An Ultrastructural Study of Alveolar Permeability to Cytochrome C in the Rabbit Lung

Effect of Exposure to 100% Oxygen at One Atmosphere

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Rabbits were exposed to 100% oxygen or to air at one atmosphere. No alterations were observed in the lung of rabbits breathing air for up to 66 hours or 100% oxygen for 24 hours; after 48 hours, inflammatory cells, chiefly neutrophils, were located in the interstitium of the lung. By 66 hours of oxygen, the number of inflammatory cells in the interstitial space was greater than at 48 hours. At 72 hours, alveolar space in focal areas of the lung was filled with edema fluid containing a lightly flocculent material, and more densely staining fibrin. In experiments for the study of alveolar permeability, cytochrome C was instilled through the tracheobronchial tree into alveoli and demonstrated ultracytochemically by its peroxidase activity. No electron-opaque reaction product was observed in control rabbits or in those breathing oxygen for 24 hours, indicating that the tracer did not leave the alveolar space. However, after 48 hours of the breathing of 100% oxygen, electron-opaque reaction product was localized to the basal lamina of alveolar capillaries in focal areas, whereas in other alveolar capillaries there was no reaction product in the basal lamina. Vesicles filled with reaction product were observed in Type 1 pneumocytes and in alveolar capillary endothelial cells within capillary loops having increased electron density in the basal lamina. After 66 hours of the breathing of 100% oxygen, virtually all alveolar capillaries showed electron-opaque reaction product in the basal lamina and in vesicles within Type 1 cells and capillary endothelial cells. Increased permeability of Type 1 pneumocytes appears as an early manifestation of oxygen-induced changes in the lung preceding pulmonary edema. The presence of numerous inflammatory cells in the interstitium and in alveolar capillaries may play some part in the pathogenesis of the oxygen-induced increase in alveolar permeability. (Am J Pathol 1981, 102:1–9)

PROLONGED EXPOSURE to 100% oxygen at one atmosphere (1 ATA) is toxic to mammalian species[1-3] and eventually causes death from respiratory failure.[1,4] The most common pathologic findings are alveolar and interstitial edema, atelectasis, presence of fibrin in the alveolar space, and loss of integrity of the blood-gas barrier.[1-3,5] Oxygen-induced alterations in the ultrastructure of the lung which precede alveolar and interstitial edema have not been well characterized nor have alterations in structure been correlated directly with the physiological effects of oxygen exposure on the cardiorespiratory system.

We have recently shown[6,7] that the alveolar epithelium of rabbits exposed to 100% oxygen for 48 hours became permeable to cytochrome C, a small lipid insoluble molecule (mol wt = 12,523; $r \sim 17$ Å) to which it is normally impermeable.[8] Exposure to oxygen for 66 hours resulted in a further increase in the permeability to this molecule and also allowed bovine albumin (mol wt = 68,000; $r \sim 35$ Å) to move across the alveolar epithelium.[6] Furthermore, these changes appeared before the onset of interstitial and alveolar edema and the development of systemic hypoxemia.

The purpose of this study was twofold: first, we wanted to determine whether or not we could establish the existence of morphologic and ultrastructural changes in the lungs of rabbits exposed to 100% oxygen for 24, 48, 66, and 72 hours that might explain

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the changes observed in alveolar permeability. Second, we utilized an ultracytochemical technique to demonstrate the pathway followed by cytochrome C instilled intraalveolarly as it crossed the blood-gas barrier in animals exposed to oxygen for 24, 48, and 66 hours.

Materials and Methods

New Zealand white rabbits, weighing between 3.5 and 4.5 kg, were quarantined for 1 week. They were then placed in a clear Plexiglas chamber with a metal roof, which permitted freedom of movement, waste removal, and administration of food and water without contamination of the environment. The chamber was flushed with air at 8 l/min^{-1}, which kept the CO_2 concentration under 0.1% and the temperature between 21 and 22 C. After 48 hours, if the animal was drinking and eating, had normal fecal and urine output, and was free of symptoms of respiratory distress, it was randomly assigned to either a control or an experimental group; 3 animals were studied in each group. Control animals continued to breathe air for an additional 66 hours; the experimental animals were exposed to 100% O_2 for 24, 48, or 66 hours. Animals were then removed from the chamber and breathed either air (control) or 100% O_2 through a plastic bag placed around their heads; rabbits were anesthetized with 30 mg of Ketamine (Ketalar, Parke, Davis & Co., Detroit, Mich) and 20 mg Sparine (Wyeth Laboratories, Philadelphia, Pa). The trachea was then exposed, a transverse incision made, and two cannulas were inserted: an endotracheal tube (2.5 mm ID was advanced to the level of the carina and connected to a source of 100% O_2, and a flexible plastic tube (10 mm ID) was wedged into a peripheral bronchus isolating its subtending air space. Two ligatures passed around the trachea maintained a tight seal. The wedged cannula was clamped so that the subtended lung segment collapsed when the oxygen was absorbed. Isotonic saline (8–10 ml) containing 18.6 mg to 24.4 mg horse heart cytochrome C (Sigma Chemical Co., St. Louis, Mo) were infused through this wedged catheter. To ensure that the fluid remained in the gas-exchanging space of the lung, 5 ml of gas was added to the wedged cannula after instillation of the tracer. The tracer was allowed to remain in place for 30 minutes. During that time the animals were breathing spontaneously. At the end of these periods, 5000 units of heparin were administered intravenously. The chest was opened and a cannula was inserted into the pulmonary artery; the lungs were then perfused with a Harvard Peristaltic Pump initially with 100–150 ml of 0.9% heparinized saline to remove blood cells, followed by 900 ml of fixative (2.5% purified glutaraldehyde and 2% para-

