Ethchlorvynol-Induced Pulmonary Edema in Rats

An Ultrastructural Study

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Studies of ethchlorvynol (ECV)-induced pulmonary edema were undertaken for determination of the structural basis of increased microvascular permeability. Rats were administered an intravenous bolus dose of 15 mg/kg ECV and killed at time intervals between 5 minutes and 72 hours. Oyster glycogen and ferritin were used as permeability probes for identification of the sites of altered microvascular permeability. Edema fluid containing ferritin begins to accumulate in the alveolar interstitium 10 minutes after ECV. Thirty minutes after ECV, marked interstitial edema fluid is present containing both permeability probes. The absence of any appreciable transendothelial movement of either probe via vesicles and the presence of open endothelial junctions led the authors to propose the latter as the principal determinant of the increase in permeability. In addition to open endothelial junctions, prominent subendothelial blebs occur. These blebs develop in an otherwise intact endothelium and increase in frequency and size with time following their appearance at 10 minutes. Ferritin and glycogen progressively accumulate within the blebs. At 15 minutes the concentration of ferritin in blebs appears to equal that in plasma, whereas glycogen is absent or sparsely present in a few blebs. At 60 minutes both permeability probes have become concentrated in the blebs. The mechanism of formation of the blebs and concentration in them of the permeability probes cannot yet be specified. The lesion caused by ECV is completely reversible, so that by 72 hours after ECV there is complete resolution of interstitial edema, disappearance of the subendothelial blebs, and closure of endothelial junctions. A small amount of exudate remaining in the alveoli is cleared by 72 hours. (Am J Pathol 1984, 115:447–457)

NUMEROUS experimental agents have been studied in efforts to develop suitable models for investigating increases in pulmonary microvascular permeability analogous to those found in adult respiratory distress syndrome. Ethchlorvynol (ECV) is a chlorinated acetylenic carbinol which has been used clinically as a relatively nontoxic sedative.[1] In several reported cases of ethchlorvynol overdose, noncardiogenic pulmonary edema has been a significant complication.[2–5]

This clinical observation has stimulated the experimental study of the edema produced by ECV.[2,6] Observations of Dearden et al[6] suggested that altered permeability in dog lung capillaries caused by ECV results from an increase in pinocytotic vesicles and channel formation in endothelial and epithelial cells. Gil and McNiff,[7] more recently studying ECV-induced lung injury in rabbits, emphasized the focal endothelial and epithelial cell necrosis associated with microthrombi.

Experiments in rats were undertaken to determine the structural basis of increased microvascular permeability caused by ethchlorvynol under controlled conditions. The studies were based on quantitative studies of the time course and dose-dependence of ECV-induced pulmonary edema and utilized conditions of moderate, reversible changes rather than severe, lethal effects. Two different size vascular permeability probes, glycogen (30 nm in diameter) and ferritin (7.5 nm in diameter), were used to trace sites of large molecule leakage through the altered microvascular, endothelial barrier. Our evidence indicates that ECV at 15 mg/kg in rats results in a reproducible lesion that is confined to endothelial cells and reversible within 24 hours.

Supported in part by SCOR, Adult Respiratory Failure Grant HL30572, and the St. Louis University School of Medicine Department of Pathology Research Fund.

Accepted for publication January 25, 1984.

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Materials and Methods

Male Sprague–Dawley rats ranging in weight from 250 to 350 g were obtained from Sasco Breeders, St. Louis, Missouri. Rats were prepared for injection of ECV under anesthesia with intraperitoneal sodium pentobarbital (30 mg/kg body weight). The internal jugular vein was cannulated, an indwelling cannula was externalized in the midline of the back, and the animal was allowed to recover for 12 hours.

Pulmonary edema was assessed quantitatively by a gravimetric method.[8] Lung blood volume was measured using ^{51}Cr-labeled red cells. The lungs and blood samples were dried to constant weight at 80 C. All values were calculated as percent extravascular water.

The ECV (Placidyl, Abbott Laboratories, Chicago, Ill) preparation used contained approximately 5% polyethylene glycol 400 (PEG 400). The ECV-treated animals in these studies received a dose of 0.015 ml/kg (15 mg/kg) delivered through the jugular cannula. The ECV was administered as a bolus and flushed through with 0.5 ml of saline. The control animals were given equal volumes of PEG 400 in place of the ECV and the same amount of saline. Lung samples were taken from both control and ECV-treated rats at 5, 10, 15, 30, and 60 minutes and 12, 24, and 72 hours. For the acute experiments up to 60 minutes, 26 control animals were used, and at least 5 animals were killed at each time interval after ECV. Two control rats at 12 and 24 hours, 1 control rat at 72 hours, 4 ECV-treated rats at 12 and 24 hours, and 3 ECV-treated animals at 72 hours were studied.

Preparation of Permeability Probes

Oyster glycogen (mol wt 475,000) was purchased from Calbiochem (La Jolla, Calif). A 15% (wt/vol) suspension of oyster glycogen in 0.9% sodium chloride was prepared just before use with mild sonication. The resulting glycogen suspension was warmed to 37 C, and 1.5 ml/100 g body weight was administered through the indwelling catheter 4 minutes before killing the animals. Cadmium-free, two-times-recrystallized horse spleen ferritin was purchased from Polysciences, Inc. (Warrington, Pa). Ferritin was dissolved in saline at 100 mg/ml and injected intravenously at 50 mg/100 g body weight 4 minutes prior to sacrificing the animal. In other experiments, tracer was given 30 minutes after ECV, the peak time for water accumulation, to ensure maximal deposition of tracer; the animals were then killed 12, 24, or 72 hours later.

