Electron Microscopic Alterations at the Alveolar Level in Pulmonary Edema

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ABSTRACT

The electron microscopic alterations of the alveolar septum in advanced hemodynamic and alloxan-induced pulmonary edema were compared. Pulmonary edema was produced in anesthetized dogs by means of increased left atrial pressure and hemodilution and by alloxan administration. Sections of pulmonary tissue from these dogs and similarly anesthetized controls were processed for and examined by light and electron microscopy. In the hemodynamic form of edema the interstitial fluid collects only in the collagen-containing portions of the septum. The endothelium, epithelium, their respective basement membranes and large portions of the air-blood barrier are unaffected. Alloxan-induced edema, in contrast, is characterized by degeneration of both endothelium and epithelium and by the appearance of fibrin within the alveoli. The hemodynamic type of pulmonary edema appears to result from an accentuation of the normal process of fluid exchange within the lung. Alloxan-induced edema, on the other hand, is a pathologic process. The functional implications of these results are discussed.

ADDITIONAL KEY WORDS

pulmonary venous hypertension
alloxan
interstitial edema
fluid exchange
fine structure
vascular labeling
ground substance
lung
dogs

There is much literature concerning altered function of the lung in acute pulmonary edema (1-4). There have been, however, relatively few morphologic studies of the edematous lung (5-11). These morphologic studies have in general described intra- and extracellular edema of the alveolar septum following the administration of various agents affecting capillary permeability. In an electron microscopic study of pulmonary edema resulting from the administration of alpha-naphthylthiourea and thiosemicarbazide, Schulz described degenerative changes in the epithelial and endothelial lining cells and swelling of their basement membranes (6). Staub, Nogano and Pearce, using the rapid freezing technique and light microscopy, showed that in both hemodynamic and alloxan-induced pulmonary edema there is swelling of the connective tissues around the vessels and airways before there is swelling of the alveolar walls (10).
The present report describes the ultrastructural alterations at the alveolar level in hemodynamic pulmonary edema produced by elevating left atrial pressure and hemodilution. These fine structural changes are compared with those induced by alloxan. In addition, the functional implications of the observations with respect to the normal exchange of fluid within the lung are discussed.

Methods

Eleven mongrel dogs weighing from 9 to 15 kg were used in these experiments. They were anesthetized with pentobarbital, 25 mg/kg iv. The animals breathed spontaneously, but the lungs were periodically hyperinflated. Experimental hemodynamic pulmonary edema was produced in 6 of the animals by the methods of Levine, Mellins and Fishman (12). Since this method has been previously described in detail, it will be only summarized here.

Pulmonary venous outflow was obstructed by a balloon attached to one lumen of a double lumen catheter introduced under fluoroscopic control into the left atrium. Left atrial pressure was measured by a pressure transducer (Statham P23Db) attached to the second lumen. The circulation was overloaded and the plasma proteins diluted by rapid intravenous infusion of isotonic saline solution using a catheter introduced via the jugular vein.

At the end of 3 hr with the dogs in advanced pulmonary edema, positive pressure artificial respiration was begun, the chest was opened, and portions of inflated lung were isolated between hemostats. Peripheral and central portions from both the superior and dependent regions of the lung were excised and fixed by immersion for electron microscopy in phosphate-buffered 6.25% glutaraldehyde. These tissues were subsequently fixed in cold, veronal-acetate-buffered, 2% osmium tetroxide with added sucrose for 4 hr, dehydrated in acetone, and embedded in araldite. Tissues from similar areas were processed for conventional microscopy.

Vascular markers (13) were injected into the pulmonary arteries of 3 dogs through a cardiac catheter during the course of edema formation. Two of these dogs received a carbon suspension (Pelikan C 11/1431a, Gunther Wagner Co.) containing approximately 100 mg/ml as a single injection in a dose of 1.0 ml/kg body weight about 1 hr before death. The other dog was infused with Thorotrast (24 to 26% thorium dioxide suspended in dextrin, Testagar & Co.) over a 30-min period, starting about 1 hr before death; the total dose was 3.6 ml/kg body weight. The carbon and Thorotrast particles measured approximately 250A and 70A respectively.

Alloxan, 100 mg/kg body weight, was injected into the pulmonary artery of 2 other dogs with catheters positioned as already described. After 1 hr the animals were killed, and the lungs processed for both light and electron microscopy.

Three other dogs served as controls. Their pulmonary tissues were sampled and processed for both light and electron microscopy as previously described after 3 hr of anesthesia.