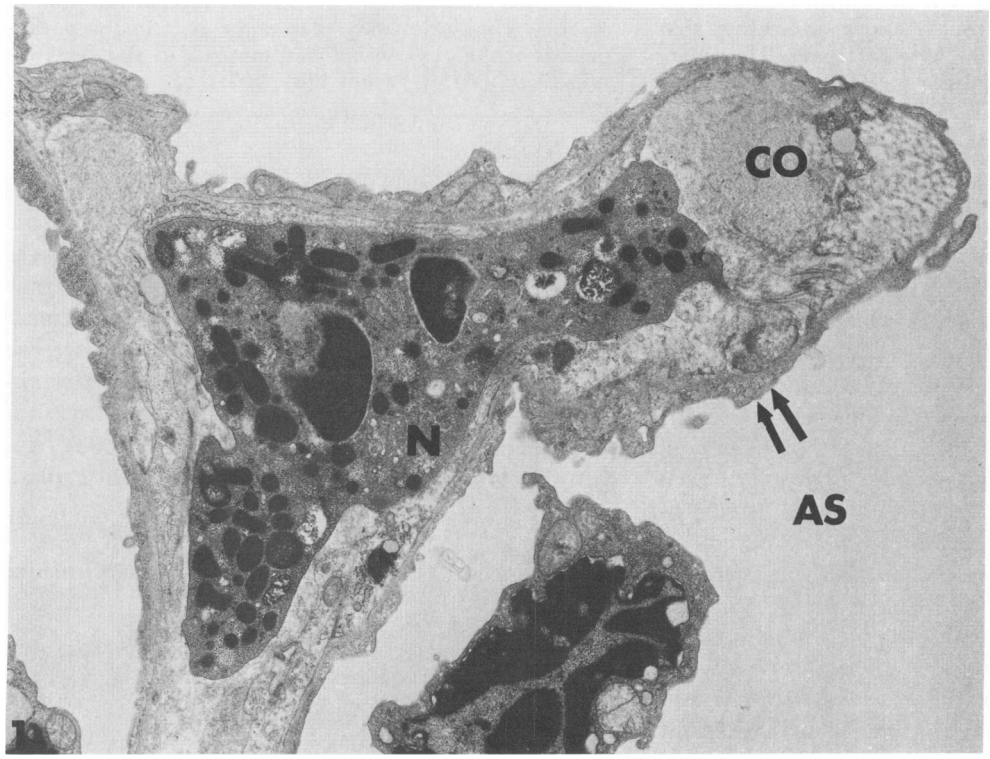


Figure 1—Lung from a rabbit that breathed 100% oxygen for 48 hours. A neutrophil (*N*) and collagen (*CO*) are observed in the interstitial space. Type 1 pneumocytes (*arrows*) border the alveolar space (*AS*). (Uranyl acetate and lead citrate, ×12,500)