Tissue Preparation for Electron Microscopy

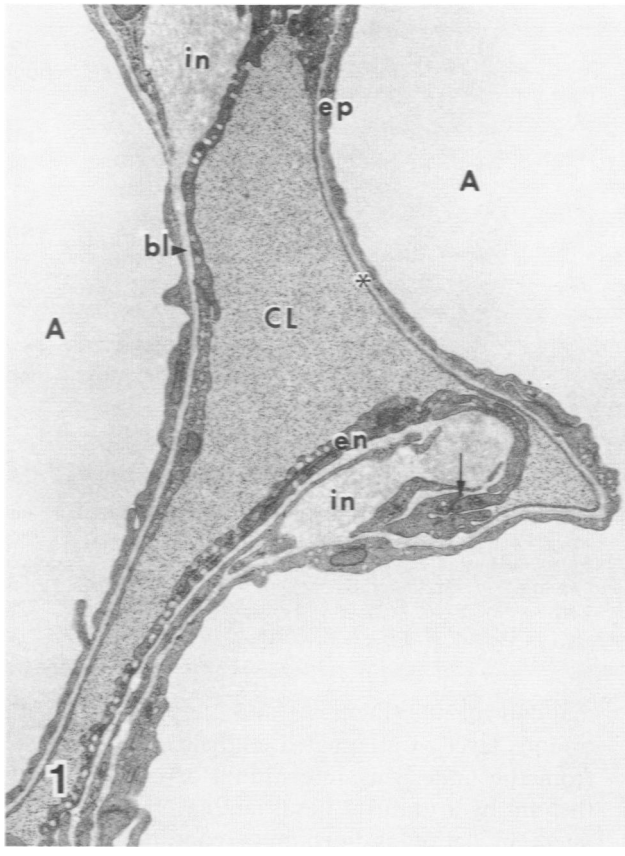
To obtain lung tissue, we anesthetized experimental and control rats with intravenous sodium pentobarbital (15 mg/kg) 1 minute before the lungs were removed. The trachea was intubated, and a tracheal tube joined to a three-way stopcock, which was in turn connected to a reservoir of fixative and a U-tube manometer. The thoracic cavity was opened, and the lungs were fixed *in situ* by intratracheal administration at 15–20 cm H_2O pressure of a fixative consisting of 1% formaldehyde and 2.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.3. The chest cavity was flooded with the same fixative prior to removal of the heart and lungs *in toto* and the submerging of these organs in fixative for 30 minutes at 4 C. Tissue samples were obtained from the right and left upper and lower lobes, diced into 1-cu mm blocks, and further fixed for 30 minutes at 4 C. Lung tissue was postfixed in 1% osmium tetroxide in 0.15 M phosphate buffer for 1 hour. Tissue was washed in the same buffer for 10 minutes at 4 C, rapidly dehydrated in graded steps of ethanol, infiltrated with resin, and embedded in Poly Bed 812 (Polysciences).

One-micron sections were cut and stained with toluidine blue for light-microscopic examination. We cut thin sections on an LKB Ultratome V with a DuPont diamond knife and stained them with either bismuth nitrate for 60 minutes to enhance the contrast of ferritin[9] or with uranyl acetate for 1 minute and lead citrate[10] for 15 minutes to optimize visualization of glycogen particles.[11,12] Sections were examined in a JEOL 100CX electron microscope.

Results

The amount of pulmonary edema in the ECV-treated animals in terms of percent extravascular

Figure 1 – Electron micrograph of control rat alveolar capillary unit (ACU). The alveolar wall consists of a layer of attenuated epithelial cells (*ep*) separated from the underlying interstitium (*in*) and capillary endothelium (*en*) by a continuous basal lamina (*bl*). Capillary endothelial cells are highly attenuated on the thin side of the ACU and separated from the epithelial cells by a single basal lamina (*). In contrast, the endothelium on the thick side abuts the supporting interstitial compartment and contains the majority of cellular organelles, vesicles, and caveolae. Endothelial cells are connected by tight junctions (*arrow*), which are distributed predominantly on the thick side of the alveolar septum. Four minutes after intravenous administration ferritin is confined to the capillary lumen (*CL*). The endothelium and epithelial cells are unaffected by the permeability probe. *A*, alveolar space. (Bismuth nitrate, × 10,400) **Figure 2** – Control rat alveolar capillary unit 4 minutes after intravenous administration of the permeability probe oyster glycogen. *A*, alveolar space; *ep*, epithelium; *en*, endothelium; *CL*, capillary lumen. (Uranyl acetate and lead citrate, × 16,600) **Figure 3** – Fifteen minutes following intravenous administration of ECV, there is accumulation of interstitial edema fluid (*E*) containing ferritin. Edema occurs primarily on the thick side of the alveolar capillary. *A*, alveolar space. The basal lamina is not well-defined in this section. (Bismuth nitrate, × 25,000)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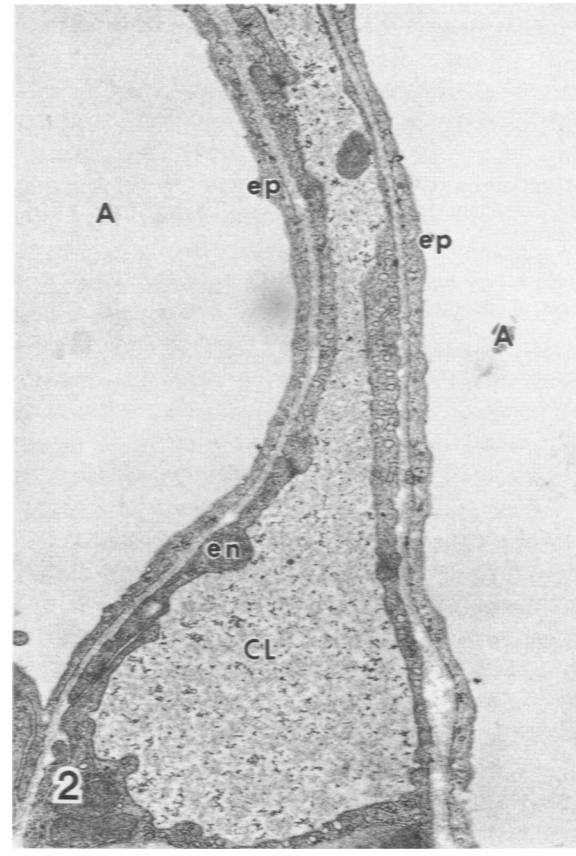
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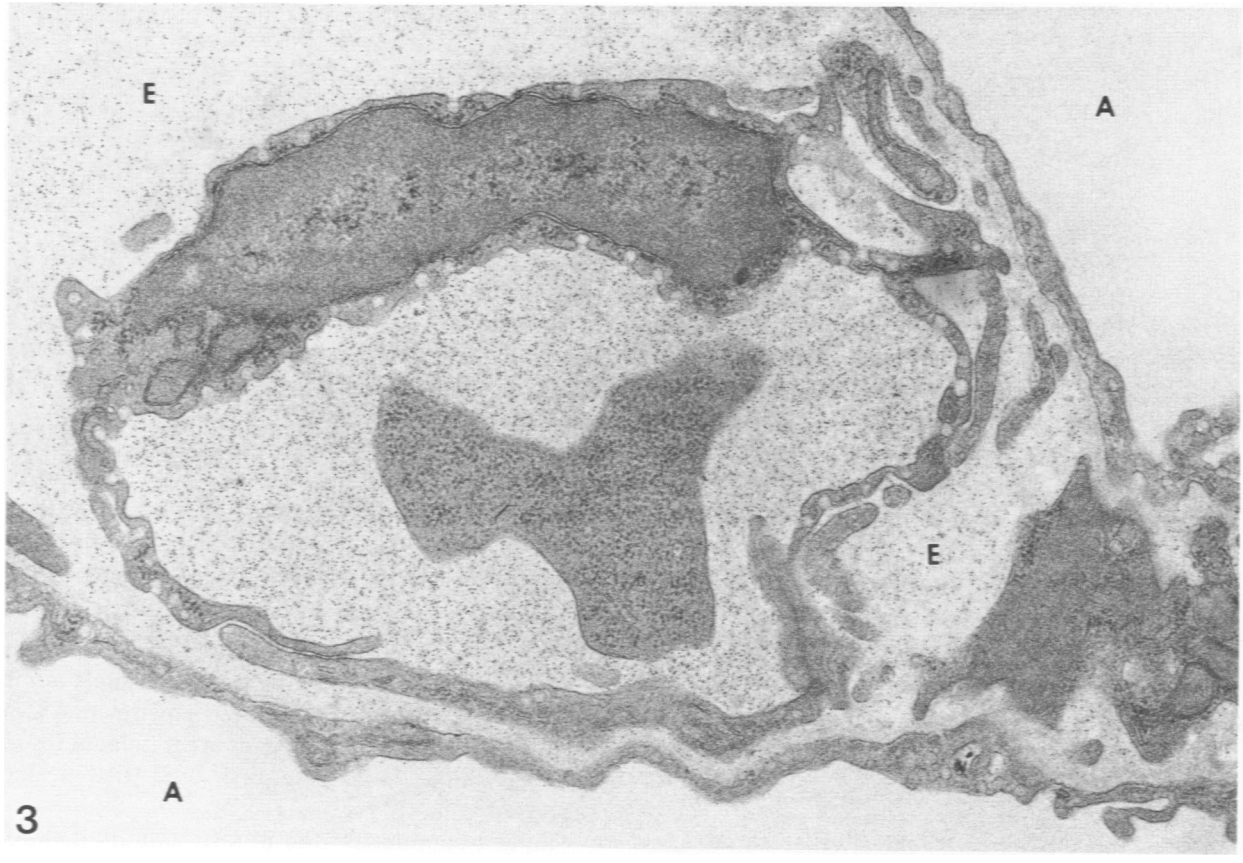
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Table 1—Summary of the Effects of ECV on Rat Lung