Thin sections were stained with lead citrate and uranyl acetate as well as with phosphotungstic acid and examined with a Siemens Elmiskop I electron microscope. Sections for conventional microscopy were stained with hematoxylin and eosin.

A semiquantitative index of pulmonary edema was obtained by comparing the weight of a sample of lung obtained immediately after death and drained passively of blood, with its weight after desiccation (12).

Results

The pulmonary venous hypertension that ensued in the animals with hemodynamic edema ranged from 30 to 40 mm Hg. The osmotic pressure of the plasma proteins in these animals was measured by a modified Hepp-Brown osmometer or calculated from the concentrations of albumin and globulin in serum determined by electrophoresis (12) and ranged between 5 and 10 mm Hg. The pulmonary arterial and left atrial pressures of the dogs receiving alloxan were normal throughout the course of the experiment except for a transient elevation for a few minutes immediately following alloxan administration.

At the end of 3 hr coarse rales were present throughout the lungs of the animals with hemodynamic pulmonary edema, and at the time of death, large amounts of pink foam were present in the trachea of each animal. At autopsy each of these animals had bilateral pleural effusions and anasarca. The lungs maintained their shapes when removed from the pleural cavities and pink frothy fluid exuded from their cut surfaces. One hour after the administration of alloxan the lungs of these test animals were deep red in color, bulky, and exuded white frothy fluid from their sectioned surfaces.
ELECTRON MICROSCOPIC STUDY OF PULMONARY EDEMA

FIGURE 1
This survey electron micrograph of control lung shows portions of four alveoli (ALV). Their surfaces are lined by membranous (I) and granular (II) pneumocytes. Numerous capillaries containing erythrocytes (R) are seen within the septa.

The ratios of wet:dry weight of the lungs of dogs subjected to pulmonary venous hypertension and hemodilution for 3 hr ranged between 6.7 and 11.7 (85 to 91% water). The ratios for the lungs from alloxan-treated animals were 8.5 and 10.5 (88% and 90% water) respectively. Ratios for control animals ranged between 4.6 and 5.0 (78 to 80% water).

CONTROLS
Electron Microscopy

The fine structure of the alveoli in the control animals was the same as that described previously by other workers (14-16). The epithelial cells lining the alveoli were of two types (Fig. 1). The type I lining cells (membranous pneumocytes) formed a layer that measured approximately 300 to 500Å in thickness (Figs. 1-4). Cytoplasmic organelles other than numerous pinocytotic vesicles were sparse (Figs. 2 and 4). These cells covered a much larger surface area than the type II lining cells (granular pneumocytes) (Fig. 1). The cytoplasm of the type II cells contained numerous, single membrane-limited inclusions measuring up to 3 μ in diameter whose contents varied in electron density. The basement membrane adjacent to the epithelial lining measured approximately 2000 to 3000Å in thickness (Figs. 3 and 4).

The alveolar capillaries were lined by a continuous layer of endothelium which measured as little as 1000Å in thickness (Figs. 2-4). Tight junctions (17) (areas of actual fusion of the outer leaflets of the unit membranes of adjacent endothelial cells) were not apparent in the alveolar capillaries. Numerous pinocytotic vesicles were present in the cytoplasm of the endothelial cells (Figs. 2 and
Another electron micrograph of control lung. Portions of several erythrocytes (R) are present within this capillary. In the left hand portion of the figure a single basement membrane (BM) lies between the endothelial (EN) and epithelial (EP) cell layers. Interposed between the endothelial and epithelial basement membranes in the right hand portion of the figure is an interstitial space containing collagen fibrils (COL) and elastic tissue (EL).

A basement membrane measuring approximately 2000 to 3000Å in thickness was adjacent to the basal aspects of these cells. In certain areas the capillary was closely apposed.
The basement membranes adjacent to the epithelial and endothelial cells are seen to fuse (arrow), forming a basement membrane measuring approximately 2000Å in thickness. Collagen (COL) fibrils and elastic tissue (EL) are seen once again in the interstitium (control).

to, and separated from, the overlying epithelium by a single layer of basement mem-
brane. Such regions may encompass up to 50% of the circumference of the capillary. In
FIGURE 4
Similar to Figure 3. The arrow points to the site of fusion of the basement membranes adjacent to the endothelial (EN) and epithelial (EP) cells (control).

Other regions the basement membranes of the endothelial and epithelial cells were not fused and were separated by an interstitial space which varied in thickness from several hundred Angstrom units to approximately 5 μ (Figs. 2 and 3). The thickness of the fused
basement membranes was approximately equal to one of the individual unfused layers (Figs. 3 and 4).

