Morpho-functional analysis of lung tissue in mild interstitial edema

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Conforti, Elena, Carla Fenoglio, Graziella Bernocchi, Ombretta Bruschi, and Giuseppe A. Miserocchi. Morpho-functional analysis of lung tissue in mild interstitial edema. Am J Physiol Lung Cell Mol Physiol 282: L766–L774, 2002; 10.1152/ajplung.00313.2001.—Mild pulmonary interstitial edema was shown to cause fragmentation of interstitial matrix proteoglycans. We therefore studied compartmental fluid accumulation by light and electron microscopy on lungs of anesthetized rabbits fixed in situ by vascular perfusion after 0.5 ml·kg⁻¹·min⁻¹ iv saline infusion for 180 min causing ~6% increase in lung weight. Morphometry showed that a relevant portion (~44%) of extravascular fluid is detected early in the alveolar septa, 85% of this fluid accumulating in the thick portion of the air-blood barrier. The arithmetic mean thickness of the barrier increased in interstitial edema from 1.06 ± 0.05 (SE) to 1.33 ± 0.06 μm. The harmonic mean thickness increased from 0.6 ± 0.03 to 0.86 ± 0.07 μm, mostly due to thickening of the thin portion causing an increase in gas diffusion resistance. Despite some structural damage, the air-blood barrier displays a relatively high structural resistance providing a safety factor against the development of severe edema. It is suggested that the increase in extra-alveolar perivascular space occurs as a consequence of fluid accumulation in the air-blood barrier. thin and thick portions of the air-blood barrier; extracellular matrix; fibrillar collagen; basement membranes; gas diffusion; fluid accumulation; proteoglycans; top and bottom lung regions

THE PRESENT STUDY WAS STIMULATED by recent results obtained from our group using the model of mild pulmonary interstitial edema. The data showed that in this condition there is partial fragmentation of matrix proteoglycans (13–16), with minor differences in the sequence of the process seen when one compares edema caused by saline loading to that caused by elastase injection; in fact, in both cases there was early activation of metalloproteases, in particular matrix metalloproteinase-9 that is of neutrophil origin. This suggested that a common inflammatory response was triggered in both the “hydraulic” and the “lesional” type of edema. On the basis of these results, we were prompted to quantify the distribution of the interstitial fluid in the thin portion of the air-blood barrier where heparan sulfate proteoglycans control microvascular permeability of the basement membranes and in the thick portion of the air-blood barrier where chondroitin sulfate proteoglycans are responsible for tissue mechanical properties. We also compared fluid accumulation in the air-blood barrier with that in the extra-alveolar tissue. We reasoned that studying the transition phase toward the development of severe edema would help us to understand the early events eventually leading to massive fluid accumulation in the extra-alveolar perivascular and peribronchial adventitial spaces and in the alveolar septa (3, 8, 17).

MATERIALS AND METHODS

Eleven adult New Zealand rabbits [2.6 ± 0.4 (SD) kg body wt] were anesthetized with a cocktail of 2.5 ml/kg 50% (wt/wt) urethane and 40 mg/kg body wt of ketamine injected into an ear vein. The animals were placed supine and tracheotomized, and the trachea was cannulated to allow spontaneous breathing. Four animals were used to induce pulmonary interstitial edema (treated group) with a protocol lasting ~3 h, as specified below; three animals were killed shortly after anesthesia and used as controls; four more animals were kept under anesthesia for 3 h and used as sham. The comparison between controls and sham allowed to evaluate the possible effect of prolonged anesthesia on the distribution of interstitial fluid.

Hydrostatic Edema Protocol

For the treated group, the right superior jugular vein was cannulated for saline infusion that was continued for 3 h at a rate of 0.5 ml·kg⁻¹·min⁻¹. Infusion of about twice the control plasma volume (3.5% of body wt) leads to a slow development of interstitial edema (11) characterized by: 1) a marked increase in pulmonary interstitial pressure (from ~10 to ~4 cmH2O), 2) a negligible increase in extravascular lung water, 3) an albumin escape rate as low as 0.05 mg·h⁻¹·g body wt⁻¹, and 4) a plasma volume expansion of 12–15%. We took a blood sample in control conditions and at the end of the experiment, before starting the perfusion procedure, to determine hematocrit.