Time	5 minutes	10 minutes	15 minutes	30 minutes	60 minutes	12 hours	24 hours	72 hours
Interstitium								
Ferritin*	0	0.4	1.1	2.4	2.8	.4	.2	ND
Glycogen*	ND	ND	1.1	2.4	3.8	ND	ND	0
Endothelial cell swelling	–	–	–	–	–	–	–	–
Subendothelial blebs	–	+	+	+	+	–	–	–
Ferritin†	–	–/1+	2+	2+	3+	–	–	ND
Glycogen†	ND	ND	–/1+	2+	3+	ND	ND	–
Epithelial Type I swelling	–	–	–	–	–	–	–	–
Probes in endothelial vesicles	–	–	+	+	+	–	–	–
Probes in epithelial vesicles	–	–	–	+	+	+	+	–
Alveolar exudate								
Ferritin	–	–	–	–	+	+	+	ND
Glycogen	ND	ND	–	–	+	ND	ND	–
Alveolar protein	–	–	–	+	+	+	+	–

* Between four and seven micrographs at each time interval with each probe were evaluated by two observers blinded with respect to the identification of the micrographs. The observers were instructed to compare the interstitial concentrations of the probe on a scale of 0 to 4+ using a 2+ standard. The two observers were in agreement 49 out of 67 times and differed by more than 1 unit only 1 out of 67 times. The values in the table are the overall mean scores.

† Permeability probe concentrations in blebs in the 15-60-minute intervals were evaluated on a scale of – to 3+, where 2+ was taken as the concentration in the plasma, – no probe molecule, 1+ less and 3+ greater than the probe concentration in the plasma. ND, not done; –, absent; +, present.

water increased rapidly from the control level of 77.9% ± 0.9% to 86.1% ± 1.4% at 30 minutes with a dose of 30 mg/kg. Over the next 90 minutes there was an insignificant increase in pulmonary edema with 87.3% ± 1.4% water at 120 minutes. At 18 hours after ECV, the treated animals' lung water of 79.45% ± 1.77% was not significantly different from the control value.

The dose-response relationship was examined 30 minutes after ECV. A dose of 7.5 mg/kg resulted in a 30-minute value of 81.6% ± 1.1% water and 15 mg/kg produced 84.7% ± 1.9% water in the lung extravascular space. Based upon these observations, 15 mg/kg was selected as a dose capable of producing a near maximal but reversible edema in the rat.

Immediately upon injection of ECV, the animals became agitated, as evidenced by their pawing at their snouts; the animals also sneezed and coughed for a few minutes but showed no other deleterious effects for the first 15 minutes. Beginning at 15 minutes, irregular breathing became apparent, and by 30 minutes the animals showed rapid shallow respiration, which slowly returned to normal over 12 hours. Grossly, the lungs of treated animals showed no remarkable differences from those of control animals prior to 15 minutes. At 15 minutes, the lungs contained scattered small atelectatic foci and exhibited boggy, rounded margins. These changes increased in severity over the next 45 minutes. By 12 hours gross differences between the treated and control animals had regressed. There were no deaths at the dose of ECV used.

As previously described,[13] the alveolar wall of normal control rats (Figures 1 and 2) consists of a continuous layer of attenuated epithelial cells separated from the underlying interstitium or capillary endothelium by a uniform basal lamina. The pulmonary microcirculation is lined by a continuous sheet of endothelium. Endothelial cells are connected by tight junctions, which are distributed predominantly on the thick side of the alveolar septum. In the part of the vasculature that overlies the interstitium, the endothelial cells are several microns thick and contain an abundance of cellular organelles. The thin segment of the capillary endothelium facing the alveolar space is reduced to a highly attenuated plasma membrane-bound sheet of cytoplasm separated from the alveolar epithelium by a single narrow basal lamina. While the endothelial layer is basically one cell thick, multiple layers are seen with some frequency, particularly in the vicinity of intercellular junctions. Serial sections indicate that the multiple layers are formed by sheetlike extensions of one cell overlying or interdigitating with those of an adjacent cell (Figures 9 and 11).

Under normal conditions, ferritin (Figure 1) and glycogen (Figure 2) are confined to the vascular lumen with only occasional probe molecules evident in endothelial cell vesicles. Endothelial and epithelial cell structure were unaffected by the permeability probes. No leakage was observed in control animals administered either probe used in these experiments. However, we have found with several other preparations of glycogen other than the one used that leakage occurred.

