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Early Morphological Alterations of the Rat Lung with Increased Intracranial Pressure

I. A Light and Electron Microscopic Study

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Summary. Ultrastructural alterations of the rat lung following marked increase in intracranial pressure involve interstitial and intraalveolar hemorrhagic and proteinous edema accumulation developing within a few minutes. Capillary endothelium reveals intracellular bleb formations, scallops, and increased pinocytic activity. There is no separation of intercellular junctions both in endothelium and epithelium of the alveolar wall. The common basement membrane of the air-blood-barrier seems to be intact, whereas type I pneumonocyte of the alveolar epithelium shows similar bleb formations as the endothelium. Type II pneumonocyte elicits imposing cytoplasmic protrusions. Edema fluid contains electron dense components and washes out surface active lining layer of lung alveoli.

The present investigation was designed to produce pulmonary edema by elevating intracranial pressure, paying particular attention to surfactant, alveolar epithelium, and endothelium of blood vessels. Using colloidal carbon as a marker, the site of fluid-leakage should be identified.

Material and Methods

Sixteen male rats of Sprague-Dawley strain, weighing from 250 to 300 g, were anaesthetised with intraperitoneal Nembutal 4 mg/100 g body weight. They were tracheotomised and allowed to breathe room air. Polyethylene catheters were placed in the left common carotid artery, the right external jugular vein, and a single femoral artery, the latter connected to a pressure transducer. Heparin in the catheters was employed to avoid clotting. Limb leads I, II, III were recorded. Colloidal carbon (Pelikan-batch C 11/1431 a, Günther Wagner Pelikan & Co., Hannover/West Germany) was administered intravenously in a dose of 0.1 ml/100 g body weight. A needle connected with a polyethylene catheter was inserted into the cisterna magna of eight rats ten minutes after the carbon injection was given. A small amount of cerebrospinal fluid was withdrawn through the needle to check the position. Then the catheters of carotid artery and cisterna magna were combined (Benassi, 1937). Prior to and during the blood infusion into the basal cisterna, systemic blood pressure, respiratory movements, ECG, and behaviour were continously recorded. From the beginning to the end of the experiment the time varied between 3 to 7 minutes. Arrhythmic bradycardia, apnea, and subnormal arterial blood pressure, eventually fallen to zero level, signalled the end of the experiment.

Eight control animals were killed by neck crushing.

After the trachea had been ligatured, the chest was opened and its contents were carefully examined. The hilum of the right lung was ligatured and the organ removed in toto. Small slices were cut from the "cortex" region of the perihilar and peripheral areas of the lung. The specimens were prepared for light and transmission electron microscopy by standard techniques (Karnovsky, 1965; Spurr, 1969).

Results

The rats with subarachnoid infusion of blood showed a characteristic behaviour: The immediate rise in intracranial pressure was followed by irregular short inspirations, the transient increase by gasping respirations terminating with apnea. Voluntary muscles of neck and back went into fibrillation, at the end of the experiment the body relaxed completely (Cameron and De, 1949). When elevating intracranial pressure no significant changes in heart rate occured, however, in the course of the experiment a distinct depression accompanied at time by supraventricular conduction block ran into extrasystolic arrhythmia. Independant of cardiac and respiratory rate were alterations in arterial blood pressure. The cerebral changes were accompanied by an increase in femoral arterial pressure from the normal range of 80–120 mm Hg to 150–170 mm Hg. Persisting at this high level for a variable time pressure fell gradually to subnormal values and finally to zero at death.

Grossly the lungs were motley with dull red patches of congestion and hemorrhages. The main arteries and veins appeared to be distended with blood. Abundant frothy fluid, pink and white, exuded from the cut surface of the lung and was present within the bronchi and the trachea.

Light-microscopically, there was intense capillary congestion. Areas of intraalveolar hemorrhage (Fig. 1a), congestive atelectasis, interstitial and intraalveolar edema changed with nearly normal lung structures. Peribronchial and perivascular lymphatic spaces were conspiciously distended (Fig. 2a). No leakage in vessel walls could be found, but many of the carbon granules appeared to lie extravascularly within the alveoli.

The *ultrastructure* of lung tissue in the normal rat had been described in detail by many authors, an extensive summary written by Meyrick and Reid (1970). To normal structure no further attention will be attributed.