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In Situ Perfused Lung Preparation

Through a midsternal splitting incision, we opened the chest to expose the pericardium, with the pleural sacs left intact, thereby preserving the physiological lung expansion. After opening the pericardium, we cannulated the pulmonary artery by advancing a catheter via the right ventricle; a ligature was passed and tied around the pulmonary artery; the left atrium was snipped to allow blood and perfusate to drain from the system. Perfusion was carried out by using a gravity perfusion apparatus that consisted of two reservoirs arranged in parallel and connected to the pulmonary artery. The reservoirs contained, respectively, saline (11.06 g NaCl/l plus 5% dextran T-70 and 1,000 U heparin/dl, 350 mosM) and fixative (phosphate-buffered 2.5% glutaraldehyde plus 3% dextran T-70, total osmolarity 500 mosM, pH 7.4). The upper level of the liquids in the reservoirs was adjusted at a height of 15 cmH2O relative to left atrium level and maintained constant during perfusion. This pressure gradient was chosen as it corresponds to that measured in previous studies (11) between pulmonary artery and left atrium pressures (~20 and 5 cmH2O, respectively) with the beating heart in the same experimental conditions. An overdose of anesthesia was given to the animal before the perfusion. The perfusion circuit was first primed with saline for ~3 min till the outflow appeared clear of blood cells. Then fixative was perfused for 25–30 min. At the end of the perfusion fixation, the lungs were removed from the chest and weighed, and their volume was measured by liquid displacement, through immersion in phosphate buffer.

Definitions of Lung Compartments

We obtained tissue samples from the uppermost (top) and the lowermost (bottom) part of the lung for light and transmission electron microscopy morphometry from control, sham, and treated animals kept in supine posture. The following definitions were used to define the pulmonary compartments: bottom region: from 0 up to ~1.5 cm lung height (total lung height in supine posture ~6 cm); top region: from 3.5 to ~5 cm height; lung tissue (structures providing weight, including blood): alveolar septa + extra-alveolar vessels + extra-alveolar airways (excluding air); lung parenchyma: alveolar septa + alveolar air; alveolar septa: alveolar tissue + capillaries; alveolar tissue: endothelial and epithelial cells + interstitium; interstitium: interstitial cells + extracellular matrix.

Tissue Sampling

The top and bottom regions of the right lungs of control, sham, and treated animals were dissected. Tissue samples were obtained according to a stratified random sampling scheme; this is a suggested method for lung tissue because it compensates for regional differences, which are known to exist in the lung (21). Four horizontal slices of equal thickness were cut from the top and four from the bottom regions, and three blocks were systematically obtained from each slice. A portion of each block was processed for light microscopic morphometry by using routine histological techniques. These samples were embedded in paraffin, cut in 8-μm-thick sections, and stained with hematoxylin and eosin. From the remaining portion of the primary blocks, 5–7 small blocks (~1 mm³) were obtained and immediately immersed in 2.5% glutaraldehyde for 4 h at 4°C to be processed for electron microscopic morphometry. After repeated washing in 0.1 M phosphate buffer, pH 7.4, they were postfixied in 1% osmium tetroxide solution in 0.1 M sodium cacodylate buffer plus 3% dextran T-70 and sodium chloride (total osmolarity 350 mosM, pH 7.4) for 2 h at 4°C. The blocks were then washed in the same buffer, dehydrated in an ethanol scale, and embedded in Epon 812. Two blocks were randomly selected per each primary block and processed for morphological and morphometric analysis; the remaining blocks were kept as reserves. Ultrathin sections (600 Å) were cut from the blocks, mounted on uncoated 200-mesh copper grids, and stained with uranyl acetate and lead citrate. The sections were examined in a Zeiss EM900 electron microscope. On a single section from each block, six micrographs of the alveolar parenchyma at a primary magnification of ×4,200 were systematically taken. They were then enlarged to a final magnification of ×10,000 on high-definition glossy paper. In addition, four micrographs per section at ×12,000 were recorded on 70-mm negative films to evaluate the density of collagen fibrils in the alveolar interstitium. To do this, we randomly selected at low-power areas of the alveolar septa containing fibrillar component and then increased the power to the magnification level at which collagen fibrils became clearly visible. The negative films were acquired and electronically transformed into positive reversals at a final magnification of ×200, to be analyzed as described below.