Table 1 summarizes the ultrastructural alterations

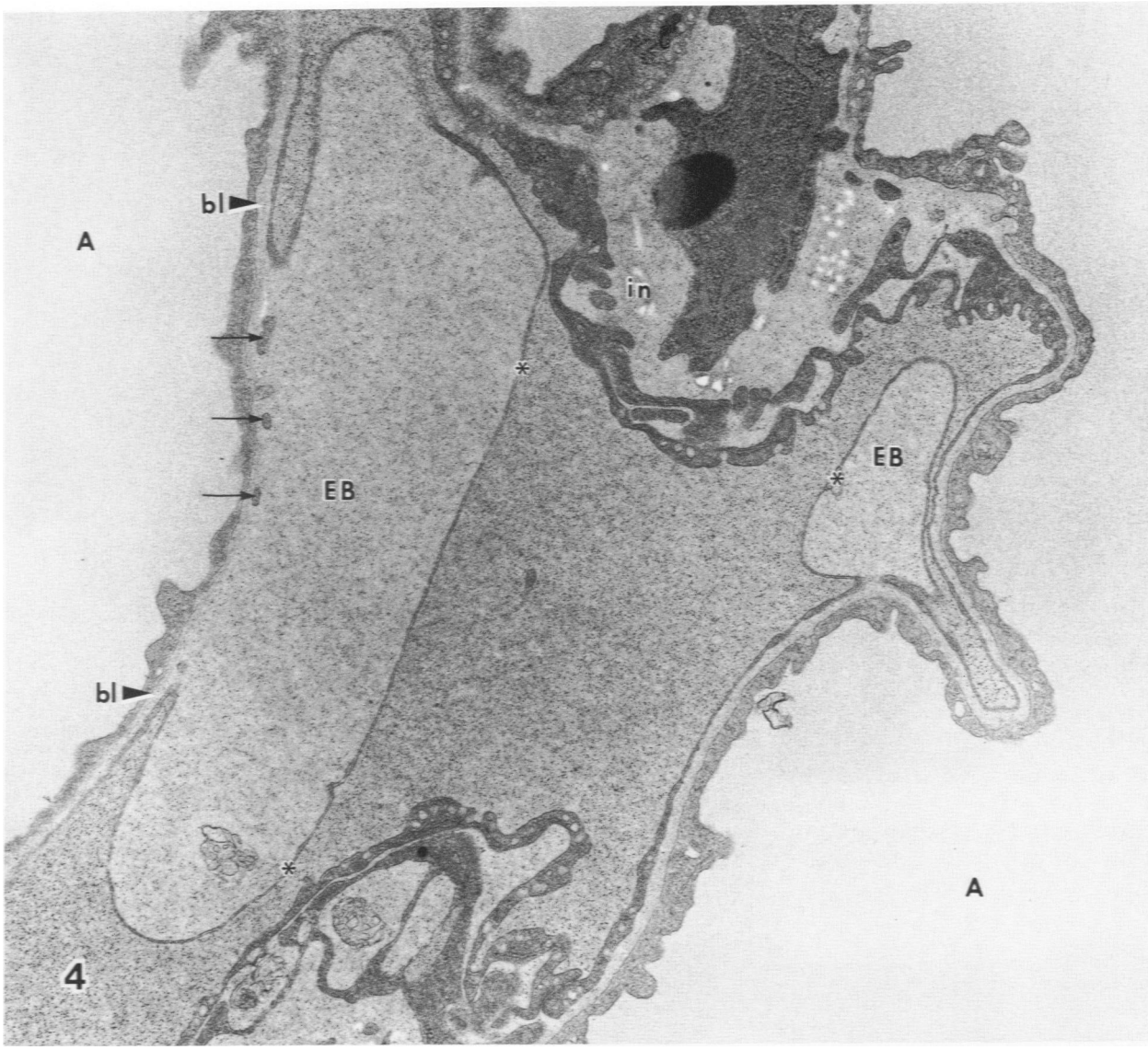
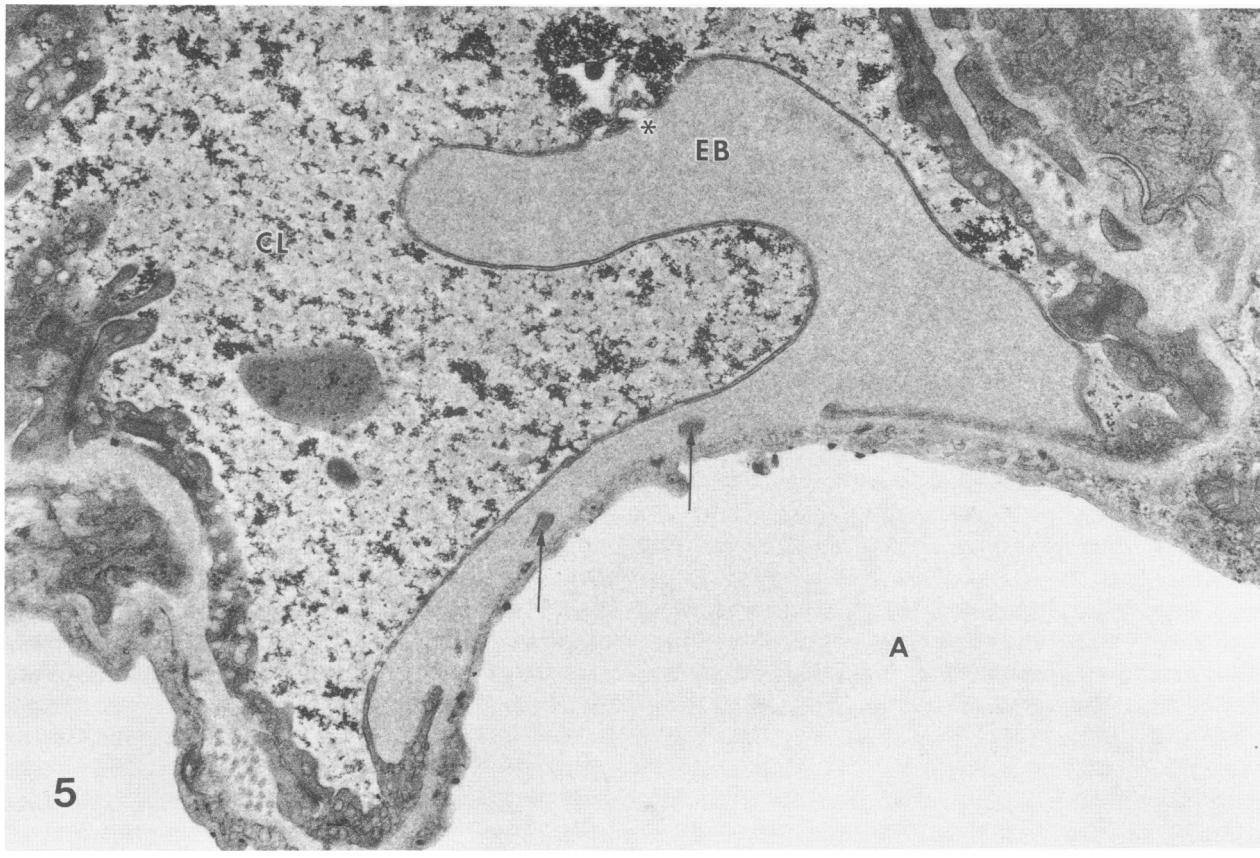


