Morphological Changes in Isolated Perfused Dog Lungs after Acute Hydrostatic Edema

DAVID O. DEFOUW AND PETER B. BERENDSEN

SUMMARY Morphometric data from stable (nonedematous) isolated dog lungs, perfused with nearly cell-free perfusates, were compared to similar stereological evaluations of isolated dog lungs after induction of severe acute hydrostatic edema. In the edematous lungs, capillary surface and volume densities were substantially increased. Alveolar surface density was also increased. Thicknesses of the endothelial and type I epithelial cellular compartments of the air-blood barrier were unchanged. Thickness of the interstitial compartment of the air-blood barrier was substantially increased and this, in turn, caused an overall increase in mean thickness of the barrier. Volume densities of the nonparenchymal connective tissue spaces surrounding the extra-alveolar vessels and airways were also increased. In both the endothelial and type I epithelial cells, cytoplasmic volume densities of pinocytotic vesicles were increased. In addition, the number of vesicles opening onto the luminal and alveolar cellular surfaces increased significantly. Transendothelial vesicular transport may contribute to interstitial edema formation, and transepithelial vesicular transport may contribute to alveolar flooding in isolated perfused dog lungs.

In a previous study of isolated dog lungs perfused under stable conditions, morphometric analyses of the air-blood barrier, alveolar and capillary surface densities, and extra-alveolar vessels and airways were presented.1, 2 The present report describes the same morphometric parameters in isolated dog lung preparations after induction of severe acute hydrostatic edema. The interendothelial junctions were unaltered; however, these observations represented the sequential pattern of fluid accumulation in acute pulmonary edema.4 Increases in capillary hydrostatic pressure with consequent increases in transendothelial transport have been suggested as a primary means of extravascular fluid accumulation in edematous lungs.6, 7 Additional evidence suggesting a contribution of extra-alveolar pulmonary vessels to edema formation has also been presented.5, 8

In the present study, established stereological techniques were employed to quantitate changes with acute pulmonary edema in air-blood barrier thickness, alveolar and capillary surface densities, and volume densities of the nonparenchymal connective tissue spaces surrounding the extra-alveolar vessels and airways, fluid accumulation in the alveolar interstitium, and finally alveolar flooding.34 It was concluded that in edematous lungs, capillary surface and volume densities were substantially increased. Alveolar surface density was also increased. Thicknesses of the endothelial and type I epithelial cellular compartments of the air-blood barrier were unchanged. Thickness of the interstitial compartment of the air-blood barrier was substantially increased and this, in turn, caused an overall increase in mean thickness of the barrier. Volume densities of the nonparenchymal connective tissue spaces surrounding the extra-alveolar vessels and airways were also increased. In both the endothelial and type I epithelial cells, cytoplasmic volume densities of pinocytotic vesicles were increased. In addition, the number of vesicles opening onto the luminal and alveolar cellular surfaces increased significantly. Transendothelial vesicular transport may contribute to interstitial edema formation, and transepithelial vesicular transport may contribute to alveolar flooding in isolated perfused dog lungs.
cytoplasm that were occupied by pinocytotic vesicles were substantially increased. Increased numbers of vesicles opening onto the luminal and alveolar cellular surfaces suggested that vesicular transport across the cellular compartments of the air-blood barrier may contribute to edema formation in isolated dog lungs.

Methods

Mongrel dogs (weight, approximately 20 kg) of either sex were anesthetized with pentobarbital (30 mg/kg, iv), heparinized (25,000 IU), and given the antihistamine, phenylhydramine hydrochloride (35 mg). The trachea was cannulated and the lungs were artificially ventilated with 5% CO₂ in humidified air by a Harvard respirator at 12 strokes/min and 400 ml/stroke. The thorax was opened via a midsternal incision and the pulmonary trunk was cannulated with 3/8-inch Tygon tubing which was connected to a gravity-feed upper reservoir containing approximately 1.5-2 liters of perfusate. The outflow connection was via 3/8-inch Tygon tubing which was inserted through the tracheal valve and sewn in place by means of a purse-string suture. The outflow connection led to a lower level reservoir from which the perfusate was roller pumped back to the upper reservoir. After the inflow and outflow tubing had been inserted, the lungs were removed from the thorax and placed on a platform which was suspended from a gram-sensitive scale. Arterial inflow and venous outflow pressures were monitored and positive pressure ventilation was maintained throughout the period of perfusion.