The thin portion of the endothelium towards the air-blood-barrier showed bleb formations (Fig. 3) and scallops, whereas localized scalloping and an increase of pinocytic activitiy were observed in the remaining areas of nearly normal endothelium. Blebs of the kind found in our experiments appeared to be an intracellular phenomena, a thin cytoplasmic layer still remained attached to the unaltered basement membrane. On the contrary scallops were separated from the basement membrane and projected into the capillary lumen. Large endothelial blisters appeared to produce occlusions of the lumen, others sectioned distal to their point of attachment lay free within the capillary. The intercellular junctions were intact and they never seemed to be involved by endothelial blebs. The blebs contained granular material distinguishable from that lying within blood vessels only by the quantity of granules. In no instance carbon particles were found within endothelial blebs, scallops, and pinocytic vesicles, nor was leakage through intercellular junctions demonstrated. Occasionally groups of tracer material were visible extravascularly in relation to the thick part of the capillary endothelium, but usually the carbon appeared to be inside the lumen adhering to the inner surface of the endothelium. This occasional observation of tracer-escape may well be an artefact.

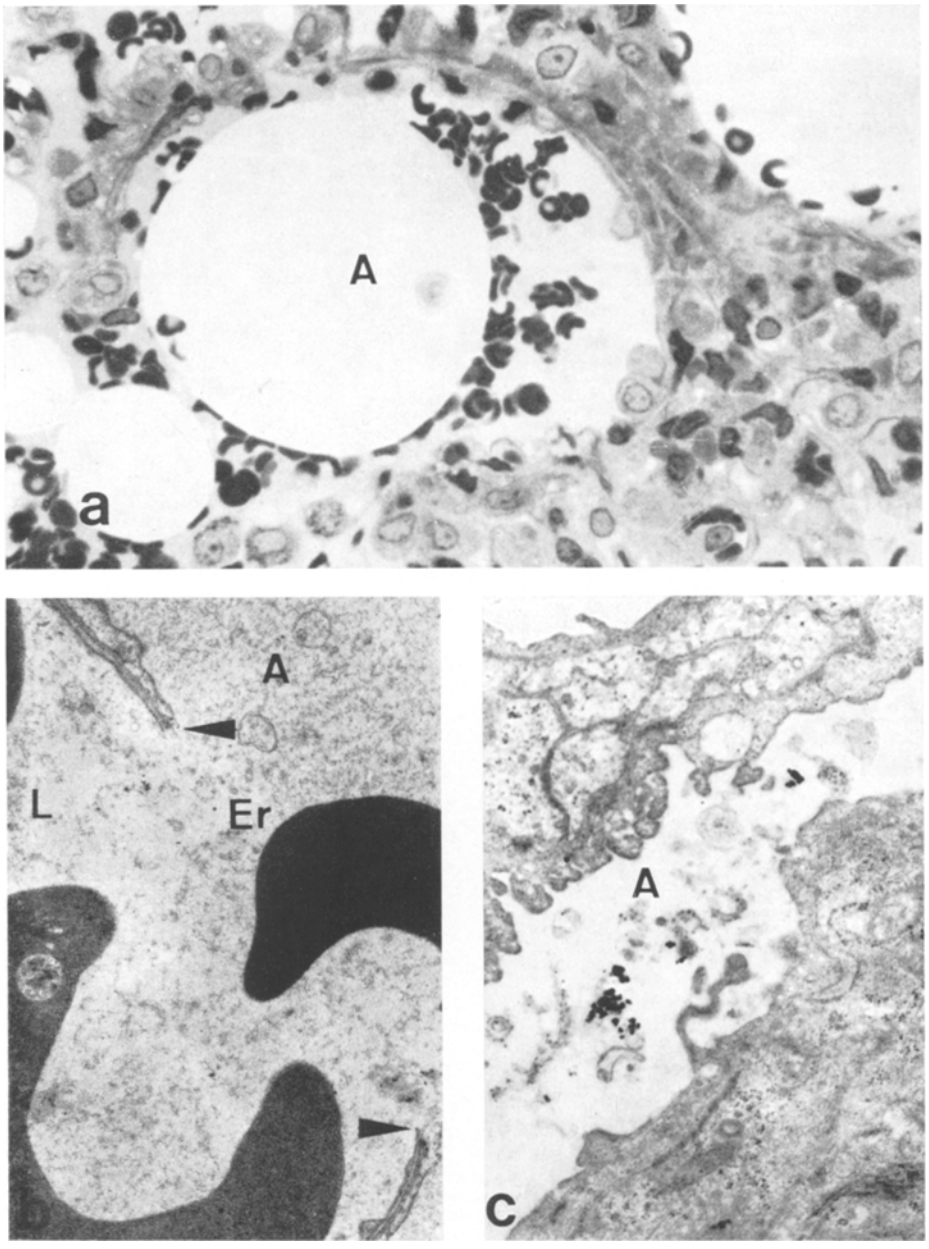

Fig. 1a—c. Hemorrhagic edema formation following increased intracranial pressure. a Intra-alveolar hemorrhage. ×730. b Rupture of the air-blood-barrier (⇄) and emigration of an erythrocyte (*Er*) from a capillary. ×9000. c Carbon granules within alveolar space. ×9000. *A* alveolar space. *L* capillary lumen

