface film properties contribute significantly to the dynamic lung stiffness only when the lung volume is cycled through large excursions (31), which was not the case in our measurements. Furthermore, since the half-life for the clearance of surfactant is far longer than the length of the experiment (15), and the absence of pulmonary perfusion leads

![Fig. 2. A–D: changes in $R_{aw}$ with altered mean transpulmonary pressure ($P_{twp}$) in unperfused lungs and in lungs perfused with blood or 8% albumin. Arrows indicate the sequence of change in $P_{twp}$. *Significant intragroup changes compared with the initial value obtained at $P_{twp} = 2$ cmH$_2$O.](image-url)
to an increase in surface tension (6), there is no reason to assume a diminished surfactant function in the unperfused lungs. It is also possible that the higher lung inflation in the perfused lungs stimulates epithelial type II cells to produce surface-active material (13); however, this is not likely to play a role, since an excess of surfactant compared with normal physiological levels makes little difference in lung mechanics. The proportional changes in G and H, indicating the lack of ventilation heterogeneities (2, 26), also confirm the absence of a surfactant dysfunction, which would have led to heterogeneous closures of peripheral airways at low lung inflation pressures (2, 26). It is also unlikely that the interstitial cells played a major role in the changes following the onset of perfusion, since perfusion with either 8% albumin or blood resulted in a similar lung mechanical response, while only blood is expected to provide full physiological nutritive support for these cells. It is therefore plausible to suggest that configurational changes in the connective tissue network were primarily responsible for the prompt improvement in the intrinsic parenchymal properties.

Collagen and elastin fibers are the principal constituents of the connective tissue network, and the interactions between these components contribute significantly to the viscoelastic properties of the parenchyma (23, 41). It has been demonstrated that the elastin and collagen fibers are connected in parallel mechanically (5), and their distributions, orientation, and interaction dominate in determining the mechanical behavior of the lung parenchyma (41). Our results can be interpreted in the context of these findings: the histological findings in Fig. 5 suggest that, in the absence of capillary pressurization, the contours of the alveoli are convoluted and there is a wide distribution of elastin fiber orientation, with some fibers running perpendicular to the direction of the basal membranes (Fig. 6). After the onset of capillary pressurization, however, the capillaries become turgid, which in turn eliminates the convoluted nature of the alveolar wall and results in a significant reorganization of the elastin fibers (Figs. 5 and 6). Reestablishment of the physiological pressures in the pulmonary capillaries therefore helps the alveoli to regain their optimal geometry.

To estimate how such reorganization of the elastic fibers affects the alveolar wall mechanics, we derived equations for Young’s modulus of a fibrous material as a function of the uniaxial stretch ratio, the elasticity of the fibers, Young’s modulus of the matrix, and the distribution of the angles of the fibers with respect to the direction of the deformation (see Eqs. A9 and A10 in APPENDIX A). Assuming that the contribution of the matrix is small, with use of the measured distributions of angles in Fig. 6, we find that Young’s modulus of the nonperfused tissue decreases from 75% of Young’s modulus of the tissue with filled capillaries to 65% when the stretch ratio increases from 5 to 50%. This can readily be understood: when an alveolus is inflated in the perfused lung, the elastin fibers running parallel to the wall experience stretching and hence contribute to the elastic resistance of the alveolus to a degree depending on the extent to which the elastin contributes to the

Fig. 3. A–D: changes in G with altered Ptp mean in unperfused lungs and in lungs perfused with blood or 8% albumin. Arrows indicate the sequence of change in Ptp mean. *Significant intragroup changes compared with the initial value obtained at Ptp mean = 2 cm H2O. # Significant intergroup differences from the corresponding values obtained during blood perfusion.
stiffness. In contrast, when the unperfused alveolus is stretched, the fibers at a large angle with respect to the wall do not contribute significantly to the elastic resistance of the alveolus.

The above calculations demonstrated that Young’s modulus of the alveolar wall is larger in the lung with pressurized capillaries. The elastance of the alveolus also depends on the prestress or tension in the wall tissue and the surface tension at the air-liquid interface. The prestress was controlled and was the same in the two conditions. On the basis of the discussion above, surfactant is not likely to contribute here, and hence the elastance of the perfused lung should be larger than that of the unperfused lung. However, our data in Fig. 1 suggest just the opposite behavior at the organ level, since both G and H dropped significantly on filling of the pulmonary capillaries.