The perfusate consisted of approximately 1500 ml of 6% dextran in normal saline plus 500 ml of a solution composed of the following solutes dissolved in deionized water, in grams per liter of solution: NaCl, 7; NaHCO₃, 2.52; CaCl₂·2H₂O, 0.026; KCl, 0.35; MgCl₂·6H₂O, 0.325; glucose, 1. Osmolarity of the perfusate was 300 mOsmol/liter and the pH was adjusted to 7.4. The flow rate and perfusate temperature were maintained at 10-20 ml/sec and 37°C, respectively.

After steady state conditions had been established, hydrostatic edema was produced by progressively elevating venous outflow pressure by 5-cm H₂O increments at intervals of 5 minutes for periods of 30-45 minutes. At each 5-minute interval, changes in lung weight and inflow and outflow pressures were recorded. The development of edema was verified by increases in lung weight and the appearance of frothy fluid in the trachea. Similar preparations have been used for studies of pulmonary endothelial reflection coefficients.⁹

Studies on the stable isolated lung preparations that remained nonedematous (weight gain less than 2-3% during 30-minute, 1-hour, and 2-hour periods of steady state isolated-perfusion) were performed in a similar fashion. Details of these stable lung preparations were presented previously.⁷

The edematous isolated lungs, like the stable isolated preparations, were fixed by tracheal instillation at 20 cm H₂O pressure of 2% glutaraldehyde in 0.15 m sodium bicarbonate buffer at pH 7.4 and 550 mOsmol/liter. The fixed lungs were then cut into 12 longitudinal slices and two tissue blocks were taken from each slice according to a stratified sampling procedure.⁹ From the primary sample of 24 blocks, 10 blocks were selected randomly, a portion of each block was prepared for electron microscopy, and the remainder was used for light microscopy. The segments used for electron microscopy were post-fixed in 1% osmium tetroxide in 0.15 m sodium bicarbonate buffer, dehydrated in graded ethanol and propylene oxide, and embedded in Epon. Twenty-five electron micrographs, taken at three sequential magnifications, were randomly obtained from thin sections (60-90 nm) of each tissue block from the stable and edematous lungs. Each micrograph was analyzed with a stereological test grid composed of test lines and test points for estimating volume and surface densities, respectively.⁹ Ten micrographs at 6100× were evaluated to estimate alveolar and pulmonary capillary surface densities, capillary volume density within the alveolar septa, and average thickness of the air-blood barrier and its cellular and interstitial compartments. Ten micrographs at 21,600× were analyzed to estimate endothelial and type I epithelial cytoplasmic organelle volume densities and the cell, fiber, and matrix (ground substance) volume densities within the interstitial compartment of the air-blood barrier. Five micrographs at 81,500× were used to estimate volume and surface densities of the endothelial and type I epithelial pinocytotic vesicles (caveolae). The interendothelial clefts from both the stable and edematous lungs were also examined and the junctions were categorized as tight (the outer two lamellae were fused) or open (the outer lamellae were separated by gaps). In the open junctions, the widths of the gaps were then measured. The sampling procedure randomly generated 50 micrographs at 81,500× from each isolated perfused lung preparation. Approximately 15-25 micrographs of the sample contained interendothelial clefts which were examined and categorized.