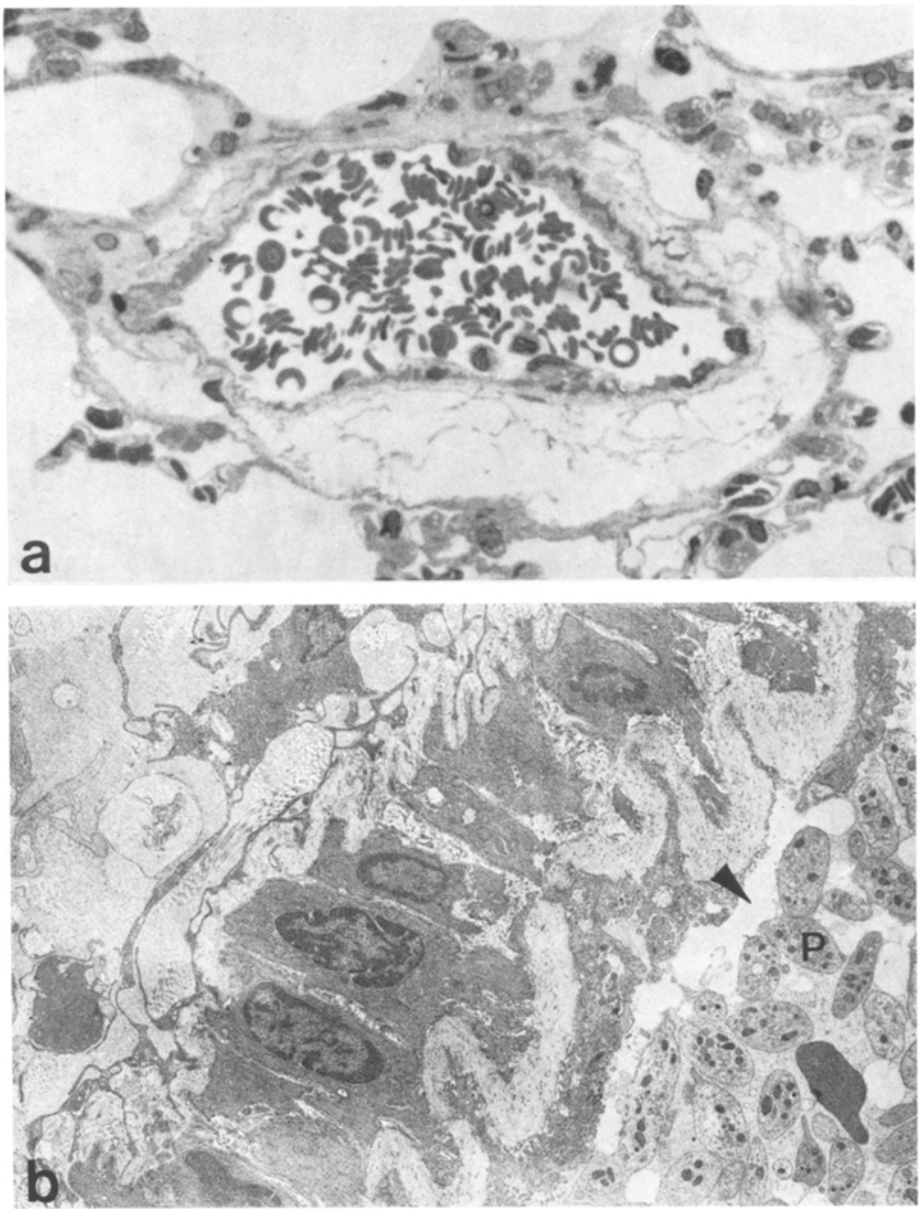

Fig. 2a, b. Perivascular edema formation. a Distension of perivascular lymphatic space. × 690. b Platelet (*P*)-aggregation adherent to the endothelium (▲) of a smaller artery. × 4000

