AN ELECTRON MICROSCOPIC STUDY OF THE ALVEOLAR–CAPILLARY WALL FOLLOWING INTRATRACHEAL ADMINISTRATION OF SALINE AND WATER

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An extensive literature deals with the physiologic responses of tissue to varying fluid environments. However, little attention has been directed to the study of specific alterations that may accompany the exposure of cells to anisotonic media.[1–3] In a previous study, it was found that the introduction of fresh water and sea water into the lungs of rats caused distinctive alterations in the fine structure of the alveolar–capillary region.[4] The hypotonic fresh water produced disruptions of the alveolar and endothelial cell membranes and caused dilatation and swelling of cell organelles. Sea water resulted in the formation of vacuoles in the alveolar epithelium and capillary endothelium.

The present study investigates in more detail the pulmonary alterations observed following the intratracheal administration of fluid. The use of pulmonary lavage in the treatment of respiratory disease emphasizes the need for examination of the response of the lung to fluid.[5]

MATERIAL AND METHODS

The study was performed on 24 healthy, male and female, adult Osborne–Mendel rats maintained on Purina® Laboratory Chow and water *ad libitum*. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed during this study. The animals were divided at random into 8 groups with a minimum of at least 2 animals in each group. Each group received one of the following solutions intratracheally: distilled water, 0.25 per cent NaCl, 0.50 per cent NaCl, 0.85 per cent NaCl, 1.0 per cent NaCl, 2.0 per cent NaCl, 2.5 per cent NaCl and 3.0 per cent NaCl in distilled water.

The technique for the introduction of the fluid was identical to that previously described.[4] Each rat was anesthetized with intraperitoneal pentobarbital sodium, 5 mg per 100 gm body weight. A glass cannula was inserted into the trachea and was secured with a suture. The test solutions were introduced into a buret, the lower end of which was connected by rubber tubing to the glass cannula. The buret was opened and the solution at room temperature was allowed to flow freely into the respiratory tract. When all respiratory motion had ceased, the thorax was opened. The interval between the opening of the buret and the cessation of respiration varied

Presented in part at the Fifty-fifth Annual Meeting of the International Academy of Pathology, Cleveland, Ohio, March, 1966.

Accepted for publication, August 18, 1966.

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from 3 to 5 minutes. An additional ½ minute was required to open the thorax and remove the tissue. A slice of lung no greater than 2.0 mm thickness was removed from both lower lobes. The tissue was immersed in ice-cold 4.0 per cent glutaraldehyde buffered with phosphate to ph 7.3.[6] Fixation was continued for 18 to 24 hours. The slices were then diced in an identical buffer, without the fixative, and rinsed for 24 hours. The tissue was postfixed for 2 hours in 1.0 per cent osmium tetroxide in a similar buffer. Dehydration was accomplished in ascending concentrations of ethyl alcohol. Epon epoxy resin was used for embedding.[7] A minimum of 6 blocks were examined from each animal. Silver and grey sections were obtained using glass knives and a Porter–Blum microtome. All sections were mounted on copper grids and double-stained with uranyl acetate and lead tartrate. Observations were made using an RCA EMU 3-F electron microscope at 50 kv.

As controls, 7 normal rats were used. The glutaraldehyde fixative, at room temperature, was introduced into the respiratory tract as described above. The pulmonary tissue was removed and prepared exactly as in the experimental groups.

Results

Controls. The ultrastructural appearance of control lungs was similar to that described in the literature.[8,9] Capillary loops filled with red cells projected into the alveolar spaces (Fig. 1); the endothelium was continuous. Alveolar spaces were completely lined by the cytoplasmic processes of alveolar epithelial cells. Scattered among the epithelial cells were granular pneumocytes with deeply osmiophilic cytoplasmic inclusions.

Of interest was the presence of microtubules in the cytoplasm of endothelial cells (Fig. 2). These were similar to those previously described in a variety of other animal cells.[10,11] They appeared as straight, moderately dense structures, 200 to 250 Å thick, surrounded by an area of cytoplasmic clearing. Microtubules were also observed in the cytoplasm of granular pneumocytes and septal cells.