Four micrographs at 467× were taken randomly from sections of the portion of each block prepared for light microscopy. The light micrographs were analyzed with a stereological grid, composed of test points only, to provide estimates of volume densities of the conducting arteries and veins and the nonparenchymal connective tissue compartments of the lungs. The differences between means of the morphometric parameters in the stable and edematous isolated-perfused lungs were evaluated by a two-tailed t-test.¹¹

Results

Morphometric results from the edematous isolated-perfused lungs were compared with morphometric evaluations of stable or nonedematous isolated-perfused lung preparations. Mean values of the morphometric parameters from the three periods of stable perfusion were determined and used for comparison with coinciding parameters from the edematous lungs. In the edematous isolated lung preparations, initial arterial (inflow) and venous (outflow) pressures were 16 cm H₂O and 8 cm H₂O, respectively. Initial lung weights were approximately 200-225 g. After induction of hydrostatic edema by progressive elevations of the lower venous reservoir,
TABLE 1  Nonparenchymal Interstitium and Conducting Vessels

<table>
<thead>
<tr>
<th></th>
<th>None edema (stable)</th>
<th>Hydrostatic edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peribronchial sleeves</td>
<td>0.33 ± 0.06</td>
<td>0.67 ± 0.13</td>
</tr>
<tr>
<td>Peribronchiolar sleeves</td>
<td>0.85 ± 0.03</td>
<td>2.77 ± 0.43</td>
</tr>
<tr>
<td>Interlobular septa</td>
<td>0.12 ± 0.02</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Pulmonary arterial contents</td>
<td>1.22 ± 0.04</td>
<td>1.50 ± 0.06</td>
</tr>
<tr>
<td>Periarterial sleeves</td>
<td>0.40 ± 0.03</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>Pulmonary venous contents</td>
<td>1.13 ± 0.11</td>
<td>1.47 ± 0.15</td>
</tr>
<tr>
<td>Perivenous sleeves</td>
<td>0.30 ± 0.05</td>
<td>0.80 ± 0.25</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

Light Microscopy

Volume densities obtained from the light micrographs provided an estimate of the lung volumes that were occupied by nonparenchymal interstitium, i.e., the interlobular septa and the connective tissue sleeves which surround conducting portions of the respiratory tree and extra-alveolar pulmonary blood vessels (external diameters greater than 50 μm). In addition, the light micrographs provided an estimate of lung volumes that were occupied by contents of the extra-alveolar vessels. Table 1 defines the substantial increases in nonparenchymal interstitial volume densities in the edematous lungs. Lung volumes that were occupied by the peribronchial sleeves ($P < 0.05$), the peribronchiolar sleeves ($P < 0.025$), the interlobular septa ($P < 0.025$), the periarterial sleeves ($P < 0.05$), and the perivenous sleeves ($P < 0.05$) were significantly increased. Lung volumes that were occupied by contents of the extra-alveolar vessels were, on the other hand, increased only slightly in the edematous lungs.

Electron Microscopy

Figure 1 defines changes that occurred in alveolar and capillary surface densities and in the proportion of the alveolar septa occupied by capillary contents during edema formation. Alveolar surface density, capillary surface density, and capillary content volume density within the alveolar septa were each substantially increased ($P < 0.05$).

The air-blood barrier is defined as the sheet of tissue that separates air and blood at the level of alveoli and capillaries in the gas exchange region of the lung. The barrier consists of a thin portion composed of endothelium, basal lamina, and epithelium and a thick portion in which ground substance, collagenous and elastic fibers, and interstitial cells separate the respective basal laminae of the endothelium and epithelium. Average thickness of the air-blood barrier (including the thin and thick portions) was significantly greater in the edematous lungs ($P < 0.05$) than in the nonedematous or stable lungs (Fig. 2). To evaluate further this increase in barrier thickness, individual thicknesses of the epithelial, endothelial, and interstitial compartments of the air-blood barrier were compared (Fig. 2). Thickness of both cellular compartments was not significantly increased, however ($0.10 <
This suggests that increases in numbers of luminal and respective surface densities were increased (P < 0.025). Since the relative percentage of cytoplasmic vesicles was both increased (P < 0.05). Since the relative percentage of cytoplasmic vesicles was decreased (P < 0.025), the increase in cytoplasmic vesicular surface density was attributed to the increase in vesicular diameters. Table 4 also indicates that production of hydrostatic edema with accompanying elevations in pulmonary perfusion pressures was not associated with increased gap widths within those interendothelial junctions that appeared open in the plane of section of the individual electron micrographs.