To resolve this apparent contradiction, we hypothesize that the decreased elasticity of the alveoli in the unperfused lung causes the alveolar ducts to lose their elasticity, and at transpulmonary pressures lower than 4 or 6 cmH2O, they are prone to closure. If a significant number of alveoli are collapsed, the lung elastance as measured by H increases. When perfusion starts at physiological vascular pressures, the convoluted walls of the alveoli become smooth, their elasticity increases, and they reopen, allowing the communication of many more alveoli with the trachea. The reopening of alveolar units, manifested in increases in EELV, results in a significant decrease in the total lung elastance or H. The presence of alveolar derecruitment and recruitment in the unperfused lungs with changes in Ptpmean can also be substantiated from the hysteresis observed in G and H (see Figs. 3 and 4). The decreases in G and H on increase of Ptpmean from 1 to 4 cmH2O are most likely due to alveolar recruitment. A Ptpmean of 6 cmH2O is sufficient to recruit the entire unperfused lung, whereas decrease of Ptpmean to <4 cmH2O leads to alveolar decruitment, indicated by the steep increases in G and H. In APPENDIX B, we derive equations for Raw and lung elastance as a function of the number of alveoli communicating with the trachea when only the last-generation airways are closed in a tree structure. The simulation results illustrated in Fig. 8 indicate that the sensitivity of lung elastance to closure of the last-generation airways is much larger than that of Raw, which provides an explanation for the finding that G and H decrease, whereas Raw remains practically constant after the pulmonary capillary filling. Both the increases in EELV and the return of the alveolar architecture to normal physiological structure, as revealed by the histology, suggest that the pressurized pulmonary capillaries contribute to maintenance of the normal physiological alveolar geometry.

Effects of perfusion on pulmonary mechanics at different transpulmonary pressures. The concept that vascular engorgement leads to an increased lung volume and lung stiffening at low lung volumes was first proposed by von Basch (38a) in 1889. This qualitative observation was confirmed by Hogg et al. (14), who demonstrated a tendency to a positive correlation between blood flow and lung expansion. Subsequent studies involving lung function measurements have provided further
evidence that pulmonary vascular congestion leads to an increase in lung size and/or stiffness at low lung volume by overstretching the capillary network in the alveolar wall (10, 29, 36, 37). It seems plausible to adapt this mechanism to explain the marked differences in the parenchymal parameters between the perfused and unperfused lungs at low lung volumes on the basis of the stabilizing role of the filled pulmonary capillaries in the maintenance of the physiological alveolar architecture. The contribution of the pressurized capillary network is essential at lung volumes around or below FRC (which corresponds to Ptp mean of 2 cmH2O in the rat), where the distending pressure alone is apparently unable to prevent the collapse of alveoli and the subsequent increase in tissue impedance, as demonstrated in Figs. 3 and 4. During the decrease of Ptp mean, a critical pressure level is reached at ~2 cmH2O, where G and H in the unperfused lungs suddenly start to increase. At higher lung inflation pressures (Ptp mean > 6 cmH2O), this mechanism loses its importance, and the tissue impedance seems to be determined solely by the transpulmonary pressure.

**Physiological implications.** The mechanism of the circulatory-respiratory mechanical interaction discussed above may be of relevance in decreasing the ventilation/perfusion mis-
match in the lungs. Assuming that our findings in whole lungs are also valid for the regional lung mechanics in vivo, lung areas with decreased or diminished pulmonary capillary pressures would impose a higher parenchymal impedance against the distension of the air spaces, which in turn would contribute to a redirection of the airflow to the more compliant parts of the lungs, i.e., to perfused alveoli with physiological capillary pressures. This adaptation mechanism may be of importance in situations where impairment in pulmonary capillary perfusion may occur (e.g., in hypovolemia or embolism). This phenomenon may also be involved in the airflow redistribution caused by hypocapnia-induced airway narrowing (28, 33) that occurs during hypoperfusion.

**APPENDIX A: UNIAXIAL STRESS-STRAIN RELATIONSHIP OF FIBROUS MATERIAL**

A general theory of the stress-strain relationship of soft tissues with wavy fibers embedded in a fluid matrix was derived by Lanir (19) using micromechanical considerations and tensor calculus. Instead of considering a special case of this general theory, here we derive a stress-strain relationship for fibrous material embedded in an elastic matrix. The calculations are based on elementary considerations that lead to a formulation suitable for easy calculation of the contribution of the fibers. We first consider a two-dimensional case, which is then generalized to three dimensions.