For light microscopic morphometry, one section was obtained from each of the three samples selected per slice, and five micrographs at a primary magnification of ×25 were systematically taken on 35-mm negative film. Morphometric measurements were performed on positive reversals of the negative film by using a projector at a final magnification of ×200.

After the tissue sampling procedure for morphometry, other samples from the top and bottom specimens were taken to determine their wet weight-to-dry weight ratio (W/D); they were weighed fresh and after being dried in an oven at 70°C for 24 h.

Thickness of the Lung Air-Blood Barrier

Both the arithmetic and the harmonic mean thickness of the air-blood barrier were estimated by using the stereological method developed by Weibel and Knight (23), which is particularly useful in the study of anisotropic tissue such as the lung. Each micrograph at ×10,000 was superimposed with a transparent sheet marked with a test grid, as shown in Fig. 1. The grid is a system of 15 test segments of equal length (z) placed at different angles producing a hexagonal network. To measure the arithmetic mean thickness (τ), we counted the number of end points (p) of the test lines lying on the air-blood barrier (Fig. 1, rectangle A) and the number of intersections (n) of the test lines with the external and internal surfaces of the barrier (Fig. 1, rectangle B). The arithmetic mean is then given by: τ = z/p/2n.

In addition, eight parallel rows of equidistal dots are necessary for the determination of the harmonic mean thickness through the measurement of the length (l) of the row of dots lying on the tissue (Fig. 1, rectangle C) given by l = d·m, where m is the number of dots and d the distance between adjacent dots. The harmonic mean thickness is defined as: τh = 2/3 l/h, where h is the harmonic mean of all intercept lengths of the probes (l₁, l₂, . . . lₙ) given by: h = n/(1/l₁ + 1/l₂ + 1/l₃ + . . . + 1/lₙ).

Thick and Thin Portions of the Air-Blood Barrier

To further investigate the morphological changes induced by mild edema and particularly the presence and distribution of interstitial fluid in lung parenchyma, we distinguished the thin and thick portions of the air-blood barrier. In the thin portion, endothelium and epithelium are separated only by
cells) and peribronchial (and peribronchiolar) adventitia. Volume densities were expressed as fractions of lung tissue. 

**Electron microscopic morphometry.** To evaluate the volume distribution of the various compartments of lung parenchyma, a point-counting orthogonal grid with 16 × 16 intersections was superimposed on the electron micrographs at ×10,000 from the top and bottom regions of both control and treated lungs. We estimated the volume density of the alveolar air, the capillaries, and the alveolar tissue within the lung parenchyma. We also evaluated the volume density of the interstitial compartments within the alveolar tissue, i.e., the interstitial cells and the extracellular matrix.

**Density of the Fibrillar Collagen in the Interstitial Matrix of the Alveolar Septa**

Micrographs at ×20,000 with cross-sectioned clusters of collagen fibrils have been evaluated through an image-processing program (Optimas v. 4.02, Optimas). We estimated the density of the collagen fibrils by evaluating both the number of fibrils per square micrometers and the interfibrillar distance. The evaluation of the number of fibrils per square micrometers was performed with the implementation of a macro in Optimas environment (ALI language). The macro works by superimposing on the image a grid whose elements are centers of circles of 8-μm diameter. The number of collagen fibrils lying in the circle was counted, and the density of fibrils was then derived. The mean distance among collagen fibrils was estimated from the segment joining the centers of adjacent cross-sectioned collagen fibrils. The macro computed the mean of 50 distances between adjacent cross-sectioned collagen fibrils per each micrograph.