Relative percentages and surface densities of the type I epithelial luminal and albuminal vesicles were increased (P < 0.025), whereas their mean diameters were increased only slightly in the edematous lungs. As in the endothelial cells, this suggests that the number of luminal and albuminal vesicles was increased. The relative percentage of cytoplasmic vesicles was decreased (P < 0.025), whereas their surface density and mean diameters were unchanged.

Discussion

Garr et al. reported that pulmonary edema increases directly with increases in capillary hydrostatic pressure, as induced by raising the venous reservoir in isolated dog lung preparations. Lung weight gains exceeding 35% of original weight on excision also have been reported as a requirement for the onset of alveolar edema in isolated dog lungs. In the present investigation, increases in capillary pressure were indicated by the 6-fold increase in outflow pressures and the resultant 3-fold increase in inflow pressures. Accordingly, the 90-100% gain in lung weight accompanied by appearance of frothy fluid in the trachea verified the presence of alveolar flooding.

Previous evaluations of isolated dog lungs suggested that weight gains of 20-30% were accompanied by perivascular and peribronchial cuffing. The present morphometric results from light microscopic analyses provide quantitative evidence of both perivascular and peribronchial cuffing after 90-100% weight gains. In addition, fluid cuffs surrounded the bronchi. The interlobular septa, which are haphazard and incomplete in the normal dog lung, also were sites of fluid accumulation after this extensive increase in lung weight. Thus, the present evidence suggests the nonparenchymal interstitial compartment provides a site of continuous fluid accumulation with massive production of edema. Extensive bronchiolar cuffing relative to the bronchial cuffing also indicates the sequential nature of fluid accumulation which has been suggested previously. That is, as the capacity of the peribronchial spaces is reached, further fluid accumulation occurs within the contiguous peribronchial spaces.

Several studies have indicated an increase in lymph flow from intact dog lungs after the production of cardio- genic edema. The condition of pulmonary lymphatics in isolated lung preparations, however, remains uncertain. Some evidence of lymph vessel engorgement in the nonparenchymal interstitial compartment was obtained subjectively in the present investigation. The substantial

Table 2

<table>
<thead>
<tr>
<th>Volume densities (% interstitium)</th>
<th>Nonedema (stable)</th>
<th>Hydrostatic edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>39 ± 0.67</td>
<td>18 ± 0.04</td>
</tr>
<tr>
<td>Fibers</td>
<td>21 ± 1.2</td>
<td>15 ± 1.1</td>
</tr>
<tr>
<td>Matrix (ground substance)</td>
<td>40 ± 2.5</td>
<td>67 ± 1.7</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

P > 0.05), whereas the interstitial compartment was substantially thickened (P < 0.025) in the edematous lungs.

Table 2 describes the more detailed morphometric analyses of the interstitial compartment. The interstitial volume that was occupied by cells was substantially reduced (P < 0.025), whereas the collagenous and elastic fiber volume density was essentially unchanged. The matrix or ground substance, on the other hand, was distributed in a greater proportion of the interstitium (P < 0.025); thus, accounting for the overall increase in interstitial thickness. Figures 3 and 4 illustrate the obvious accumulation of fluid in the thick portion of the air-blood barrier's interstitial compartment in the edematous lungs.