Interstitial edema of the alveolar wall, the most common appearance in all experimental animals, involved separation of collagen fibres and cellular elements. The edema material revealed an electron density similar to that of plasma. The basement membranes both of the capillary endothelium and alveolar epithelium

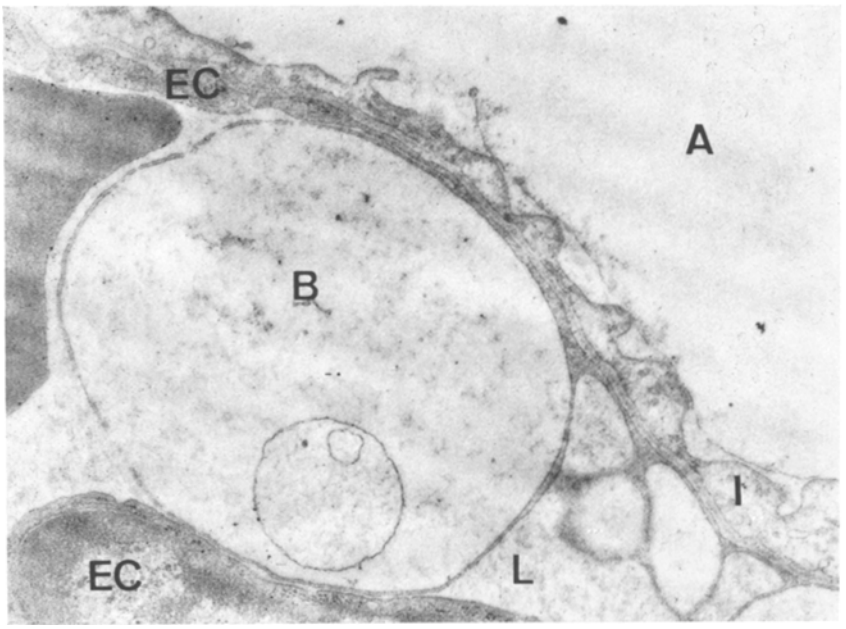

Fig. 3. Capillary endothelium of the air-blood-barrier showing intracellular bleb formation. *A* alveolar space. *B* bleb. *EC* endothelial cell. *L* capillary lumen. *I* (peripheral portion of) type *I* pneumonocyte. × 19900

surprisingly did not show any abnormality. As in light-micrographs an extensive perivascular edema of larger vessels was demonstrated; more markedly affected were the smaller arteries and venules. In arteries where occasionally aggregations of platelets lay adherent to the inner surface of the wall the endothelial cells exhibited marked swelling with pale cytoplasm and disorganization of organelles (Fig. 2b). There were no separations of their intercellular junctions. A similar endothelial damage without cellular adsorption had also been noted in venules.

In addition, ultrastructural examination of *alveolar lining cells* revealed morphological alterations of type I and type II alveolar cell, whereas type III pneumonocyte was often difficult to identify. If the latter is a result of debatable significance, it may still remain unnoticed; in experimental animals, if discovered, alveolar brush cells appeared entirely normal in structure. The pathological changes in type I cell ranged from distension of rough endoplasmic reticulum, swelling of mitochondrias with loss of their cristae to overall disintegration both of cellular organelles and cellular matrix (Fig. 4a). Furthermore intraepithelial bleb formation could be seen in those areas, where an accumulation of intra-alveolar edema was a common feature. Blebs contained granular material, less electron dense than that of intraalveolar fluid or blood plasm; similar to the endothelial blisters the unaltered basement membrane was still adjacent to a thin cytoplasmic layer (Fig. 4b). It must be emphasized that the apparent alterations