This interstitial space contained plates of elastic tissue and bundles of collagen fibrils that were embedded in amorphous, slightly electron opaque, ground substance (Figs. 2 and 3). Occasional fibrocytes, smooth muscle cells and macrophages were also seen within the interstitium. Collagen was the most prominent interstitial element, however, and occurred as interwoven bundles of tightly packed fibrils that displayed their characteristic 640A periodicity (Fig. 3). The plates of elastic tissue were usually associated with these large aggregates of collagen. Small numbers of collagen fibrils were also found adjacent to the basement membranes (Figs. 2 and 4). No vessels thought to be lymphatics were seen within the septa.

HEMODYNAMIC PULMONARY EDEMA

Light Microscopy

The veins and capillaries within the pulmonary parenchyma appeared distended with blood. The only evidence of interstitial edema was marked swelling of connective tissues around some, but not all, of the lobar and segmental pulmonary arteries and veins (Fig. 5). No tracer particles, however, were seen around these vessels. When compared with the controls, there was a scant amount of pink granular fluid within the alveolar spaces. Erythrocytes and macrophages were also seen within these spaces.

Electron Microscopy

The major alterations in the edematous state were confined to the interstitial portions of the septum. The collagen-containing areas were expanded, measuring as much as 15 μ in thickness, and the fibrils were widely separated from one another (Figs. 6-9). The portions of the septum devoid of collagen, where the air-blood spaces are in closest proximity, were not enlarged (Figs. 6-8). Numerous erythrocytes in various stages of degeneration, but no fibrin, were found both within the interstitium and the alveolar spaces (Fig. 8). Cytoplasmic fragments containing variable amounts and forms of membranes were also seen within the interstitium (Fig. 6) of the edematous but not control animals. The sections from animals that were injected with vascular markers exhibited colloidal particles within capillary lumina (Fig. 9) or within cytoplasmic vacuoles of macrophages or within both. Neither the expanded nor unexpanded regions of the interstitium contained such particles.

The enlarged interstitial spaces did not encroach upon the capillaries (Figs. 6, 8, and 9). In fact, by inspection the capillaries appeared distended in the edematous lungs, and had generally larger luminal spaces around the contained red blood cells. The capillaries in the edematous lungs were otherwise indistinguishable from those of the control animals. The endothelial and epithelial basement membranes were intact (Fig. 9); the endothelial cells were not swollen (Figs. 6-9).
This electron micrograph from a lung with hemodynamic edema exhibits widening of the interstitial spaces (IS) and separation of the collagen fibrils. The area of the septum devoid of collagen (arrow) is not expanded. The endothelial and epithelial linings are unaltered. Membrane containing cytoplasmic fragments (CF) are seen within the interstitium. ALV = alveolus.

and there were no separations of their intercellular junctions (Fig. 10). The lining layer of the alveoli was similarly intact and its cells were unaltered (Figs. 6-10).

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FIGURE 7
Similar to Figure 6. The expanded interstitial space (IS) contains dispersed collagen fibrils.

ALLOXAN EDEMA
Light Microscopy

The morphologic picture was similar to that seen in hemodynamic pulmonary edema, with apparent distention of veins and capillaries by blood and swelling of perivascular connective tissues (Fig. 11). In addition, focal areas displayed amorphous and fibrillar acidophilic material, but no erythrocytes, within the air spaces.
Another electron micrograph from an animal with hemodynamic pulmonary edema. The enlarged interstitial spaces (IS) contain an erythrocyte (R) and a cytoplasmic fragment (CF). Two other erythrocytes are present within the alveolar capillary.

Electron Microscopy

In contrast to hemodynamic pulmonary edema where the cellular elements were intact, there was marked disruption of both endothelial and epithelial cells (Figs. 12 and 13), making it impossible to assess by inspection the relative diameters of the capillaries. The endothelial cells of the capillaries exhibited marked swelling and disorganization as well as numerous discontinuities of their surface membranes (Figs. 12 and 13). Large areas of the capillaries were devoid of an endothelial lining (Fig. 12). Similar alterations were seen in the epithelial cells lining the alveoli. These epithelial cells were frequently detached from their basement membrane.
Electron micrograph from an edematous animal injected with Thorotrast. The electron opaque particles of Thorotrast are present within the vascular spaces (CAP) but are not found in the edematous interstitial space (IS).

membranes (Figs. 12 and 13). Despite the presence of severe degenerative changes in epithelial and endothelial cells, numerous intercellular junctions were found (Fig. 13).

Both the endothelial and epithelial basement membranes appeared intact, and their width was similar to that of the controls.