Table 3 presents the morphometric comparisons of organelle volume densities within the epithelial and endothelial cytoplasm in the stable and edematous lungs. Volume densities of the rough endoplasmic reticulum, smooth endoplasmic reticulum, and mitochondria were not substantially changed in either the epithelial or endothelial cells. The cytoplasmic volumes occupied by vesicles were, on the other hand, substantially increased in both cellular compartments of the air-blood barrier. In the edematous lungs, pinocytotic vesicles occupied nearly half of endothelial cytoplasmic volume as compared to a quarter of cytoplasmic volume in nonedematous lungs. Likewise, vesicular volume density in the epithelial cytoplasm was more than doubled during production of edema. Figures 5 and 6, which are representative examples from the large sample of micrographs required in a morphometric study, illustrate this substantial increase in volume densities of the endothelial and epithelial vesicles.

The endothelial (Table 4) and epithelial (Table 5) vesicular populations were further defined according to their distributions within the cells, i.e., those attached directly to the luminal or albuminal plasma membranes and those contained freely within the cytoplasm. In endothelial cells of edematous lungs (Table 4), relative percentages of vesicles opening onto the luminal and albuminal cellular surfaces were increased (P < 0.05), whereas the relative percentage of cytoplasmic vesicles was decreased (P < 0.025). Mean diameters of the luminal and albuminal vesicles were unchanged, whereas their respective surface densities were increased (P < 0.025). This suggests that increases in numbers of luminal and albuminal vesicles were responsible for their increased relative percentages. Mean diameters and surface density of the cytoplasmic vesicles were both increased (P < 0.05). Since the relative percentage of cytoplasmic vesicles was decreased (P < 0.025), the increase in cytoplasmic vesicular surface density was attributed to the increase in vesicular diameters. Table 4 also indicates that production of hydrostatic edema with accompanying elevations in pulmonary perfusion pressures was not associated with increased gap widths within those interendothelial junctions that appeared open in the plane of section of the individual electron micrographs.

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FIGURE 3. An interalveolar septum demonstrating the air blood barrier from an isolated lung preparation perfused for 1 hour under stable conditions. The interstitial compartment of the air-blood barrier is divided into a thick and thin segment. The thin segment is composed of fused basal lamina from the adjacent type I epithelial (EP) and endothelial (EN) cells. The thick segment (INT) contains densely arranged collagenous fibrils which separate the basal lamina of the cellular compartments of the barrier. AS = alveolar space. 21,600x.
increase in volume density of the nonparenchymal interstitium coupled with evidence of some lymph vessel engorgement indicates that any degree of lymphatic transport capacity retained in the isolated lung preparations had been exceeded.

Increases in capillary surface density and in capillary volume density within the alveolar septa suggest an increase in perfusion of the capillary networks in the edematous lungs. Observations of the stable isolated-perfused lung preparations suggested a widespread incidence of collapsed capillary beds. The marked increase in capillary surface and volume densities in the edematous lungs suggests that underperfused capillary beds were opened after the onset of edema. In addition, those capillary beds...
slightly in the edematous lungs and, thus, indicated a lack of arterial and venous contents were increased only extensively engorged after edema production. Lung volume densities during production of edema.

Results are expressed as mean ± 1 SE.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Volume densities (% cytoplasm)</th>
<th>Volume densities (% luroplasmin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough endoplasmic reticulum</td>
<td>2.14 ± 0.11</td>
<td>1.72 ± 0.30</td>
</tr>
<tr>
<td>Smooth endoplasmic reticulum</td>
<td>2.14 ± 0.11</td>
<td>3.10 ± 1.3</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>4.18 ± 0.15</td>
<td>4.18 ± 0.15</td>
</tr>
<tr>
<td>Pinocytotic vesicles</td>
<td>40.0 ± 3.4</td>
<td>40.0 ± 3.4</td>
</tr>
</tbody>
</table>

Epithelium

Average thickness (μm) 0.32 ± 0.01 0.35 ± 0.01

Volume densities (% cytoplasm)

Rough endoplasmic reticulum 1.55 ± 0.15 1.67 ± 0.39
Smooth endoplasmic reticulum 1.96 ± 0.08 1.87 ± 0.30
Mitochondria 4.23 ± 0.50 46.0 ± 3.4
Pinocytotic vesicles 4.23 ± 0.50 46.0 ± 3.4

Results are expressed as mean ± 1 SE.