Changes Following Distilled Water Administration. Long finger-like projections of epithelial cytoplasm extended into alveolar lumens. The epithelium was markedly swollen (Fig. 3). Nuclei in all cells exhibited a diffuse homogeneous granular appearance (Fig. 4) and there was marked dilatation of the perinuclear space particularly where it was continuous with the endoplasmic reticulum. Dilated portions of the endoplasmic reticulum were very prominent (Figs. 3 and 4). The endothelium was focally disrupted by areas of extensive swelling. Erythrocytes in capillary lumens showed advanced lysis of hemoglobin with only cell membranes remaining (Fig. 3). Mitochondria were distinctively altered; separations appeared between the inner and outer mitochondrial membranes with large projections of the outer layer extending into the adjacent cytoplasm (Fig. 5). Mitochondrial matrices exhibited a reduction in electron density and cristae were focally swollen. Large electron-lucid areas separated the collagen fibers and microfibrillary material in the interstitial area (Fig. 3, I).

Changes Following Saline (NaCl in distilled water) Administration. Following the intratracheal administration of saline, certain ultrastructural features appeared to be directly related to the concentration of saline while others were present in all the groups (Table I).

The alveolar epithelial swelling induced by distilled water was almost completely absent after 0.25 per cent saline was given. At this concentration, only scattered mitochondria contained swollen cristae and exhibited decreased matrix density. At concentrations above 0.50 per cent saline, no mitochondrial abnormalities were detected.

Extensive dilatation of the endoplasmic reticulum observed following distilled water was only slightly evident after 0.25 per cent saline and no changes could be found at the 0.50 per cent saline level or above.

The homogeneous and finely granular appearance of nuclear chromatin following distilled water was present to some extent after 0.25 per cent saline. A return to the normal pattern was seen at 0.50 per cent and higher concentrations showed no nuclear changes.

Extensive lysis of red cells as observed with distilled water was still apparent with 0.25 per cent saline; it could not be detected with 0.50 per cent saline or above. No alterations in the contour or density of red cells were present at higher concentrations.

Microtubules were maintained in the endothelium following all concentrations of saline. They appeared to be more prominent as the concentration increased to hypertonic levels. This was apparently due to an increased clearing of the cytoplasm immediately surrounding the microtubules, as no increase in their size was noted.

The overall density of the cytoplasm appeared to increase in all cells with the hypertonic saline solutions, so that at the 2.0 to 3.0 per cent concentrations it was difficult to visualize cytoplasmic organelles (Fig. 8).

Certain ultrastructural features occurred following all concentrations of saline. These may best be categorized as alterations in the endothelium, in the basement membrane and in the interstitium, and changes in the alveolar epithelium.

Portions of the endothelium were elevated from the basement membrane forming double-walled projections into the capillary lumens (Figs. 6 and 7). These appeared occasionally after 0.25 per cent saline administration. With 0.50 per cent saline the endothelial folds were more frequent and continued to increase in number to the 3.0 per cent level. The extensive projections of the folds into the capillaries resulted in a variety of planes of sectioning so that numerous double-walled structures were encountered in many portions of the capillary lumens. The regions underlying the endothelial folds were invariably electron-lucid.

TABLE I

MORPHOLOGIC CHANGES FOLLOWING IN VIVO EXPOSURE OF THE LUNG TO WATER AND SALINE

Morphologic alteration	Control	Distilled water	NaCl test solutions						
			0.25%	0.50%	0.85%	1.0%	2.0%	2.5%	3.0%
Alveolar epithelial swelling	−	++++	++	−	−	−	−	−	−
Dilatation of endoplasmic reticulum	−	++++	+++	−	−	−	−	−	−
Nuclear chromatolysis	−	++++	+++	+	−	−	−	−	−
Mitochondrial swelling	−	++++	+++	++	−	−	−	−	−
Hemolysis	−	++++	+++	−	−	−	−	−	−
Increase in overall cytoplasmic density	−	−	−	−	+	+	++	+++	++++
Presence of electron-lucid areas in interstitium	−	+++	+++	++	++	+++	+++	++++	++++
Endothelial vesiculation and folding	−	++	++	++	++	+++	+++	++++	++++
Focal alveolar epithelial vesiculation	−	−	−	+	+	+	+++	++++	++++

−, not observed; + to ++++, occasionally to frequently observed.