Figure 4 — This electron micrograph illustrates a large subendothelial bleb (*EB*) 15 minutes after ECV administration. This bleb as is typical, is present in the thin portion of the alveolar capillary unit. The endothelium has lifted off its underlying basal lamina (*bl*). Endothelial cell cytoplasm overlying the blebs is highly attenuated (*) and lacks caveolae and vesicles. Beneath the blebs, endothelial islands (*arrow*) remain adherent to the basal lamina. Ferritin is present in the bleb at a concentration below that found in the plasma and is also present within the interstitium (*in*). (Bismuth nitrate, × 18,500)

in animals treated with ECV. Examination of lung samples 5 minutes after ECV injection shows no detectable morphologic alterations in either endothelial or epithelial cells. By 10 minutes, modest interstitial edema is present, with moderate accumulation of ferritin predominantly on the interstitial side of the alveolar capillary unit. By 15 minutes, marked interstitial edema fluid collects, containing modest amounts of ferritin (Figure 3). The accumulation of ferritin is primarily restricted to the thick side of the pulmonary microvasculature at 15 minutes. At this time, glycogen is also only sparsely present in the interstitium. Trace amounts of ferritin and glycogen are present in the intact basal lamina on the thin side of the alveolar septum at this and later times.

Ten minutes after administration of ECV, a distinctive lesion appears in the form of subendothelial blebs. The blebs develop in otherwise seemingly intact endothelium where a portion of the cell lifts off the basal lamina or occasionally the surface of a second underlying endothelial cell and bulges into the lumen. The endothelial cell cytoplasm comprising the blebs 15 minutes after ECV administration (Figure 4) is highly attenuated and typically lacks caveolae and channels; the cell membranes generally appear intact. We interpret occasional smudged out regions of the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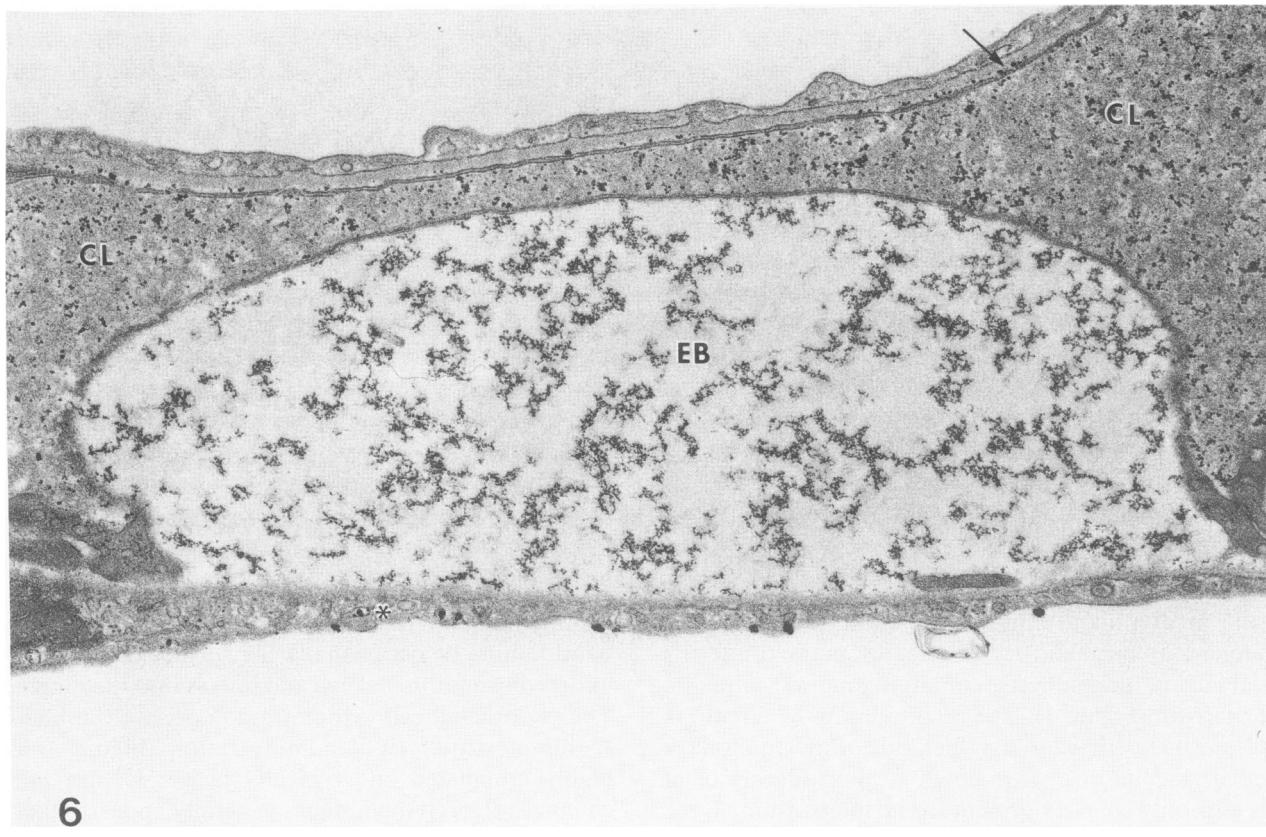
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cell membrane as artifacts, because no transendothelial movement of probe was associated with these sites (Figure 5), and frequently, on close observation, an unstained or irregularly stained membrane bilayer can be found. The blebs are located predominantly in the thin portion of the alveolar septum and increase in extent and frequency between 10 and 60 minutes after ECV. At the base of the blebs, sparsely distributed small islands of membrane-bound cytoplasm remain in close proximity to the basal lamina (Figures 4–6), which in turn maintains its normal intimate relationship with the epithelial cells. Ferritin and glycogen progressively accumulate within the blebs. At 10 minutes, ferritin is absent or else sparingly present within a few small blebs. By 15 minutes, the concentration of ferritin in the blebs usually appears equivalent to the concentration of ferritin in plasma (Figure 4). At this time little or no glycogen is present in blebs (Figure 5). Five blebs have been mapped by subserial sectioning. In two instances the blebs extended at least 2 μ; in the other three instances the capillary deviated out of the plane of sectioning. In no case was a bleb followed to its termination.

Extensive subserial sectioning of lung tissue obtained 15–60 minutes after ECV administration reveals the presence of a small number of open endothelial cell junctions. Glycogen (Figures 7 and 8) and ferritin (Figure 9) can be found in the open junctions. No evidence of endothelial or epithelial cell swelling, disruption, or denudation has been observed, and there is no concentration of ferritin or glycogen in endothelial vesicles.