Increased barrier thickness in edematous dog lungs. To our knowledge, these are the first quantitative estimates of changes, induced by edema, within the cellular and extracellular (interstitial) compartments of the barrier. The accumulation of fluid within the ground substance of the interstitial compartment (thick portion of the air-blood barrier) was primarily responsible for overall increases in air-blood barrier mean thickness. This pattern of preferential enlargement of the thick portion of the barrier typified the appearance of the air-blood barrier after hydrostatic edema production. Thus, the interstitial edema within the thick portion of the air-blood barrier increased the proportion of the interstitium occupied by matrix or ground substance and concomitantly decreased the interstitial cellular volume density. Cells of the interstitium are not fixed in position. Thus, fluid influx into the interstitial space tended to force the cells into the nonparenchymal connective tissue spaces which surround the extra-alveolar vessels and airways and are continuous with the interstitium of the air-blood barrier.

Further evidence of cellular migration was provided by a decrease in number of cell nuclear profiles in the alveolar interstitium of the edematous lungs.

Morphological evidence of alterations in transendothelial transport via the interendothelial junctions in the edematous lungs was not obtained. Recent ultrastructural evidence from freeze-fractured preparations suggested that occasional discontinuities in the rows of intramembranous junctional particles could represent gaps in the intercellular portion of the junctions and thus serve as sites of passage across the capillary endothelium. Schneeberger and Karnovsky did not, however, observe a discernible widening or lengthening of these discontinuities after perfusion fixation at hydrostatic pressures of 140 cm H2O. Likewise, the present results obtained by the measurement of distances between adjacent endothelial plasma membranes failed to detect widening of the gap widths. Additional morphological evaluations of pulmonary endothelial junctions are required, however, as cytotoxic tracer studies have provided results interpreted as indications that permeability of the junctions can be increased by elevating intravascular fluid volume or by raising hydrostatic pressure.

The role of vesicles in transport across the endothelium remains unsettled. Data have been presented which are interpreted as suggesting that vesicles provide passage across the entire endothelium and thus represent the morphological equivalent of both the large-pore system and the small-pore system. Although still a controversial concept, the proposal has been made that, at normal pulmonary capillary pressures, molecules the size of serum proteins are transported chiefly by the pinocytotic vesicles. Moss et al. on the other hand, suggested that pinocytotic vesicles were the primary means of sodium transport across the pulmonary endothelium during hemorrhagic shock.

The present morphometric results provide a basis for the interpretation that vesicular transport across the endothelium may contribute to edema formation in isolated dog lungs. Vesicular volume densities in endothelial
cells of the stable isolated lungs were consistent with previous estimates from systemic capillaries. Vesicular diameters also corresponded to previous measurements on pulmonary endothelial cells. In the edematous lungs, the number of luminal and albuminal vesicles was increased while the cytoplasmic vesicles were increased in size. Greater numbers of vesicles opening onto both luminal and albuminal sides of the endothelium suggest greater vesicular uptake and discharge during edema formation. Karnovsky and Shek suggested that enumeration of vesicle profiles in electron micrographs without prior vesicular labeling with tracer materials would provide an underestimation of those vesicles that were connected with the plasmalemma directly or by fusion with another vesicle. Presently, only those vesicles that opened directly onto the cellular surfaces were classified as luminal or albuminal and the vesicles had not been labeled with electron opaque tracers. Thus, the percentage of vesicles classified as cytoplasmic may be overestimated; however, the counting and classification procedures were applied to both the stable and edematous lungs to establish the comparative stereological analyses.