Tubular bodies (Fig. 7, arrows) and round vesicles (Fig. 6) were observed in the electron-lucid areas adjacent to and in the endothelium. The morphologic similarity between many of these structures and the microtubules seen in normal endothelium suggested that they represented altered microtubules. Endothelial changes of this type were found in all lungs following saline administration.

The interstitium of the lung contains bundles of collagen fibers, microfibrillary material and the cytoplasmic processes of septal cells. Following saline administration, electron-lucid areas were observed with great regularity throughout the interstitium (Fig. 6). They were found to connect with the subendothelial clear areas forming continuous electron-lucid spaces between the septal and subendothelial regions.

The last category of changes involved the alveolar epithelium. Elevations and focal areas of clearing were seen in the alveolar epithelium following the administration of the higher concentrations of saline (2.0 to 3.0 per cent) (Figs. 8a to 8g). These projections were associated with vesicular structures similar to the bodies described in endothelial vacuoles. The vesicles in the alveolar epithelium did not exhibit the elongated tubular appearance observed in endothelium, but rather appeared more round (Figs. 8c, 8f and 8g, arrows). Considerable variation existed in the structure of the epithelial swellings with clusters of vesicles lying adjacent to and within the alveolar epithelial cytoplasm.

Discussion

It has been established that there is movement of water from the alveoli into the capillaries following the intrapulmonary introduction of hypotonic fluid, and that fluid moves from the capillaries into the alveoli after the introduction of hypertonic fluid.[12] Roberts, Taub, Liebow and Aperia[13] recently demonstrated that if the fluid introduced into alveoli is isotonic and buffered, and hypoxia is avoided, an exchange of water between the blood and alveolar fluid will still occur. It appears that exposing the alveolar epithelium to any aqueous solution induces rapid movement of water across the cell membranes, the net direction of movement depending upon the relative osmolarity. By increasing the salinity of intra-alveolar fluid from a hypotonic to a hypertonic solution, as in the present experiment, the alveolar–capillary wall would be exposed to extensive movements of water and electrolytes.

Under physiologic conditions, the mammalian cell may be considered freely permeable to water.[14,15,20] Movements of water across the cell membrane are effected in a passive manner since no evidence exists for the active secretion of water in animal cells.[14] It has been shown that over 80 per cent of cell water may move across the cell membrane in

response to an osmotic gradient.[16] Although the cell membrane may be considered freely permeable to water under physiologic conditions, the osmotic gradients imposed in the present experiment may have resulted in an excessive load on the water transport capabilities of the plasma membranes resulting in accumulations of fluid. These foci of fluid accumulation were felt to be represented morphologically as electron-lucid areas in the basement membranes, by the elevations of the endothelium, and by vacuoles in the cytoplasm of pulmonary epithelium.

The literature contains little information concerning morphologic study of cells undergoing controlled osmotic stress. Schrek[3] examined different tissues by light microscopy after exposure to distilled water and found that some cells showed no change in staining reaction while others varied in the degree of nuclear staining and cytoplasmic swelling. He also noted lysis of endothelium in small blood vessels after exposure to hypotonic fluid. Naito[17] observed changes in the cellular ultrastructure of leukocytes exposed to hypotonic solutions. The nuclei became irregular in contour, the outer nuclear spaces were dilated, and the mitochondria were completely vacuolated. Hypertonic saline resulted in the formation of cytoplasmic projections. In a study of tumor cells treated with distilled water, Herdson and Kaltenbach[18] described narrowing of the endoplasmic reticulum, increased density of mitochondrial matrices, and an absence of generalized cell swelling. Fibroblasts have been shown to swell and rupture within 3 minutes after exposure to distilled water.[19]

In the present study, mitochondria and endoplasmic reticulum were the cytoplasmic organelles exhibiting the most severe alterations when the cell was exposed to hypotonic solutions. Distilled water resulted in the formation of a wide separation between the inner and outer mitochondrial membranes. Similar effects have been obtained in isolated mitochondria by exposure to hypotonic sucrose solutions.[21] Although osmotic stress does not appear to produce a specific mitchondrial alteration, separation and rupture of the outer envelope may be characteristic of primary osmotic injury in contrast to that resulting from toxic or anoxic damage. The propensity of the endoplasmic reticulum to undergo extensive dilatation in response to hypotonic saline and water was clearly evident. No explanation can be offered to account for this at the present.