Thirty and 60 minutes after ECV, the subendothelial blebs are more prominent than earlier. By 30 minutes, both ferritin and glycogen (Figure 6) are widespread within blebs, and by 60 minutes the particulate probes generally are concentrated in blebs so that their density within the blebs exceeds that in the capillary lumen.

The movement of the particulate probes appears to be unimpeded by the basal lamina on the thick side. However, on the thin side of the alveolar septum, there is accumulation of tracers on the endothelial aspect of the basal lamina as well as in the blebs. Ferritin and glycogen begin to penetrate the basal lamina and appear in epithelial cell vesicles and the alveolar space by 60 minutes.

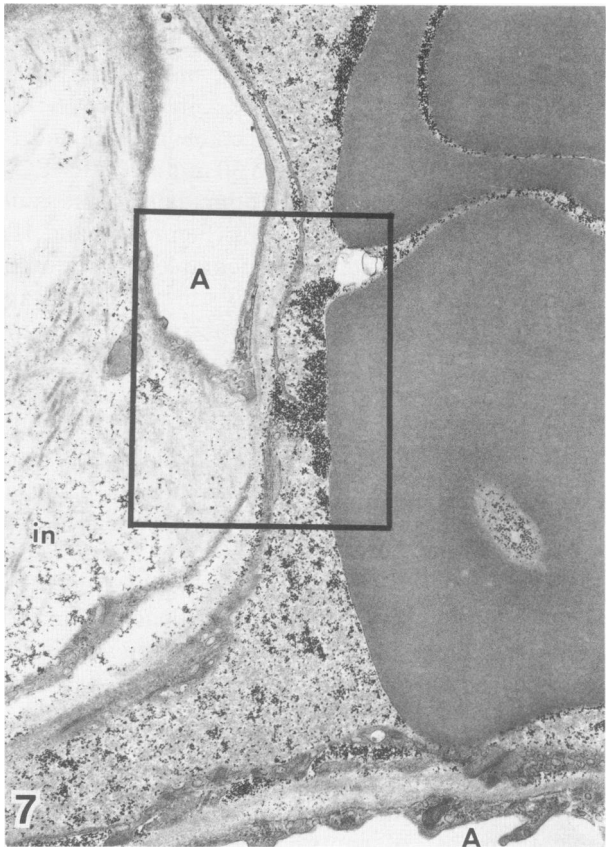
Ferritin injected into otherwise untreated rats 12 and 24 hours, or glycogen, 72 hours before sacrifice, causes no detectable morphologic alterations within the pulmonary microvasculature. Circulating ferritin in low concentration is still detectable after 12 and 24 hours. When ferritin is injected 30 minutes after ECV and the animals are killed 24 hours later, there is no evidence of any remaining blebs (Figures 10 and 11), and the deposited ferritin is completely removed; whereas at 12 hours a rare bleb is still present. Disappearance of interstitial edema is accompanied by the collection of ferritin in phagosomes of interstitial cells (Figure 10). Type I epithelial cell vesicles still contain some small amounts of ferritin at this time. There is a heterogeneous exudate in the alveoli, consisting of fibrin strands, tubular myelin, lamellar bodies, and some ferritin (Figure 11). Alveolar macrophages contain phagocytosed exudate components, including ferritin. During the progression of ECV-induced edema, no morphologic evidence of neutrophil sequestration or platelet aggregation within the pulmonary vasculature has been observed at any time.

Three days after intravenous injection of ECV and oyster glycogen, given 30 minutes after ECV, the pulmonary microvascular endothelium is indistinguishable from that of control animals. Subendothelial blebs and any previously accumulated glycogen have disappeared, and the intraalveolar exudate has completely resolved.

Discussion

Agents inducing experimental lung injury with edema formation can be classified into two categories: those which produce delayed pulmonary damage and those which induce acute lung injury. Alpha-naphthylthiourea (Antu),[14–20] dihydromonocrotaline (DHM) in rats,[21–23] oxygen toxicity,[15,24] and staphylococcal endotoxin B[25] fall in the first class. Antu-induced edema begins within 1 hour after administration, produces its maximal effects at 3 to 4 hours, and either causes death or resolves in 24 to 48 hours. DHM has a latency of 6–8 hours before lung injury develops, and edema does not reach a maximum until 18–24 hours. If not acutely fatal, DHM-induced edema resolves within 48 hours, but the ani-

Figure 5 – Alveolar capillary subendothelial bleb (*EB*) 15 minutes after administration of ECV and 4 minutes after oyster glycogen. The oyster glycogen, in contrast to ferritin (see Figure 4), is still excluded from the bleb 15 minutes after ECV. The bleb contains amorphous material similar in electron density to that found within the capillary lumen (*CL*). Beneath the bleb, endothelial islands (*arrows*) remain adherent to the basal lamina. Apparent defects in the portion of the endothelial cell overlying the bleb (*) are not associated with any transendothelial movement of glycogen and are probably artifacts of preparation. *A*, alveolar space. (Uranyl acetate and lead citrate, ×24,900) **Figure 6** – Subendothelial bleb (*EB*) 30 minutes after intravenous administration of ECV. Oyster glycogen is concentrated within the bleb, exceeding the concentration of glycogen within the capillary lumen (*CL*). On the thin side of the alveolar capillary unit, glycogen has accumulated on the endothelial side of the basal lamina (*arrow*). Some glycogen is present in epithelial cell vesicles (*). Endothelial cell islands remain adherent to the basal lamina. (Uranyl acetate and lead citrate, ×23,100)