**Statistical Analysis**

Data of lung volumes and weights and hematocrit are expressed as means ± SD. Morphometric data are instead presented as means ± SE. Because of the large variation in the composition of the micrograph content, we pooled point and intersection counts and estimates of air-blood barrier thickness with electron microscopy, and point counts with light microscopy over all the micrographs derived from each section. The data relative to the density of collagen fibrils were instead obtained by averaging the estimates from individual micrographs. The significance of the differences among groups was determined by using one-way ANOVA and the Student-Newman-Keuls method for multiple comparison tests.

**RESULTS**

**Lung Volume and Weight and Hematocrit**

Table 1 reports the mean data of volume and weight (normalized to body wt) of right fixed lungs; no signif-

<table>
<thead>
<tr>
<th></th>
<th>Total Lung Volume (tissue + gas)</th>
<th>Lung Weight, g/kg body wt</th>
<th>Lung Gas Volume, ml/kg body wt</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.8 ± 0.1</td>
<td>4.49 ± 0.6</td>
<td>4.31 ± 0.6</td>
<td>44.1 ± 1</td>
</tr>
<tr>
<td>Treated</td>
<td>8.83 ± 0.2</td>
<td>4.65 ± 0.4</td>
<td>4.18 ± 0.3</td>
<td>36.8 ± 2.4*</td>
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</table>

Values are means ± SD. Volume and weight were measured on right lungs extracted from the chest after in situ vascular perfusion fixation and normalized to body weight. Control group also includes data from sham animals. *P < 0.05 vs. control group.
significant changes were found between control and sham animals, so the corresponding data were averaged. In treated animals, we found no significant change in total lung volume and a slight nonsignificant increase in lung weight. If we assume lung tissue density to be equal to 1 g/ml, the difference between total lung volume and lung weight yielded gas volume that was found to decrease slightly, although not significantly, in treated animals. Hematocrit was significantly decreased due to plasma volume expansion.

The W/D of lung specimens obtained from control and sham lungs were 4.97 ± 0.26 (SD) and 5.26 ± 0.36 for top and bottom regions, respectively; the corresponding values from treated lungs were 5.08 ± 0.13 and 5.73 ± 0.21, the latter value being significantly higher than the other values (P < 0.05).

**Thickness of the Air-Blood Barrier**

Figure 2 shows the appearance of the air-blood barrier in control and treated lungs (Fig. 2, A and B, respectively) consisting of thin and thick regions. The morphological organization of the air-blood barrier in control lungs appeared normal (Fig. 2A): the thin portion consisted of an extremely thin layer of fused basement membranes between endothelium and epithelium, whereas in the thick portion, the interstitial layer was mainly occupied by cytoplasm of interstitial cells. Treated lungs (Fig. 2B) showed widening of the air-blood barrier both in the thin and in the thick regions. However, no disruptions, such as breaks of endothelium and epithelium or epithelial bleb formations, were observed as reported in conditions of frank edema (2, 3, 24). In the thin portion of the barrier, the basement membranes appeared irregularly enlarged with some focal more pronounced swelling (Fig. 2B, asterisks). In the thick portion, treated lungs displayed a predominance of extracellular matrix with respect to the cellular compartment (Fig. 2B). With regard to the morphology of the barrier cell layers, no major alterations, such as epithelial blebs or discontinuities, affected treated lungs. Vacuoles were frequently observed both in endothelial and epithelial cells in the air-blood barrier of treated lungs.

Table 2 shows the arithmetic and the harmonic mean thickness of the air-blood barrier, which provide a weighted mean from thin and thick regions of the barrier. The arithmetic mean thickness reflects the mass of the tissue that builds the barrier; the harmonic mean thickness reflects the resistance to gas diffusion (23). No significant differences were found when corresponding regions of control and sham lungs were compared. Furthermore, neither control nor sham lungs showed significant differences between top and bottom regions. In treated lungs, the arithmetic mean thickness increased significantly by 27% (top and bottom average), whereas the harmonic mean thickness increased by 50%. Furthermore, in treated lungs, a significantly higher value was found in harmonic mean in the bottom relative to the top. The ratio of arithmetic to harmonic mean decreased in the treated compared with the control lung, mainly when one compares the bottom regions.