Pulmonary endothelium frequently detaches from the basement membrane under abnormal conditions.[22–24] This may be related to the constantly changing shape of the alveolar–capillary wall in the course of normal respiration. The large capillary projections and elevations appearing after intratracheal fluid administration may be a reflection of a loose attachment between the endothelium and the basement membrane

so that fluid would tend to accumulate in this area and stretch the endothelium into the configurations observed. In addition, this would result in an increase of cell surface for the interchange of water and electrolytes. An example of structural cell modification to an osmotic load is found in the salt gland of marine birds where excretion of concentrated saline appears to be associated with large infoldings of the cell membrane.[25]

Microtubules, similar to those described in a variety of other mammalian cells, were noted in the endothelium in control rats.[10,11] They appeared to be more prominent in the endothelial cytoplasm in animals treated with saline, particularly at hypertonic levels. In addition, the tubular bodies found in association with the elevation and vacuolization of the endothelium bore a considerable resemblance to the normal microtubules except for irregularity of outline and larger size. It is interesting to note that microtubules may be associated with shunting of ions and water within cytoplasm in response to increased functional loads.[11] If this theory is correct, the alterations observed after saline administration may be a morphologic reflection of increased fluid movement in the face of excessive osmotic loads.

Hypertonic saline was associated with alveolar epithelial vacuolization in contrast to the effects of isotonic and hypotonic solutions in which epithelial vacuoles were infrequent or absent. A direct irritative action of the saline on the epithelium might be postulated. Alterations in alveolar epithelium have been observed following the intratracheal administration of foreign material.[26] The appearance of the epithelium, however, was unlike that observed in this study. It may be pertinent to note that the net exchange of water would be into the alveoli following the administration of hypertonic fluid. The preponderance of epithelial changes may be related to this flow of fluid into the alveolar spaces.

No increase in the size or number of endothelial pinocytotic vesicles was observed in any of the animals. With exposure to saline, some morphologic change might be expected if these vesicles were concerned with fluid transport. The lack of such alterations is in accord with the work of Brandt[27] suggested that pinocytosis may be more important in the movement of large molecules than in the transfer of hydrated inorganic ions.

The influence of anoxia in the production of the changes encountered deserves mention. Hypoxia has been associated with vacuolization and edema of alveolar epithelium.[8,28] The large endothelial folds and the tubular bodies seen in this study, however, have not been reported following hypoxia. In addition, the controls in this experiment were anoxic for periods of time comparable to the experimental groups but lacked

the extensive changes seen in the experimental animals. It is therefore unlikely that anoxia alone was responsible for the changes observed. It is possible, however, that the combination of excessive fluid load and hypoxia produced the structural alterations. The present experiment did not attempt to separate these two effects.

SUMMARY

The introduction of varying concentrations of NaCl in water into the respiratory tracts of rats produced changes in the structure of the alveolar–capillary wall. These alterations were to some extent related to the concentrations of the NaCl. Hypotonic saline produced mitochondrial swelling, nuclear chromatolysis, dilatation of the endoplasmic reticulum and perinuclear space and lysis of erythrocytes. Vesicles in the alveolar epithelium were frequently observed following the administration of hypertonic saline. Elevation and vacuolization of the endothelium were relatively constant features in all the animals examined. It is postulated that the alterations described resulted from osmotic stress and excessive fluid load in pulmonary tissue.