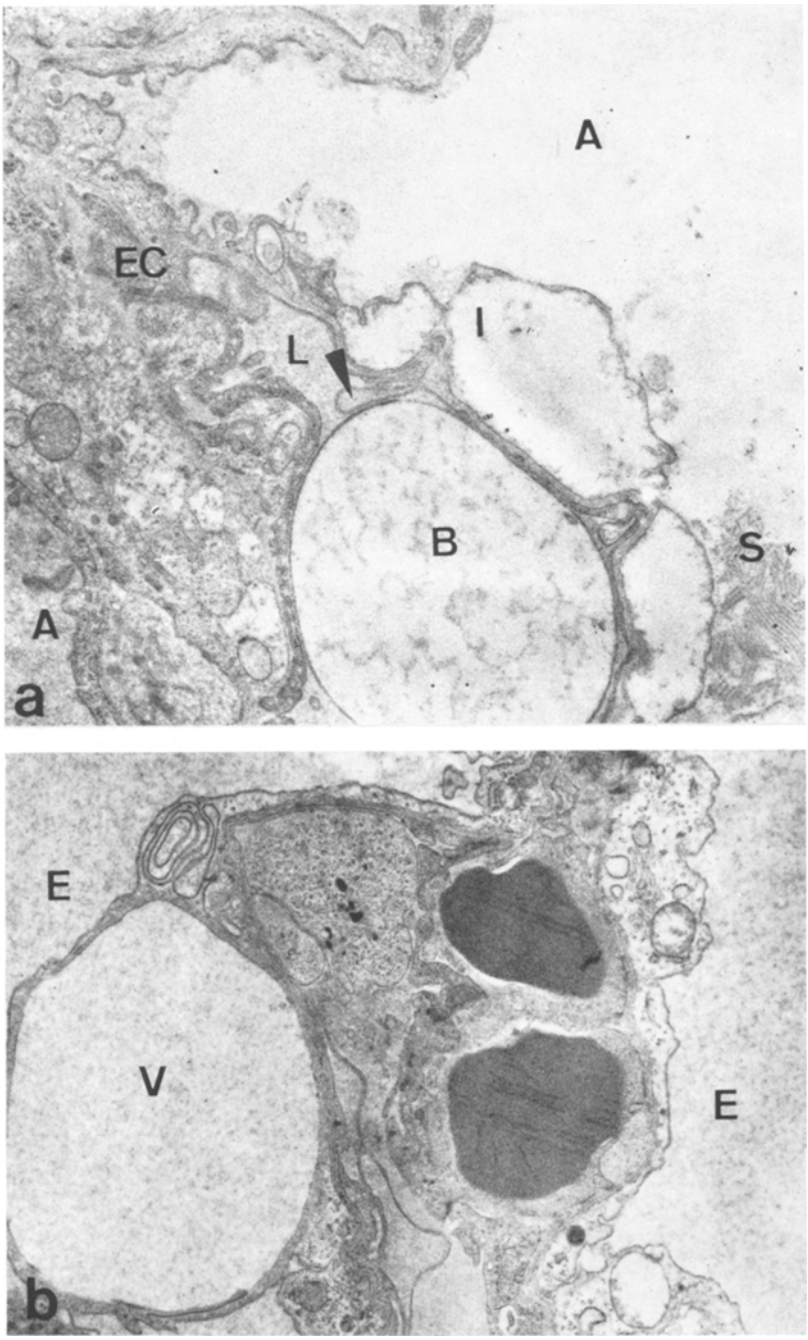

Fig. 4a, b. Intraalveolar edema. a Type I pneumonocyte (*I*) showing swelling and homogenisation of the cell contents. ▲ scalloping of the endothelium. *A* alveolar space. *B* bleb. *EC* endothelial cell. *L* capillary lumen. *S* surfactant components. ×12800. b Gigantic granular vacuole (*V*) within a type I pneumonocyte. The alveolar space contains edema fluid (*E*). ×8700