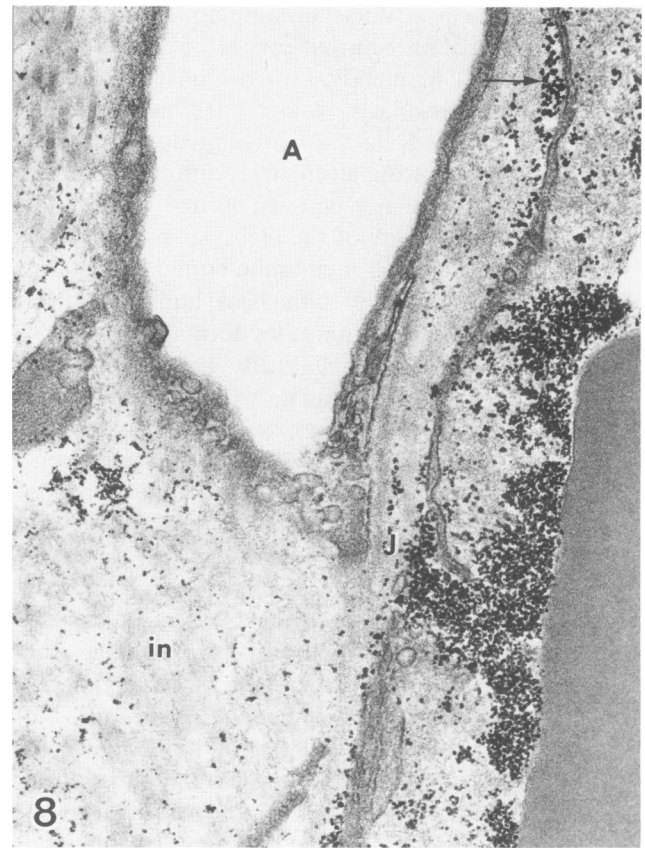


Figure 7—Sixty minutes after administration of ECV an open interendothelial cell junction is present. Glycogen escapes through the open endothelial junction and is present within the interstitial (*in*) compartment. *A*, alveolar space. (Uranyl acetate and lead citrate, × 10,500) **Figure 8**—Enlarged view of the area within the box in Figure 7. Glycogen particles fill the open endothelial junction (*J*). There is an accumulation of the permeability probe at the endothelial surface of the basal lamina (*arrow*); glycogen is also present within the interstitium (*in*). (Uranyl acetate and lead citrate, × 33,800).

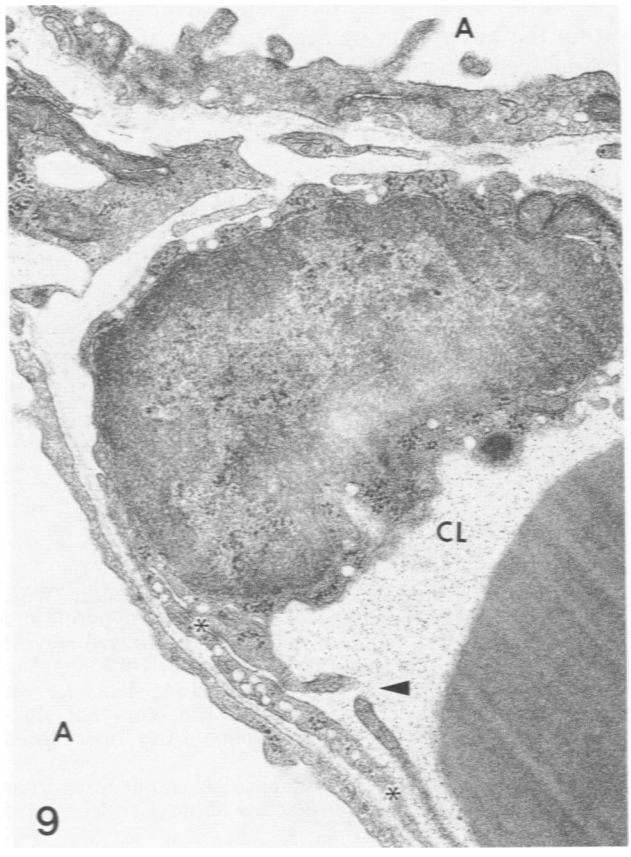
mals characteristically die weeks later.[23] Staphylococcal endotoxin B has a latency of 40–48 hours before respiratory distress ensues.

ECV belongs to the group of acutely effective experimental edema-producing agents, which includes ammonium sulfate,[17,26,27] alloxan,[28] anthrax toxin,[29,30] and intratracheal saline or water.[31] Each of these agents evokes an acute injury with edema detectable within 1 hour.

In their original studies of ECV in dogs, Dearden et al[6] found alveolar edema and septal alterations at 5 minutes, becoming maximal 60 minutes after ECV administration. The endothelial cell alterations they described included cell swelling and increased numbers of plasmalemmal vesicles at 5 minutes, subendothelial bleb development at 15 minutes, and endothelial cell channel formation at 30 minutes. Vacuolization and swelling of epithelial Type I cells with fusion of vesicles and channel formation were described at 30 minutes. No alterations in either endothelial or epithelial cell junctions were reported.

More recently, studying rabbits, Gil and McNiff[7] used an intravenous dose of 40 mg/kg ECV and observed a severe focal injury with loss of endothelial and epithelial cell continuity at 15 minutes. They observed intracapillary plugs of platelets and thrombin and an intraalveolar exudate containing fibrin and RBCs; they did not comment on open capillary