Figure 3 shows a detailed analysis of the thin and thick portions of the air-blood barrier and reports the width of epithelial, endothelial, and interstitial layers. No significant differences between sham and control were found when we compared the three layers; therefore, the corresponding data were averaged with the control group. In the thin portion, no differences were found between top and bottom regions in the control
The density of collagen fibrils in the interstitial space of lung parenchyma was not significantly different in the top and bottom of control lungs (Table 5). In interstitial edema, the density of fibrils significantly decreased in top and bottom regions (significantly more in the latter), indicating that the increase in volume density of the extracellular matrix is due to fluid accumulation. The ultrastructural appearance of clusters of collagen fibrils in the parenchyma interstitium of both control and treated lungs is shown in Fig. 5.

**Fluid Distribution in the Lung Parenchyma and in the Extra-Alveolar Compartment**

Figure 6 presents the fluid contents of the capillaries, the interstitial matrix of the parenchyma, and the extra-alveolar perivascular adventitia, namely the compartments displaying differences in regional vol-
LUNG MORPHOMETRY IN INTERSTITIAL EDEMA

DISCUSSION

The relative “dryness” of the pulmonary interstitium results from the matching between a powerful lymphatic drainage and a low microvascular permeability (18) that maintains a very low capillary filtration rate. Furthermore, the low interstitial compliance (11) provides a strong “tissue safety factor” against the development of edema. Despite these features, the morphometric data from this study clearly show that a relatively modest fluid perturbation in lung fluid balance already entails significant changes in compartmental water distribution. We will evaluate the impact of such changes on microvascular permeability, interstitial tissue compliance, and gas diffusion, also taking into account data from previous studies indicating that mild interstitial edema causes a damage to interstitial matrix (13–16).

Methodological Considerations

Experiments were done in anesthetized spontaneously breathing rabbits, an obvious advantage over passive mechanical ventilation, which is known to alter the parenchymal stresses and is, in fact, a possible cause of lung lesions. Furthermore, we performed the perfusion-fixation procedure by keeping the pleural sacs intact, ensuring that the lungs were exposed to a physiological transpulmonary pressure (alveolar pressure being equal to atmospheric and pleural pressure to subatmospheric). The choice of the perfusates (saline and fixative), ensuring optimal tissue preservation and yielding minimal structural alterations to the lung architecture, was derived from previous works (1, 19). Lung volume, measured by liquid displacement after fixation, was well within the physiological values. The top to bottom alveolar volume density ratio averaged 1.35 in control (Table 4); this value is in good agreement with the ratio calculated from regional alveolar architecture, was derived from previous works (1, 19).

Table 3. Volume density of lung tissue components

<table>
<thead>
<tr>
<th>Component</th>
<th>Control Top</th>
<th>Control Bottom</th>
<th>Treated Top</th>
<th>Treated Bottom</th>
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<tbody>
<tr>
<td>Extra-alveolar airways</td>
<td>0.034 ± 0.005</td>
<td>0.028 ± 0.004</td>
<td>0.044 ± 0.006</td>
<td>0.046 ± 0.008</td>
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<tr>
<td>Airways wall (epithelium + smooth muscle cells)</td>
<td>0.006 ± 0.0008</td>
<td>0.005 ± 0.0006</td>
<td>0.01 ± 0.002</td>
<td>0.008 ± 0.001</td>
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<tr>
<td>Peribronchial adventitia</td>
<td>0.17 ± 0.008</td>
<td>0.15 ± 0.006</td>
<td>0.16 ± 0.005</td>
<td>0.14 ± 0.012</td>
</tr>
<tr>
<td>Lumen</td>
<td>0.105 ± 0.004</td>
<td>0.08 ± 0.004</td>
<td>0.11 ± 0.009</td>
<td>0.085 ± 0.004</td>
</tr>
<tr>
<td>Wall (endothelium + media)</td>
<td>0.015 ± 0.002</td>
<td>0.017 ± 0.002†</td>
<td>0.044 ± 0.006*</td>
<td>0.056 ± 0.008‡</td>
</tr>
<tr>
<td>Perivascular adventitia</td>
<td>0.67 ± 0.007</td>
<td>0.720 ± 0.007</td>
<td>0.635 ± 0.03</td>
<td>0.665 ± 0.018</td>
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Values are means ± SE. Volume densities are expressed as relative volumes in the lung tissue. Alveolar septa includes alveolar ducts.