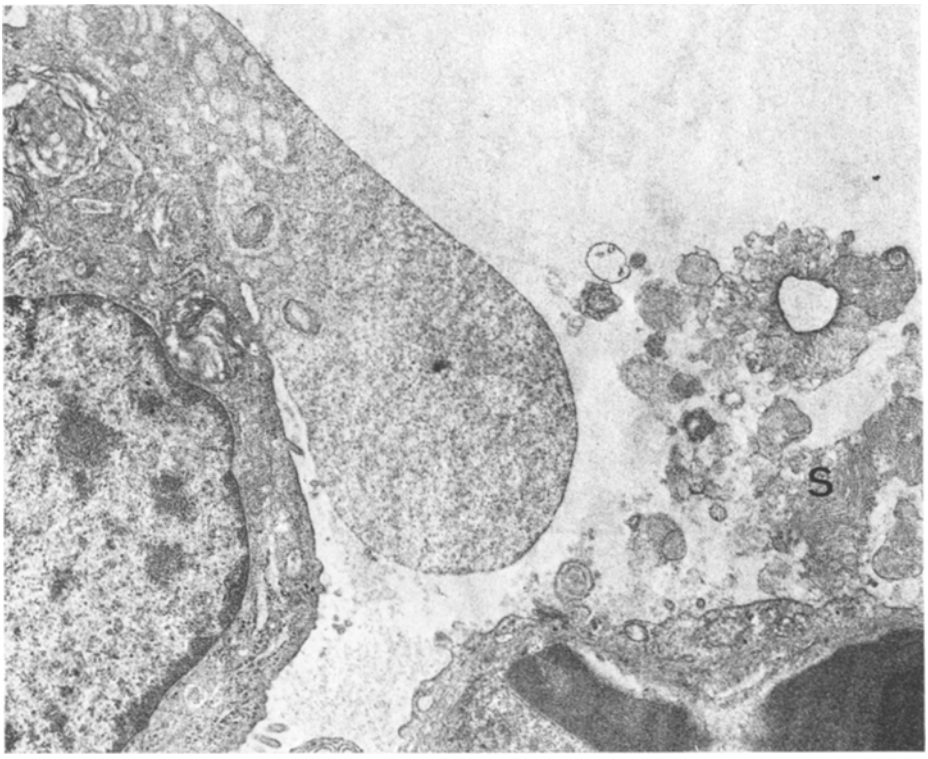

Fig. 5. A cytoplasmic protrusion of type II pneumonocyte bulging into the alveolar space. *S* surfactant components. × 9500

were localized in that portion of type I cell which belongs to the air-blood-barrier; perinuclear cell body seemed to be unchanged both in size and structure. In no case there was evidence to suggest leakage of edema fluid through interepithelial cell junctions with distension of zonula occludens. Type II pneumonocytes appeared normal save that there were occasionally cytoplasmic protrusions of granular contents without any typical organization of cell organelles (Fig. 5). As a common feature intraalveolar edema fluid seemed to be repelled by the free cell surface, whereas a comparable electron lucent border lining the type I pneumonocyte was nowhere to be found.

In our experiments immersion fixation allowed preservation of *intraalveolar edema*. The edema fluid contained masses of myelin figures and phospho-lipid lattices, red blood cells with fibrin strands, and marcrophages by accident (Fig. 6). If stabilized, electron lucent blebs within the edema fluid showed a clearly defined double osmiophilic line at their surface film.

Discussion

The reflex stimulation of the cardiovascular system and the respiration following increased intracranial pressure was demonstrated by physiologists since