Figure 9—Fifteen minutes after administration of 15 mg/kg ECV an open interendothelial cell junction is present (*arrow*). Note the multiple layering of capillary endothelium (*) on the thin side of the alveolar capillary unit. Ferritin freely exits the capillary lumen (*CL*) through the open junction and dissects between the endothelial cell layers. This lesion is a candidate for the initial event in the progressive development of the subendothelial bleb. *A*, alveolar space. (Uranyl acetate and bismuth nitrate, × 24,000) **Figure 10**—Twenty-four hours after ECV and ferritin given together, ferritin has accumulated within lysosomes of interstitial cell (*). The alveolar epithelial cells (*ep*) appear unaffected. *A*, alveolar space. (Uranyl acetate and bismuth nitrate, × 8500) **Figure 11**—Twenty-four hours after ECV and ferritin administration the endothelium exhibits its normal architecture of continuous cells held together by interendothelial junctions. No subendothelial cell blebs are present, and any ferritin that had accumulated in the blebs has been removed. Note the normal overlapping of endothelial cells (*). Alveolar spaces (*A*) accumulate fibrin (*F*), some ferritin, and flocculent material (inset × 31,840), probably protein in nature. (Uranyl acetate and bismuth nitrate, × 14,490)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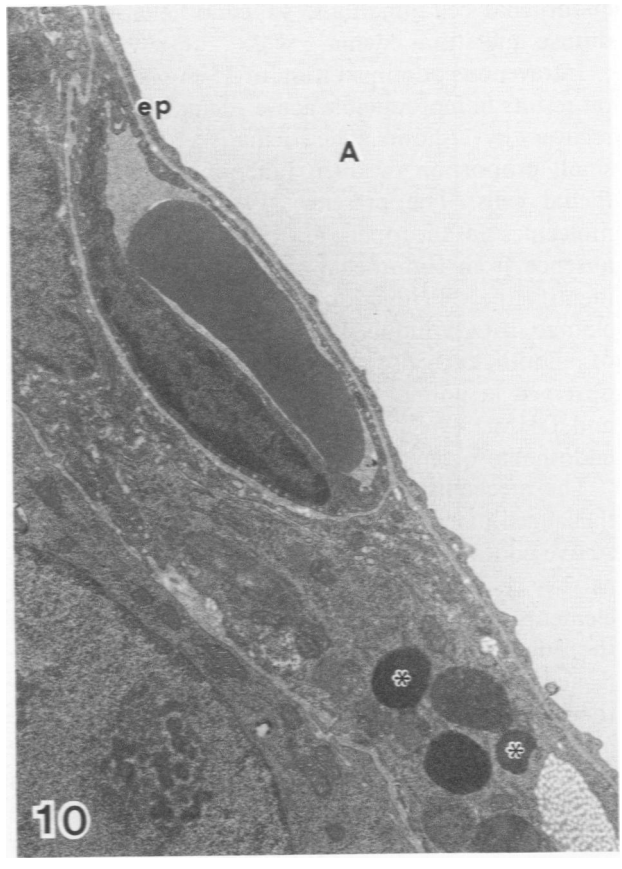
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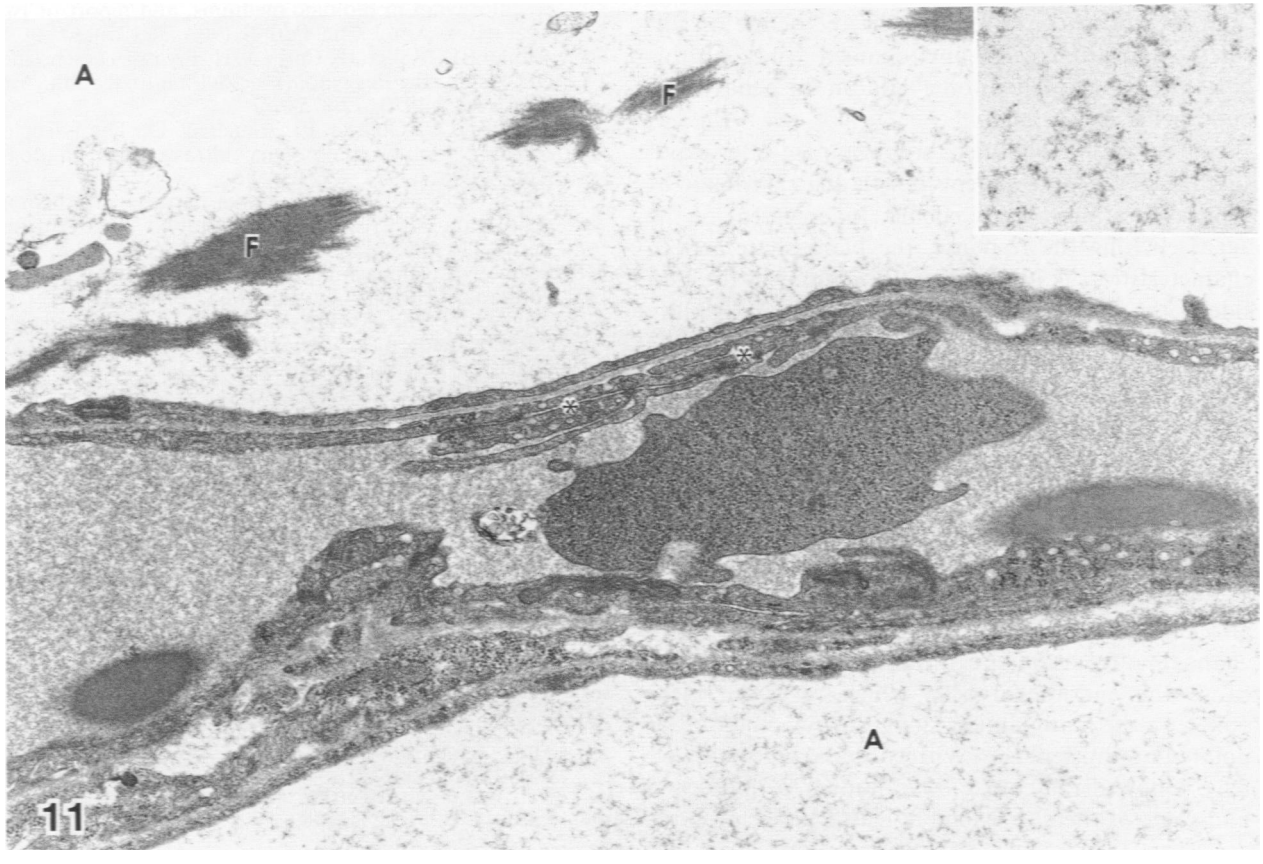
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endothelial cell junctions, subendothelial blebs, or diffuse interstitial edema.

Intravenous administration of 15 mg/kg ECV to a rat results in reproducible acute edema with complete recovery by 72 hours. The edema is associated with a small proportion of open junctions between endothelial cells. The presence of permeability probe molecules in the open junctions and their virtual absence from endothelial cell vesicles permits us to identify the cell junction as the site of permeability change in ECV-induced edema. Openings at the site of endothelial cell junctions have previously been observed in pulmonary edema induced with Antu[16] and DHM.[21] We have not noted any loss of either endothelial or epithelial cell integrity.

The mechanism accounting for opening of endothelial cell junctions in pulmonary edema is unsettled. Active contraction of endothelial cells, well-endowed as they are with potentially contractile cytoskeletal elements,[32] is an obvious possibility. However, in a thorough review, Hammarsen[32] has seriously questioned the evidence supporting contractility of endothelial cells. The loss or modification of one or more elements of the cytoskeleton with consequent shape change is another possibility. In support of this mechanism, Shasby et al[33] have been able to produce pulmonary edema with cytochalasin B, an agent that disrupts the integrity of actin-rich stress fibers. ECV does have a marked effect on the cell shape of cultured endothelial cells, and detailed study of this effect *in vitro* may shed some light on the behavior of endothelial cells *in vivo*.[34]

The subendothelial blebs so prominent after exposure to ECV are a frequent morphologic correlate of experimental pulmonary edema. Independent of the agent eliciting the response, the blebs share certain characteristics: 1) preferential formation on the thin side of the capillary, 2) concentration of permeability probes in the blebs equal to or exceeding that in the plasma, and 3) the presence of intact, membrane-bound cytoplasmic processes adherent to the basal lamina at the base of the blebs.

Even though subendothelial blebs are commonly seen in experimental edema, their mode of formation remains elusive. There is no evidence to support direct transport of plasma across the endothelial cell,[30] because neither vesicles nor channels are evident. It is also difficult to accept the proposal of edema fluid channeling from the interstitium to the blebs,[18,20] because the endothelial cells which roof the blebs adhere to the basal lamina along the bleb margins, as pointed out by Teplitz.[19] With subserial sectioning we have followed the course of a number of blebs and find some of them to extend at least 2 μ. We propose, without yet being able to produce definitive evidence, that the blebs form by the dissection of edema fluid from cell junctions between two overlapping cells (Figure 9). The islands of cytoplasm that remain attached to the basal lamina at the base of the blebs are, we propose, cross-sections of long, fingerlike processes extending from the main portion of the cell. We have seen closely analogous processes develop in cultured endothelial cells exposed to ECV *in vitro*.[34] This drastic change in cell configuration may contribute significantly to the formation of the blebs by perturbing normal cell–cell associations. We have no explanation or even reasonable conjecture as to why the blebs are distributed so predominantly over the thin portion of the alveolar wall.

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