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[*Illustrations follow*]

Legends for Figures

Key:

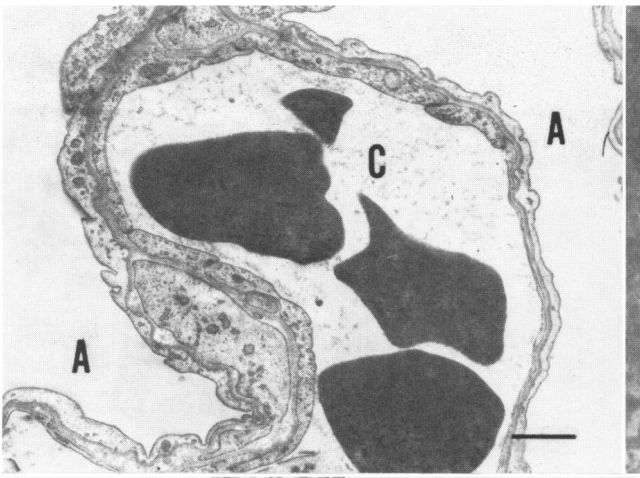
A = alveolar space	ER = endoplasmic reticulum
C = capillary lumen	I = interstitial area
En = capillary endothelium	N = nucleus
Ep = alveolar epithelium	R = red cell

All electron micrographs are of sections stained with uranyl acetate and lead.

Fig. 1. Control rat. The capillary lumen contains red cells and is lined by a continuous layer of endothelium. The cytoplasmic processes of alveolar epithelium completely line the alveolar spaces being separated from the endothelium by a thin basement membrane. × 4,100. The line indicates 2 μ.

Fig. 2. Control rat. Microtubules (arrows) are observed in an area of normal endothelial cytoplasm. × 46,000. The line indicates 0.2 μ.

Fig. 3. Distilled water. Distilled water results in alveolar epithelial swelling, clear areas in the interstitial tissue and lysis of red cells. Dilatation of septal cell endoplasmic reticulum is manifest. × 4,900. The line indicates 3.0 μ.



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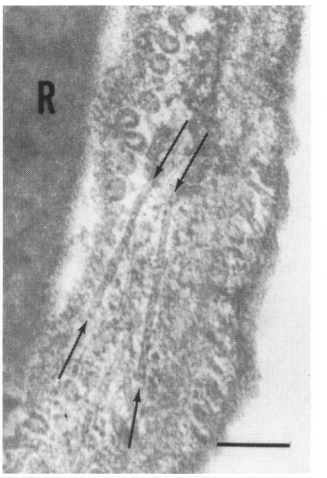

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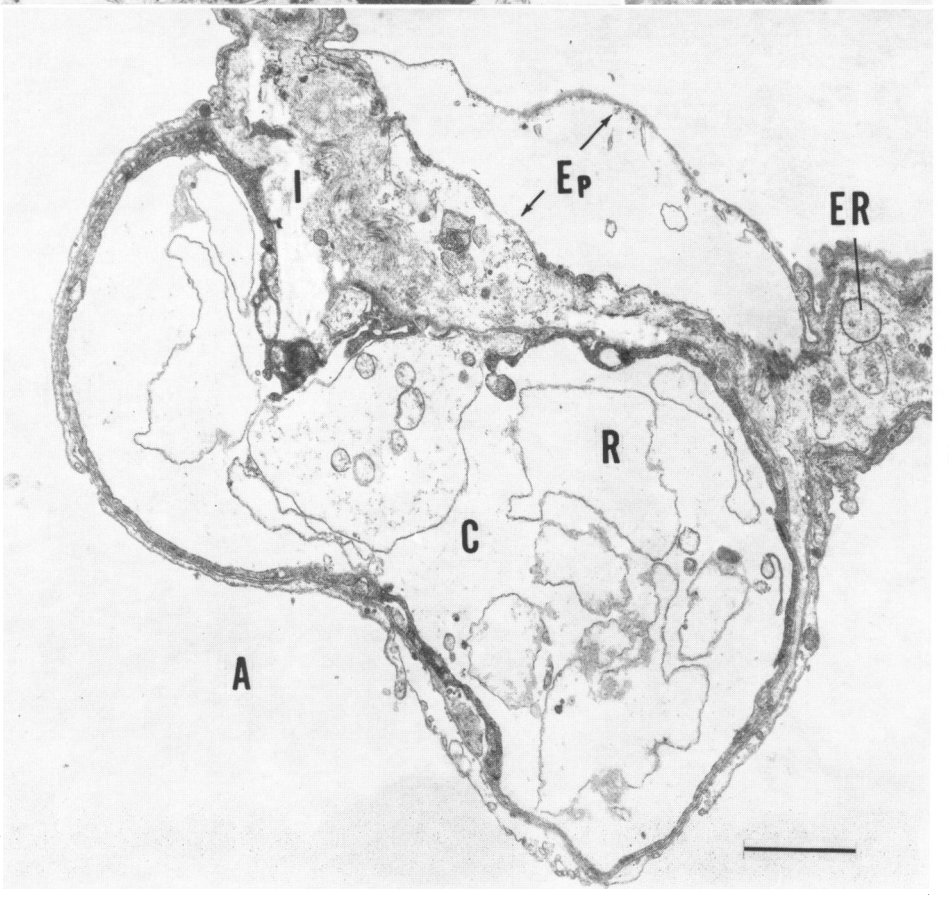