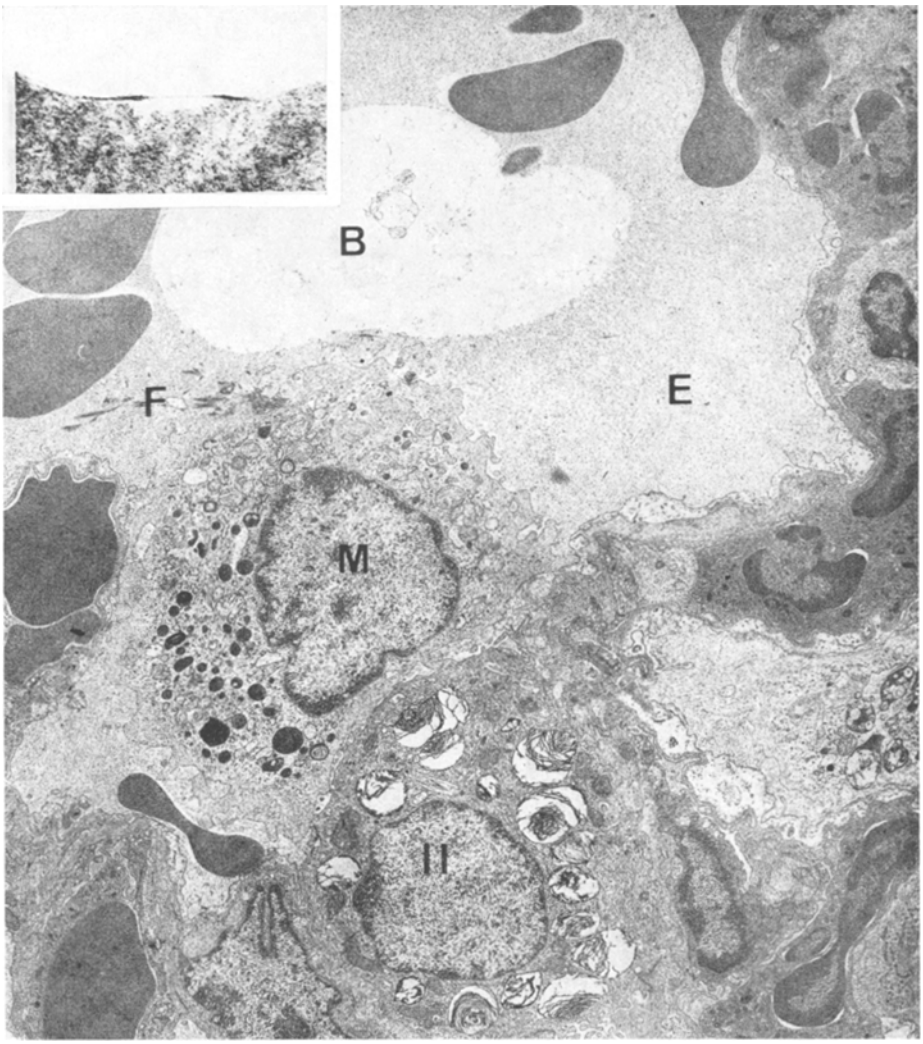

Fig. 6. Intraalveolar hemorrhage. *B* electron lucent bleb. *E* edema fluid. *F* fibrin strands. *M* macrophage. *II* Type II pneumonocyte. × 5200. Inset: Osmiophilic surface film of an electron lucent bleb within edema fluid. × 28000

1881, starting with Naunyn and Schreiber. Central nervous system tends to maintain brain perfusion by elevating diastolic blood pressure at levels greater than cerebral pressure (Cushing, 1901). Gradations of sympathetic discharge depending on the degree of intracranial pressure elevation result in somatic and splanchnic arteriovenous shunting associated with myocardial inotropic response, venoconstriction, and peripheral arterial constriction. This enlarged Cushing reflex, in protecting the brain, seems to be the result of a predominant beta-receptor stimulation. On the contrary fulminant pulmonary edema, heart failure, and total peripheral vasoconstriction may be caused by an excessive alpha-stimulation mediated by marked elevations of intracranial pressure (Berman and Ducker,

1969). In our experiments the massive elevation of intracranial pressure is associated with an increase in arterial blood pressure, heart failure, and pulmonary edema. The hemodynamic and respiratory changes resemble those described by many authors (a.o. Horst *et al.*, 1950; Riechert, 1951; Sarnoff and Sarnoff, 1952a, b; Maire and Patton, 1956; Wood *et al.*, 1964; Ducker and Simmons, 1968; Berman and Ducker, 1969). The subarachnoid blood infusion should favour an intracranial pressure elevation as uniform as possible, and fluid administration into the basal cisterna should not be limited with the intention to produce fulminant pulmonary edema. No clear time-sequence in the development of pulmonary edema is appreciated in this investigation; as lesions are present within 3 to 7 minutes after intracranial pressure elevation, we suggest that the pathogenetic mechanism is set in motion immediately after the cerebral manipulation. According to the results of Sarnoff and Sarnoff (1952a, b) we tend to define this type of edema as "neurohemodynamic" pulmonary edema. With marked increase in peripheral resistance and left ventricular decompensation the pulmonary venous pressure overwhelms elevated pulmonary arterial pressure and promotes the development of pulmonary edema. Add to this, sympathetic stimulation elicits an intense pulmonary vasomotor-activity of the venous branch (Fishman, 1972).

Ultrastructural examination of the capillary wall shows surprisingly significant abnormality. The alterations range from increased pinocytic activity to bleb formation within the endothelium. Whereas artificially raised pulmonary venous pressure, the hemodynamic form of pulmonary edema, is not capable of damaging the capillary endothelium (Cottrell *et al.*, 1967), our results do favour a hemodynamic mechanism for endothelial damage. But if a chemical mediator rather than physical plays an important role in these pathological events, the present investigation lacks any illustrative material. Platelet accumulations adhering to the inner surface of small arteries seem to be responsible for the alterations in the underlying endothelium. Blebbing of the endothelium, scalloping, and an increased pinocytic activity are a common feature in experimental pulmonary edema, thus including the administration of substances as ANTU (a.o. Meessen and Schulz, 1957; Böhm, 1961; Teplitz, 1968; Cunningham and Hurley, 1972; Meyrick *et al.*, 1972), Alloxan (Cottrell *et al.*, 1967), ammonium sulphate (Hayes and Shiga, 1970), adrenalin (Gil, 1971), acid solutions (Alexander, 1968), and bacterial toxin (Finegold, 1967; Dalldorf *et al.*, 1969; Coalson *et al.*, 1970). Other methods producing pulmonary edema include intratracheal water instillation (Reidbord and Spitz, 1966) and induction of hypoxia (Kisch, 1965) and hyperoxia (Kistler *et al.*, 1967; Kapanci *et al.*, 1969; Gil, 1971). The ultrastructural observations are similar to those described above, and the most common appearance in these experiments involves separation of the endothelium from the basement membrane during bleb formation. Our findings suggest that bleb formation appears as a cytoplasmic event, because a thin cytoplasmic layer is still adjacent to the basement membrane. Whether blebs represent a membrane bounded accumulation of intravascular components or a vesicular shaped damage of the cell, former as a result of increased capillary hydrostatic pressure and increased capillary permeability, cannot be ruled out in this investigation. The passage of large molecules is stopped by the capillary endothelium. Fluid-escape out of capillaries seems to be related to complicated structural features, as it is described by many authors. Using colloidal carbon as a tracer, Cunningham and Hurley (1972) observed a reversible