*P < 0.001 vs. control top; †P < 0.05 vs. control top; ‡P < 0.05 vs. treated top.

Fig. 4. Light microscopic appearance of lung tissue from bottom regions of sham (A) and treated animals (B). In sham animals, the adventitial space of extra-alveolar vessels appears normal, whereas it is clearly thickened in treated animals. However, no apparent widening of the connective tissue around airways was observed in treated lungs. Hematoxylin-eosin stain. V, vessel; Ar, airway. Scale bar = 150 μm.
rabbids (9). The corresponding alveolar volumes (as %maximum volume), derived from the volume-pressure curves of the rabbit lung, assuming isotropic mechanical behavior (5), were 50 and 35%, respectively, yielding indeed a top-to-bottom alveolar volume ratio of 1.43.

Fluid Distribution in the Alveolar and Extra-Alveolar Lung Tissue

The alveolar compartment includes the thin and the thick portions of the air-blood barrier and the capillary network that represents the “exchanging unit” of the lung for fluid and gases. A novel finding of this study is that, in mild interstitial edema, a relevant portion of the extra-vascular fluid accumulation occurs at the level of the interstitial space of the exchanging unit (44%), the remaining portion being found in the extra-alveolar perivascular adventitia. Within the air-blood barrier, fluid was being mostly confined to the thick portion. As far as regards the thin portion, namely the basement membranes, water did not accumulate in the top region of the lung (Fig. 3A), despite a capillary recruitment (Fig. 6); however, fluid swelling was observed in the bottom region (70% increase in thickness, Fig. 3A). This represents a new notion, as a top-to-bottom gradient of interstitial fluid accumulation was so far described only in more severe states of pulmonary edema (2, 24). To our knowledge, an early functional involvement of the basement membranes in the development of interstitial edema has not been detected in previous studies. Another interesting result from the present study is the significant thickening of the endothelial cells in the thin portion of the air-blood barrier, suggesting that they may actively respond to interstitial fluid loading. The present data allow us to extend the generally accepted notion that the first site of fluid accumulation in pulmonary edema is the extra-alveolar adventitial perivascular and peribronchial space (4, 17). In fact, although we can confirm fluid accumulation in mild interstitial edema at least in the perivascular space, it ought to be regarded as a consequence of fluid accumulation at the level of the exchanging unit. One possible reason why a widening of the parenchymal interstitium was overlooked with fluid dynamic perturbations largely exceeding those caused by mild interstitial edema may be the fact that a massive increase in volume density of the perivascular and peribronchial cuffs would have rendered relatively smaller water accumulation in the air-blood barrier.

The in situ fixation procedure also allowed us to evaluate the finding that ~70% of the total increase in extravascular fluid accumulates in the bottom region of the lung, which appears more vulnerable to edema compared with the top, also as far as mild interstitial edema is concerned.

Compartmental Fluid Accumulation and Interstitial Matrix Structure

We attempt to discuss the compartmental fluid accumulation, recalling data from our previous studies that showed proteoglycan fragmentation in mild hydralic and a lesional type of interstitial edema (13–16). Although we do not provide immunostaining of these molecules, it is tempting to relate the increase in thickness of the thin portion of the air-blood barrier to fragmentation of heparan sulfate proteoglycans of the basement membrane; since these molecules act as a selective permeability barrier, their degradation should lead to an increase in microvascular permeability. Furthermore, the degradation of chondroitin sulfate proteoglycans is compatible with the observed increase in interfibrillar distance in the thick portion of the air-blood barrier and should result in some loss of mechanical resistance to compressive and tensile stresses normally provided by these molecules (6, 13).