Let us assume that we have a two-dimensional body of incompressible elastic material with length ($L$) and width ($b$) before deformation. The body contains linearly elastic fibers with arbitrary orientation ($\Theta$), length ($l$), and spring constant ($k$). We attach a rectangular coordinate system to the body as shown in Fig. 9. Without loss of generality, we assume that a fiber originates from the center of the coordinate system and makes an angle $\Theta$ with the x-axis. The length of the fiber is $l$ and its end point is $P$. When the body is stretched in the x-direction, its length becomes $L'$. Due to incompressibility, the volume $V = Lb$ does not change after deformation ($L'b' = L'b$) and hence the new width is $b' = bL/L'$. The point $P$ with coordinates ($x$ and $y$) will move to $P'$ with coordinates ($x'$ and $y'$) given by the following transformations

$$x' = \frac{L'}{L}x = \frac{L'}{L}l \cos \Theta$$

and

$$y' = \frac{L'}{L}y = \frac{L'}{L}l \sin \Theta.$$  \hspace{1cm} (A2)

The new length $l'$ of the fiber is given by

$$l' = (x'^2 + y'^2)^{1/2} = \frac{L'}{L}Q$$

where

$$Q = \left(\cos^2 \Theta + \frac{l^4}{L'^2} \sin^2 \Theta\right)^{1/2}. \hspace{1cm} (A3)$$

The force $F$ on the fiber is the displacement $u = l' - l \times k$

$$F = kl'(l' - l) = kl'\left(\frac{L'}{L} - 1\right). \hspace{1cm} (A5)$$

To calculate the true stress ($\sigma_t$) in the direction of the strain, we first calculate the x-component of the force on the fiber and normalize it with the cross-sectional area $b' = bL/L'$ of the body after deformation. Because the orientation of the fiber after deformation is $\Theta'$, the stress contributed by a fiber with original length $l$ and angle $\Theta$ is

$$\sigma_t(l, \Theta) = \frac{kL'}{b'L} \left(\frac{L'}{L} - 1\right) \cos \Theta. \hspace{1cm} (A6)$$

Eq. A6 still contains the unknown $\cos \Theta'$ that, from Fig. 9, can be written as $x'/l'$. With the use of Eqs. A1 and A3, the $\cos \Theta'$ can be written as $\cos \Theta/Q$ and Eq. A6 becomes

$$\sigma_t(l, \Theta) = \frac{kL'}{bLQ} \left(\frac{L'}{L} - 1\right) \cos \Theta. \hspace{1cm} (A7)$$

It is interesting to consider the special cases when the fiber is parallel ($\Theta = 0$) or perpendicular ($\Theta = \pi/2$) to the direction of the macroscopic strain. In the former case, $Q = 1$ and Eq. A7 reduces to Eq. A5 normalized by the cross-sectional area, whereas in the latter case, the stress contributed by the fibers is 0.

Let us now consider the case when there are many fibers with a distribution of their orientation and length. Let the number of fibers with length between $l$ and $l + dl$ and angle between $\Theta$ and $\Theta + d\Theta$ per unit volume be $n(l, \Theta)d\Theta$ in the undeformed body. The total stress due to all fibers and the matrix can be expressed as

$$\sigma = \int_{l}^{L} \int_{0}^{\pi/2} \frac{kL'}{bL} l \left(\frac{L'}{L} - 1\right) \cos \Theta n(l, \Theta)d\Theta d\Theta + \frac{Y_m}{L} \left(\frac{L'}{L} - 1\right) \hspace{1cm} (A8)$$

where $Y_m$ is Young’s modulus of the matrix. For small deformations, $Q$ is nearly unity, and the term $(QL'/L - 1)$ is essentially the uniaxial strain $\epsilon = (L'/L - 1)$, which can be taken out of the integral. Eq. A8 is then written in the form of a Hook law or linear stress-strain relationship with an equivalent Young’s modulus that depends on the

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**Fig. 8.** Relative increases in $R_{aw}$ and lung elastance (EL) as functions of open terminal units in the lungs. Note the logarithmic scale on the y-axis. See APPENDIX B for further details.

**Fig. 9.** Deformation of a 2-dimensional body and a fiber inside the body during uniaxial stretching along the x-axis.
elastance of the matrix and the fibers as well as the length and orientation distribution of the fibers

\[ Y = \frac{kL'}{bL} \int_{l-o}^{l+b} \frac{1}{Q} \cos \Theta n(l, \Theta) dl d\Theta + Y_w. \quad (A9) \]

The above equations are readily extended to the case of a three-dimensional body. If we denote the angle between the z-axis and the fiber with \( \Lambda \), then assuming that the dimension of the body in the z-direction is also \( b \), Eq. A9 becomes

\[ Y = \frac{kL'}{b^2L} \int_{l-o}^{l+b} \frac{1}{Q} \cos \Theta \sin \Lambda n(l, \Theta, \Lambda) dl d\Lambda + Y_w. \quad (A10) \]

where

\[ R = \left( \frac{\cos^2 \Theta \sin^2 \Lambda + L_1^3 \sin^2 \Theta \sin^2 \Lambda + L_3^3 \cos^2 \Lambda}{L_1^3 \sin^2 \Theta \sin^2 \Lambda + L_3^3 \cos^2 \Lambda} \right)^{1/2}. \quad (A11) \]

It is important to note that because of the gradual folding of the fibers into the direction of the macroscopic strain, the equivalent Young's modulus is no longer constant but increases with increasing stretch ratio \( L'/L \). Finally, Eqs. A9-A11 are now suitable to evaluate the relative contribution of fiber orientation to the Young's modulus of the alveolar wall.