"gap" formation at the interendothelial junctions in ANTU-edema. Similar gaps formed by active contraction of actomyosin within endothelium are known in pulmonary venules after histamin-, bradykinin-, and mast-cell dischargers application (Majno *et al.*, 1969; Pietra *et al.*, 1971). Neither gap formation nor any visible distension of the macula occludens do account for this way of fluid-escape in our experiments; but carbon does not seem to be qualified for these tracer studies because of its large diameter. Schneeberger-Keeley and Karnovsky (1968) demonstrated the permeation of horse-radish peroxidase through "slits" of the interendothelial junction, and under elevation of intracapillary pressure Szidon *et al.* (1972) confirmed these findings. Another theory is based on a preponderant transcellular fluid transport (Bruns and Palade, 1968; Hayes and Shiga, 1970).

Perivascular edema of larger vessels following increased intracranial pressure is proposed to be the result of a delay between the transient excess of fluid accumulation in the alveolar interstitial space and the lymphatic drainage capacity. Fluid outlet in the larger lymphatics or in the interstitial spaces around larger vessels towards the hilum is mediated primarily by the respiratory movements (Fishman, 1972). The irregularity of breathing of the experimental rats could favour edema accumulation in this site of the drainage system.

Alveolar epithelium represents the critical barrier to the entry of solutes into the alveolar space. Forming "zonulae occludentes" by fusion of the outer cell membranes, the interepithelial clefts are tightly obliterated towards the alveolar space. There is no disorganization of the epithelial junctions in our experiments, whereas structural alterations, bleb formation, and swelling appear in the cytoplasmic extensions of type I pneumonocyte. According to Weibel (1969) we suggest that the large distance between the perinuclear metabolic centre and the peripheral portions of the type I cell predisposes the latter to vulnerability. Whether the epithelial damage arises from the intraalveolar fluid accumulation or from a hypothetical epithelial fluid transport, is not clear. In ANTU-edema Meyrick *et al.* (1972) demonstrated a fluid-leakage through the epithelium before there was any damage in the cell. As our results reveal a very different quality of intraalveolar edema, localized in foci—alveolar spaces contain blood with fibrin strands, protein rich and poor exudates, and possibly transudates—epithelial changes could be considered as subsequent colloid osmotic damage, too. Protrusions of the type II pneumonocytes bulging into the alveoli may perhaps be explained in this term. The type II pneumonocyte is usually regarded as the source of surfactant of which the most surface-active component is dipalmitoyl-lecithin (see for review: Scarpelli, 1969). The apparent electron lucent border within intraalveolar fluid, lining only the free surface of this cell, is attributed to similar hydrophobic material, probably membrane bounded.

Concerning the quality of edema the nature of pulmonary damage following increased intracranial pressure seems to be polyvalent. The occasional observation of a total rupture of the whole air-blood-barrier stands for the hemorrhagic edema formation, and these findings are confirmed by the appearance of carbon particles within alveoli (Fig. 1 b, c). The accumulation of intraalveolar proteinous solutions decreases the "Surface Tension Stability Index (STSI)" (Levitzky *et al.*, 1971), and the surfactant detorioration leads to continued fluid outpouring. The inactivation of surfactant caused by pulmonary edema has been shown by Said

et al. (1965) and Weibel and Gil (1968), and perhaps as a result of changes in osmolarity (Scarpelli, 1969) the alteration initially takes place at the "hypophase" of the alveolar lining layer. Surface active material appears to be lifted from the epithelium by edema fluid, and occasionally osmiophilic material is observed incorporated into the films of large and small electron lucent bubbles.

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