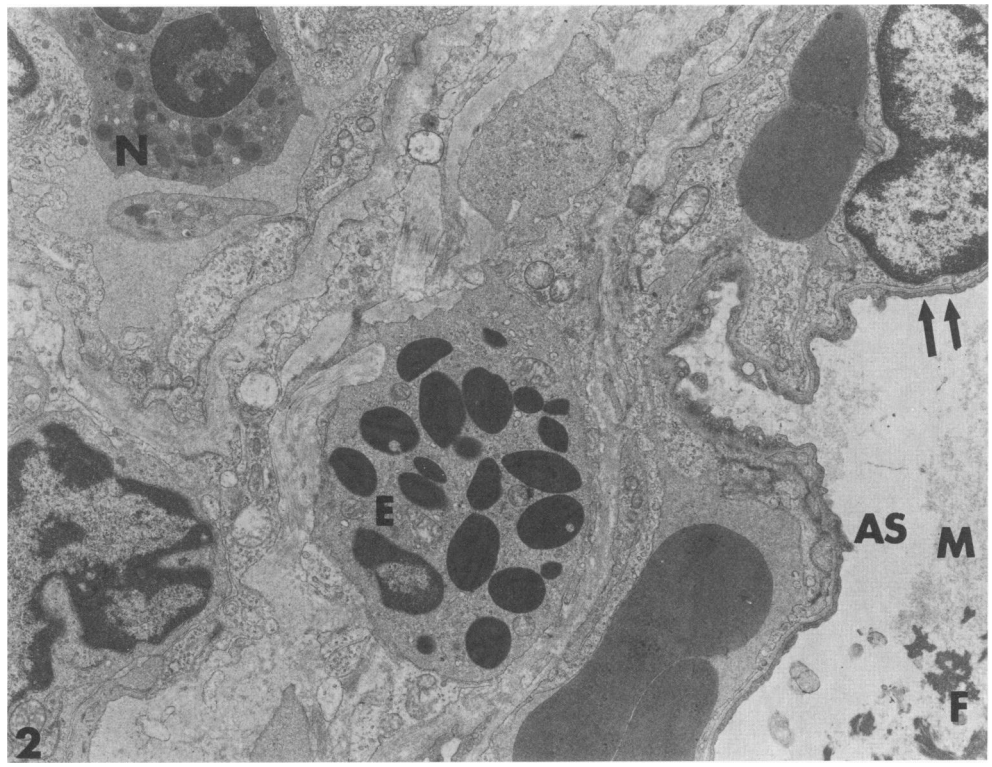


Figure 2—Lung from a rabbit that breathed 100% oxygen for 72 hours. An eosinophil (*E*) is present in the interstitial space, and a neutrophil (*N*) is seen in a capillary. Amorphous material (*M*) with a light density and electron-opaque fibrin (*F*) occupies the alveolar space (*AS*). Type 1 pneumocytes (*arrows*). (Uranyl acetate and lead citrate, ×10,000)

formaldehyde in 0.1 M cacodylate) (pH 7.2). The flow rate was kept at 150–200 ml/min^{-1} at a pressure of 20 cm H_2O as measured with a P23AC Statham pressure transducer and a 2-channel Grass polygraph throughout the perfusion; the left atrium was slit to provide an outflow track.

At the end of the perfusion, the lobe containing cytochrome C was identified, and tissue from this lobe as well as from other lobes without cytochrome C were immersed in fixative for an additional hour at room temperature. Tissues were washed in 0.1 M cacodylate buffer (pH 7.6), processed according to techniques for demonstration of peroxidase activity of cytochrome C,[9,10] dehydrated in a graded series of ethanol followed by propylene oxide, and embedded in Epon 812–Araldite.[11]

The following procedure was followed for the demonstration of cytochrome C. After washing the tissue in several changes of 0.1 M cacodylate buffer (pH 7.6) for 30 minutes, 30-μ-thick sections were cut on a Smith-Farquhar TC-2 tissue chopper (Ivan Sorvall Inc., Newtown, Conn). Peroxidase activity was demonstrated by the method of Karnovsky and Rice.[9,10] Sections were infiltrated with 3, 3′-diaminobenzidine (DAB, Sigma Chemical Co., St. Louis, Mo) in 0.1 M cacodylate buffer for 3 hours and then incubated with 0.001 M H_2O_2 and 5 mg DAB in citrate buffer (pH 3.9, 0.05 M) for 10–30 minutes at 37 C. After rinsing in 0.1 M cacodylate buffer (pH 7.6), the tissue was postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer and processed for electron microscopy as described above. Controls for the ultracytochemical procedure included omission of the substrate (H_2O_2) or capture reagent (DAB). Sections of lung without cytochrome C were also incubated in complete medium as an additional control. To eliminate the possibility that oxidation of DAB and subsequent binding influenced our results, DAB was oxidized in sunlight[10,12] and sections incubated in medium with the oxidized DAB.