Some degree of interstitial swelling was also
present (Figs. 12 and 13). Large amounts of fibrin as well as granular, electron opaque material were present within the alveoli. In addition, similar granular material, but not fibrin, was seen within the expanded portions of the septa (Fig. 13). Erythrocytes were not found either in the interstitium or alveolar spaces.

**Discussion**

The present study indicates that the major morphologic alteration at the alveolar capillary level in hemodynamic pulmonary edema is interstitial in location and focal in nature. The apparent discrepancy between the lung weight ratios and the light microscopic appearance of the lungs is because the edema fluid in this preparation has a low protein content and hence is not stained by hematoxylin or eosin. The expanded collagen-containing regions of the septum seem to represent sites of fluid accumulation in the tissues even though transudates per se cannot be visualized by current electron microscopic techniques. This focal change is in direct contrast to the diffuse changes observed after alloxan in the present study and after a variety of other toxic substances (5-11, 18). With these toxic agents the edema fluid is not limited to the collagen-containing regions of the interstitial spaces but is found in all portions of the septum and is associated with degenerative changes in the endothelium and epithelium.

In terms of Starling’s hypothesis for the exchange of fluid across capillary walls, the formation of edema in the present experiments involving increased capillary hydrostatic pressure and decreased colloid osmotic pressure may be regarded merely as an accentuation of the normal process of fluid exchange in the lungs rather than a pathologic one (19, 20). It is then conceivable that the collagen-containing interstitial areas of the alveolar septum could serve as a reservoir to
FIGURE 12

Electron micrograph of lung from an animal treated with alloxan. There is degeneration of the epithelial (EP) and endothelial (EN) cell layers. Their respective basement membranes (BM) appear unaltered. The collagen (COL) containing interstitial space (IS) is expanded. Strands of fibrin (F) and granular, electron opaque material are present within the alveolus.

Electron micrographic studies show that, as excess fluid collects in the interstitial space of the lung, the interstitial connective tissue could function as a "sponge" to bind fluid in a state of low potential energy. This prevents fluid from accumulating in the alveoli and maintains the lung in a dry state. As a corollary, free fluid in an alveolus or accumulation of fluid in the interstitial space would imply prior saturation of the local fluid-binding sites. Observations by Uhley et al. (21) on the delay before lymph flow from the lung increases in hemodynamic pulmonary edema are consistent with this concept. Diffusion across the alveolar-capillary membrane is not severely compromised by pulmonary edema, and arterial oxygenation follows accumulation of fluid in small bronchioles and alveoli rather than from diffusion limitation (22, 23).

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Similar to Figure 12. There is separation of the epithelium (EP₁) from the fused portion of the basement membrane (BM). Intercellular junctions (J) are present between the degenerating epithelial (EP) and endothelial (EN) cells.

has shown that the thin and thick portions of the alveolar capillary differ markedly with respect to the accumulation of edema fluid. The possibility then arises that the good oxygenation reflects the effective operation of the thin part of the capillary in gas exchange even though diffusion through the opposite wall may be somewhat impeded by thickening.

The focal collections of fluid observed in the present study of hemodynamic edema provide no new information concerning the pathway of fluid movement from capillary lumen to collecting sites. The vascular markers used were invariably found within capillaries and never in association with the edema fluid. The fact that both the endothelial cells and their intercellular junctions retained their normal structure in hemodynamic edema does not preclude fluid movement across these cells or their junctions (24). In fact, it is even conceivable that the interstitial fluid may not come from the capillaries. For example, as has been suggested (10), it may originate from larger vessels as perivascular edema which then extends down to the alveolar level. However, this possibility remains to be examined by electron microscopy, since the resolution available with light microscopy may be inadequate to demonstrate the earliest collections of edema fluid within alveolar septa. Also, the presence of numerous red blood cells within the interstitium of the lung and the alveolar spaces in hemodynamic pulmonary edema, even though vascular markers do not seem to escape, has still to be explained.

In any case, the pink color of the edema fluid in these animals is due to the escape of erythrocytes into the alveolar spaces, while the white edema fluid of the alloxan-treated animals reflects the retention of the erythrocytes within the vascular spaces. Finally, the origin and significance of the cytoplasmic fragments within the interstitium is unclear although they may represent fragments of endothelial or smooth muscle cells. Although the present

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observations have provided some insight into the morphological aspects of hemodynamic pulmonary edema, they have also emphasized that much remains to be learned concerning the routes of exchange for fluids and particulate matter between the blood vessels, the interstitium, the alveolar spaces and the lymphatics of the lungs.

References
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Circ Res. 1967;21:783-798
doi: 10.1161/01.RES.21.6.783

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
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