Time-sequence studies using electron-opaque tracers have suggested bidirectional pinocytotic transport in systemic capillaries. If bidirectional vesicular transport exists in pulmonary capillaries (it has not been directly demonstrated), the determinants of net directional transport must also be elucidated. Vesicular transit time has
been estimated to be 1-1.5 seconds between the luminal and albuminal cellular fronts. One-dimensional Brownian motion was assumed to be responsible for vesicular transport through the endothelial cytoplasm. More recently, Simionescu et al. reported fusion of two or more vesicles to form patent transendothelial channels, thus suggesting a more rapid means of vesicular transport. The determinants of net directional transport either via a vesicular shuttle or patent channels remain uncertain. Smith and Ryan described the existence of hydrolase enzyme systems along the membrane surfaces of luminal vesicles (caveolae) of pulmonary endothelial cells. Perhaps, other enzyme systems on the luminal or albuminal vesicular membranes could contribute to initiation or control of directional transport across the endothelium.

It has also been suggested that chemical and physical characteristics of migrating molecules could regulate their selective transport via the pinocytotic vesicles. Friedman suggested (without direct evidence) that increased capillary hydrostatic pressures in isolated gracilis muscle preparations may stimulate an increase in endothelial vesicular transport to facilitate an increase in protein transport. The present results provide evidence of increased vesiculation after elevations of capillary hydrostatic pressure in isolated lung preparations. Additional evidence is required, however, to establish direct correlation between capillary hydrostatic pressure or the other suggested variables and regulatory mechanisms of vesicular transport.

Recently, Chinard and Ritter found filtration coeffi-

FIGURE 6 The air-blood barrier after edema production. The endothelial and epithelial vesicular volume densities are both increased. The endothelial junction (EJ), on the other hand, remains unchanged. C = capillary lumen; INT = thick segment of interstitial compartment; AS = alveolar space. 81,500x.
Thus, increased coupling of vesicles could diminish numbers of luminal and albuminal vesicles coupled with exit of sodium from the cytoplasm. In addition, increased albuminal surfaces or between osculating vesicles, are increased size of cytoplasmic vesicles created many more could account for the increased filtration coefficients in edematous isolated lungs. The present evidence of increased capillary surface area was available for exchange and thus could contribute to these changes in endothelial permeability. The increased endothelial vesiculation also contributed to the altered endothelial permeability. Further evidence of vesicular transport under normal and edematous conditions must be provided, however, to elaborate on this possible correlation between increased vesicular volume and surface densities and the altered transport characteristics across the endothelium during edema formation. In addition, the behavior of other solutes and, particularly, of macromolecules in such systems must be determined. The onset of alveolar flooding suggested increased transport across the alveolar epithelium. Previous studies have suggested that protein is transported across the alveolar epithelium, probably via the epithelial vesicles,21,34,35 Vreim et al.36 also suggested that with edema due to increased microvascular pressure in intact dog lungs the alveolar epithelial membrane became freely permeable to protein. The present results suggest increased vesicular activity in type I epithelial cells with hydrostatic edema production; however, the specific solutes involved in vesicular transport were not identified. The increased alveolar surface densities coupled with increased surface densities of the luminal and albuminal epithelial vesicles would increase epithelial surface available for exchange and thus contribute to overall increases in transport across the epithelium. As in the pulmonary endothelium, patent transepithelial channels formed by vesicular fusion were not observed; however, greater numbers of fused vesicles were noted in epithelial cells of the edematous lungs. Fluid accumulation in the interstitium of the air-blood barrier could be associated with an increase in interstitial fluid pressure which, in turn, might induce the increased epithelial vesiculation. Further studies are required, however, to validate this and other potential mechanisms that could control the patterns of vesicular transport across the alveolar epithelium.

The present results describe the endothelial and type I epithelial vesicular populations after acute severe edema. To examine sequential changes in the endothelial and epithelial vesicles, we currently are completing a subsequent morphometric examination of acute moderate edema formation in isolated perfused dog lungs. This should attempt to establish whether vesicles participate only in severe edema production or whether sequential increases in vesiculation contribute to progressively rising levels of pulmonary edema.

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References

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