Table 5. Density of interstitial collagen fibrils

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<tr>
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<th>Control Top</th>
<th>Control Bottom</th>
<th>Treated Top</th>
<th>Treated Bottom</th>
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<tr>
<td>Number of fibrils/μm²</td>
<td>531 ± 9.2</td>
<td>507 ± 9.9</td>
<td>396 ± 12.3*</td>
<td>245 ± 10.6†‡</td>
</tr>
<tr>
<td>Interfibrillar distance, nm</td>
<td>59.3 ± 1.7</td>
<td>62.5 ± 1.8</td>
<td>82.0 ± 3.1*</td>
<td>103.7 ± 5.5†‡</td>
</tr>
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Values are means ± SE. Intebrillar distance was calculated as the segment joining the centers of adjacent cross-sectioned collagen fibrils. *P < 0.001 vs. control top; †P < 0.001 vs. control bottom; ‡P < 0.001 vs. treated top.
Alveolar Air-Blood Barrier and Gas Diffusion Properties

Harmonic mean thickness is weighted in favor of the thin portion of the air-blood barrier and therefore is considered to be proportional to resistance to gas diffusion (20); indeed, the flow of gas (Q) diffusing at the alveolar level down a pressure gradient (∆P) through an alveolar surface area (S) is given by

\[ Q = \frac{D ∆P S}{h}, \]

where D is the diffusion coefficient and h is the harmonic mean thickness (23). Therefore, the increased harmonic mean thickness value we found in treated animals, 50% relative to control, suggests that mild interstitial edema causes a proportional increase in resistance to alveolar gas diffusion. Furthermore, the arithmetic-to-harmonic mean thickness ratio is relatively high in control, reflecting the nonuniformity of the thickness of the air-blood barrier (7, 23). The ratio decreased in treated animals by 7 and 16% in top and bottom lung regions, respectively, indicating a greater increase in the harmonic mean thickness. This finding is in line with the increased thickness of the thin portion of the air-blood barrier, especially in the bottom region of the lung, as shown in Fig. 3, once more indicating a shift of the gas diffusion process away from its physiological optimization point.

Resistance of the Lung to Development of Severe Edema

The lungs are functionally exposed to conditions of increased capillary perfusion that in turn may lead to greater microvascular filtration; this may occur in a variety of conditions, like physical exercise or hypoxia exposure, or in microgravity as a consequence of increased cardiac output and vascular perfusion (12). Therefore, except for microgravity of course, a condition of mild interstitial edema could be relatively common.

The present study shows that, despite incredible resistance of the lung to the development of edema, some disruption of normal morphology and microarchitecture of the thin and thick portions of the air-blood barrier occurs, even with a modest perturbation of the physiological fluid balance. The present data suggest that, despite partial damage, the relatively high structural resistance of the thin and the thick portions of the...
air-blood barrier still provide low microvascular permeability and low interstitial compliance (11).

This interpretation is in keeping with two pieces of evidence coming from previous studies: 1) the marked increase in interstitial pressure from the physiological value of about $-10 \text{cm}_2\text{H}_2\text{O}$ up to $-5 \text{cm}_2\text{H}_2\text{O}$ (11) in interstitial edema; 2) the development of frank edema when interstitial pressure drops to $-0 \text{cm}_2\text{H}_2\text{O}$ due to massive matrix fragmentation (13–16). The increase in interstitial pressure in mild interstitial edema serves two functions: on one side it counteracts further filtration at capillary level (so-called tissue safety factor); on the other it may provide a pressure gradient to allow fluid drainage toward the perivascular and peribronchial adventitial spaces, representing a site of relatively high compliance and capacity, as demonstrated by fluid accumulation in severe edema (3, 8, 17).

Therefore, the control of extravascular lung volume results from the delicate equilibrium between tissue damage and repair. The involvement of cells in such a critical balance relating the reparative to the lesional process deserves, of course, further study; however, one should again mention the possible role of proteoglycans as they are involved in a number of biological processes, including cytokine activities and inhibition of protease activities and extracellular matrix deposition and organization (7).

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