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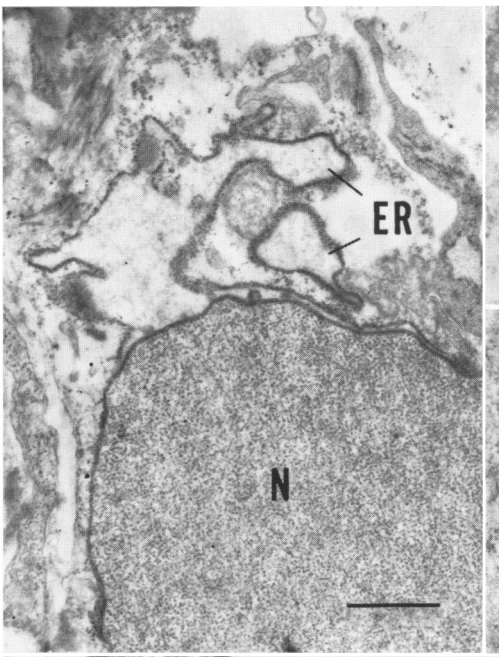
FIG. 4. Distilled water. The diffuse nuclear granularity seen here is abnormal following glutaraldehyde fixation. The perinuclear space is dilated particularly where continuous with the endoplasmic reticulum. × 12,400. The line indicates 1.0 μ.

FIG. 5. Distilled water. A large vacuole is continuous with the outer mitochondrial membrane (arrow). The matrix is less dense than normal and cristae are swollen. These changes are felt to be suggestive of osmotic injury. × 32,900. The line indicates 0.5 μ.

Inset. Control rat, normal mitochondrion. × 24,400.

FIG. 6. Saline, 1.0 per cent. This micrograph is representative of the alterations observed in the saline treated rats. A large clear area underlies an elevation of the capillary endothelium (En). Electron-lucid areas in the interstitium were frequently continuous with these subendothelial clear areas. The endothelium also extends in folds (double arrow) into the vessel lumen. The central portion of the fold is electron-lucid and contains small vesicular bodies (V). × 18,200. The line indicates 1.0 μ.



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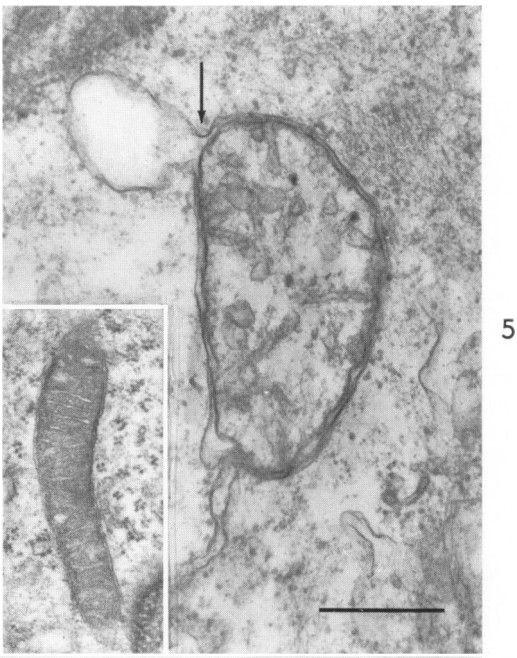