**APPENDIX B: RESISTANCE AND LUNG ELASTANCY AS A FUNCTION OF TERMINAL AIRWAY CLOSURE**

We model the lung as a symmetric binary tree with elastic alveoli attached to the terminal airways. The diameter and length of the trachea are denoted by \( D_0 \) and \( L_0 \), respectively. We introduce a scaling relationship as: the diameter and length of the airways at generation \( k + 1 \) (\( D_{k+1} \) and \( L_{k+1} \), respectively), scaled versions of the diameters and length of the parents \( D_k \) and \( L_k \)

\[ D_{k+1} = dD_k \quad \text{and} \quad L_{k+1} = lL_k \quad (B1) \]

where \( d < 1 \) and \( l < 1 \). Because we assume that \( d \) and \( l \) are constant scaling factors, airway dimensions can also be written as

\[ D_k = d^kD_0 \quad \text{and} \quad L_k = l^kL_0. \quad (B2) \]

The resistance of airway segments is modeled by the Poiseuille flow resistance. Taking into account Eq. B2, the resistance at generation \( k \) is given by

\[ R_k = C_l \frac{L_k}{D_k^4} = \frac{1}{d^k} C \frac{L_0}{D_0^4}. \quad (B3) \]

where \( C \) is the product of a numerical factor and air viscosity. Introducing the scaling factor \( \beta = l/d^4 \) and realizing the \( CL_0/D_0^4 \) is the resistance \( R_0 \) of the trachea, we obtain

\[ R_k = \beta^k R_0. \quad (B4) \]

We can now calculate the total resistance of the tree starting from the left bottom corner. If the maximum generation number is \( N \), the parallel combination of two terminal segments is \( R_{N/2} \), which is in series with a resistance of \( R_{N-1} \) so that the resistance becomes \( R_{N/2} + R_{N-1} \). We have two segments with resistance \( R_{N-1} \) in parallel connected to \( R_{N-2} \) in series. We can continue with this process and roll up the entire tree. Thus the total resistance \( R_{aw} \) is

\[ R_{aw} = \ldots (R_{N/2} + R_{N-1})/2 + R_{N-2}/2 + \ldots )/2 + R_{aw}. \quad (B5) \]

Multiplying with the one-half factors and rearranging Eq. B5 leads to

\[ R_{aw} = R_0 + \frac{1}{2} R_1 + \frac{1}{2} R_2 + \ldots + \frac{1}{2} R_N. \quad (B6) \]

By introducing \( \delta = \beta/2 \) and substituting Eq. B4 into Eq. B6, we obtain

\[ R_{aw} = R_0(1 + \delta + \delta^2 + \ldots + \delta^{N-1}). \quad (B7) \]

Eq. B7 is a geometric series that has the following closed form solution

\[ R_{aw} = R_0 \frac{1 - \delta^{N+1}}{1 - \delta}. \quad (B8) \]

Let us partition Eq. B7 into two terms. The first contains the geometric series up to \( N \) and the second term is the \( N \)th term from the series. Adding up the series to \( N \) and substituting \( \delta = \beta/2 \) into the \( N \)th term, we obtain the following expression

\[ R_{aw} = R_0 \frac{1 - \beta^N}{1 - \beta} + \frac{\beta R_0}{2^N}. \quad (B9) \]

For a given tree with fixed \( l, d, \) and \( N \), the first term in Eq. B9 is constant. If we start closing the terminal airways one by one, then the denominator \( (2^N) \) in the second term becomes less and less while the nominator and the first term do not change. If \( p \) denotes the percent of the terminal airways at generation \( N \) that are open, then while \( p \) decreases from 1 to 0, the number of open terminal airways decreases from \( 2^N \) to 0. In the limit when \( p = 0, R_{aw} \) is infinite. For computational purposes, Eq. B9 can simply be written

\[ R_{aw} = C_1 + C_2 \frac{1}{p}. \quad (B10) \]

With regard to the elastance of the model, the alveoli are modeled as elastic elements in parallel. Thus the total elastance \( (E) \) of the model as a function of \( p \) is given by

\[ E = E_0 \frac{p}{p}. \quad (B11) \]

where \( E_0 \) is the elastance of a single alveolus. In the numerical simulations, we chose \( N = 16 \). The values of \( l \) is 0.8 and \( d \) is 0.8 were obtained based on the work of Kitaoka and Suki (17), which result in \( \delta = 0.976 \).

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