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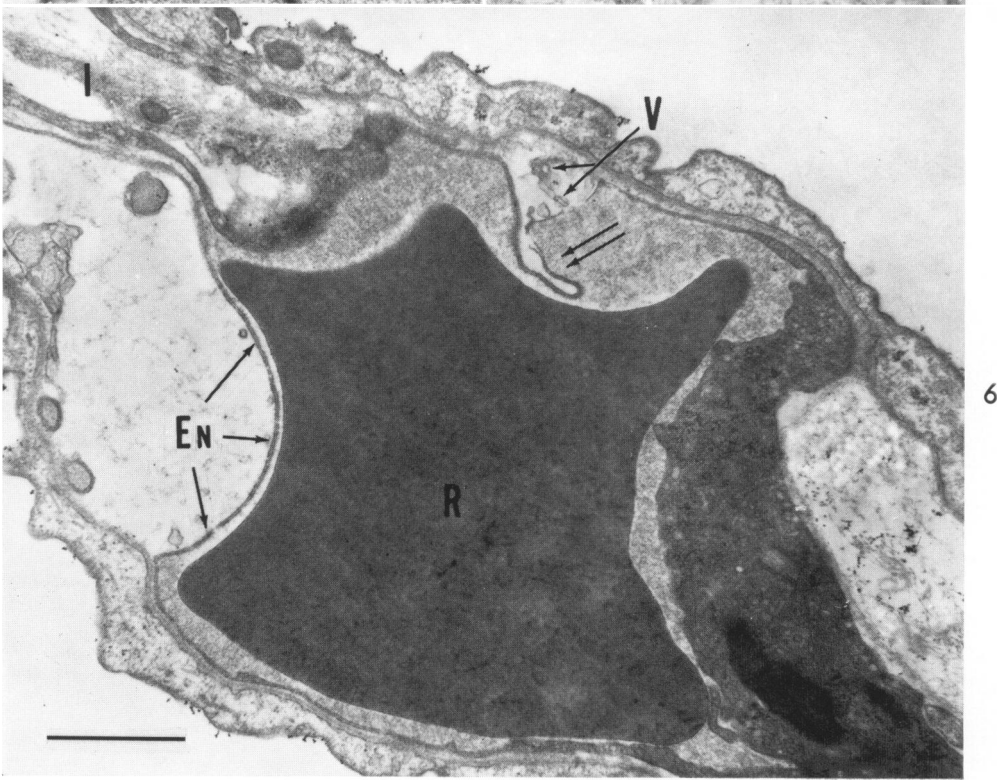

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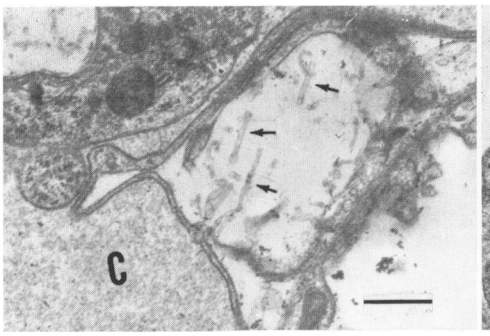
FIG. 7. Saline, 0.85 per cent. An endothelial fold demonstrates the presence of altered microtubules (arrows) in the electron-lucid area. × 17,600. The line indicates 0.5 μ.

FIGS. 8a to 8g. Saline, 2.5 to 3.0 per cent. This series of micrographs represents the alterations seen in the alveolar epithelium following exposure to hypertonic saline solutions. Alveolar space is on the upper right in each. The most salient feature is the considerable variation in the structure of the vacuoles. Many of the larger vacuoles contain clusters of small, irregularly round vesicles (arrows). The impression was gained that these clusters were being released into the alveolar spaces (Fig. 8g). The increased electron density of the cytoplasm is characteristic of exposure to hypertonic saline. × 24,300. The line indicates 0.5 μ.



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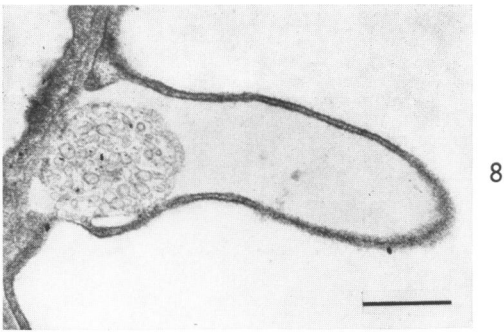

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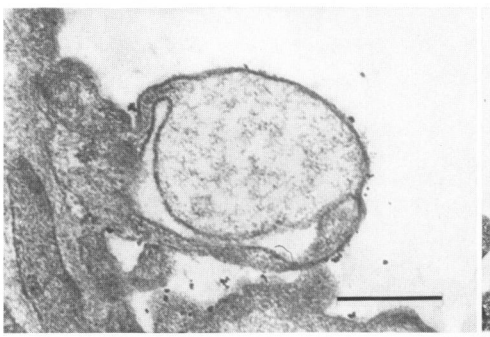

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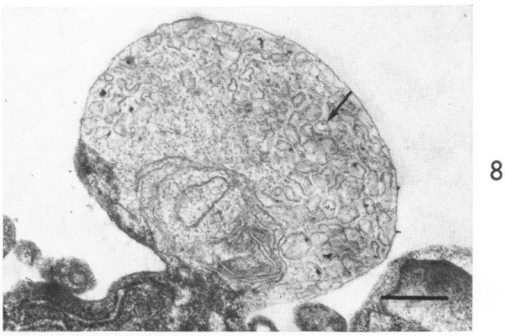

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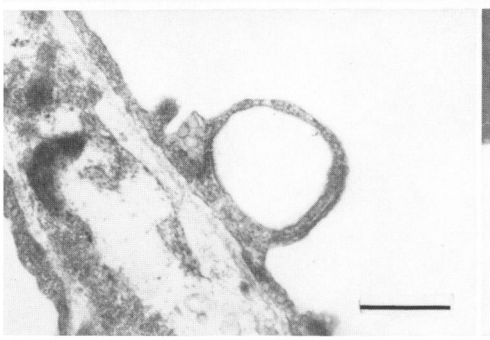

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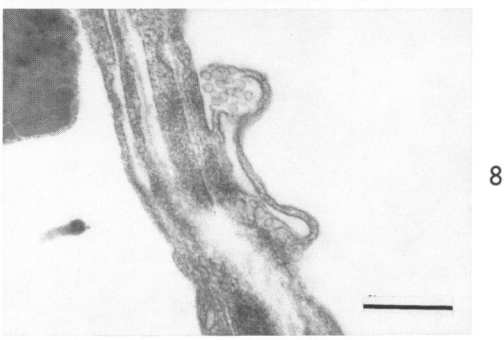

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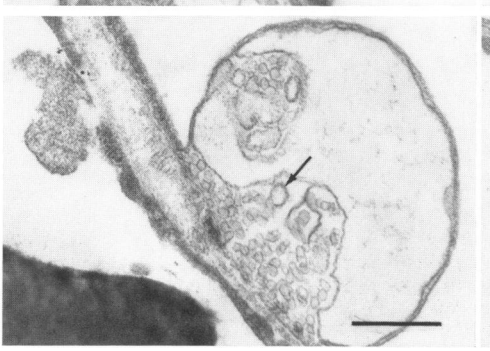

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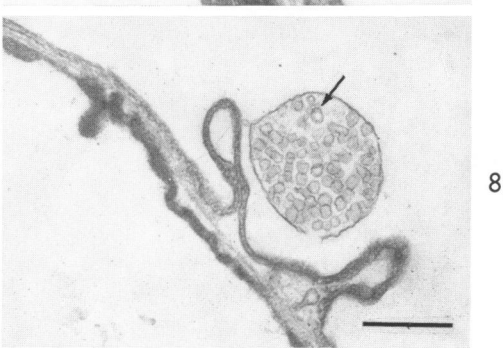

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