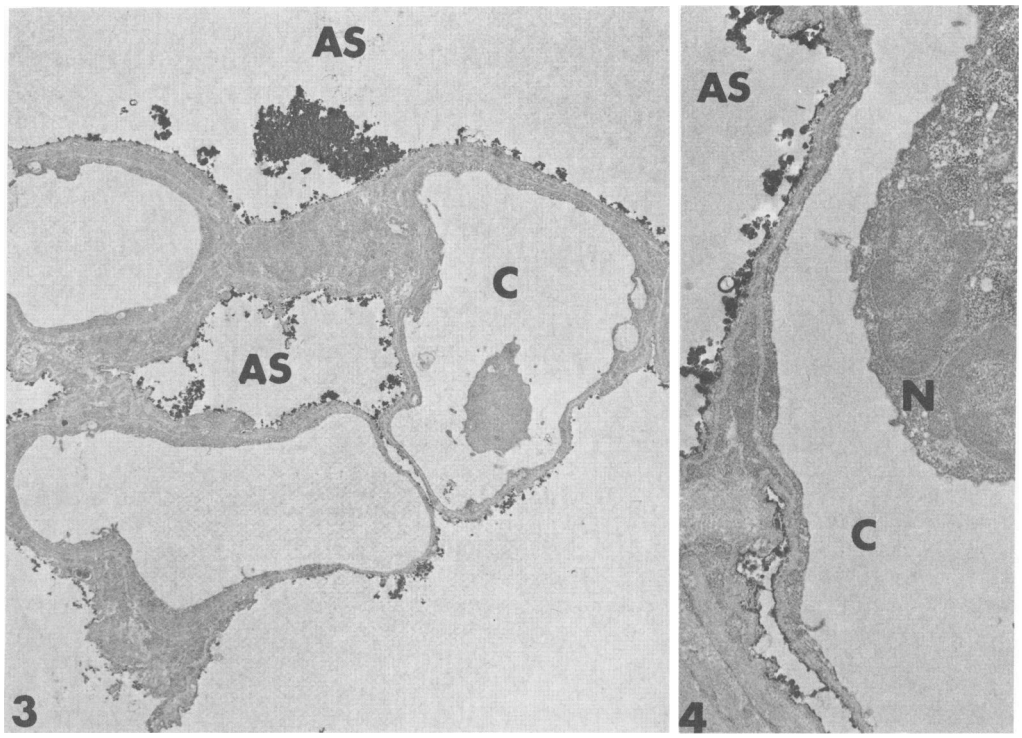
Rabbits breathing 100% oxygen for 24, 48, 66, and 72 hours were anesthetized, and pieces of lung were fixed by immersion in 3% glutaraldehyde buffered to pH 7.4 with 0.1 M phosphate. Tissues were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) and processed for electron microscopy as described above.

Sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and examined unstained or stained with uranyl acetate[13] and lead citrate[14] with a Siemens 101 electron microscope.

Results

Morphology—Controls

The ultrastructure of lung tissue from control rab-



Figures 3 and 4—Lung of a control rabbit that breathed air for 66 hours. Electron-opaque reaction product is confined to the alveolar space (*AS*), and no product is observed along the alveolar basement membrane between Type 1 pneumocytes and the capillary endothelium. Capillaries (*C*) are devoid of red blood cells. Absence of electron-opaque reaction product is confirmed at higher magnification (**Figure 4**). A portion of a neutrophil (*N*) is observed within the capillary lumen of **Figure 4**. (Unstained section reacted for peroxidase activity of cytochrome C.) (Figure 3, ×6720; Figure 4, ×10,200)

bits breathing air was identical to that described in the literature[15,16] and will not be reviewed here inasmuch as micrographs from our experimental groups document the basic structure of the lung and the adequacy of fixation. No inflammatory cells were observed by electron microscopy within the interstitium, although neutrophils, eosinophils, and lymphocytes were identified in alveolar capillaries. The alveolar epithelium and capillary endothelium were intact.

24–66 Hours of Hyperoxia

The lung tissue of rabbits breathing 100% oxygen for 24 hours resembled closely that of control animals. After breathing 100% oxygen for 48 hours, inflammatory cells, predominantly neutrophils, were observed in alveolar capillaries as well as in the interstitium of the lung (Figure 1), and the number of inflammatory cells increased by 66 hours. At all periods Type 1 cells lined the alveolar space, and no discontinuity or breakdown of adjacent cells was present. No edema was observed in the interstitial space or in the alveolar space. Endothelial cells of the alveolar capillaries were identical to those in control animals.

72 Hours of Hyperoxia

By 72 hours, focal areas of the lung were red and atelectactic upon gross examination. Morphologic alterations were more severe at 72 hours than at earlier periods. In addition to numerous neutrophils and an occasional eosinophil in the interstitium, focal alveoli were filled with a homogeneous, occasionally slightly flocculent electron-opaque material. Some of these alveoli also contained an especially electron-opaque, stringy material similar to fibrin (Figure 2). The epithelium and endothelium of the lung were intact, even in alveoli showing pulmonary edema.

Cytochrome C Tracer Studies

When introduced through the tracheobronchial tree into alveoli, cytochrome C was identified in the right lower lobe of the lung by its pink-red color in contrast to the whitish areas of lung from which blood had been removed by perfusion fixation.

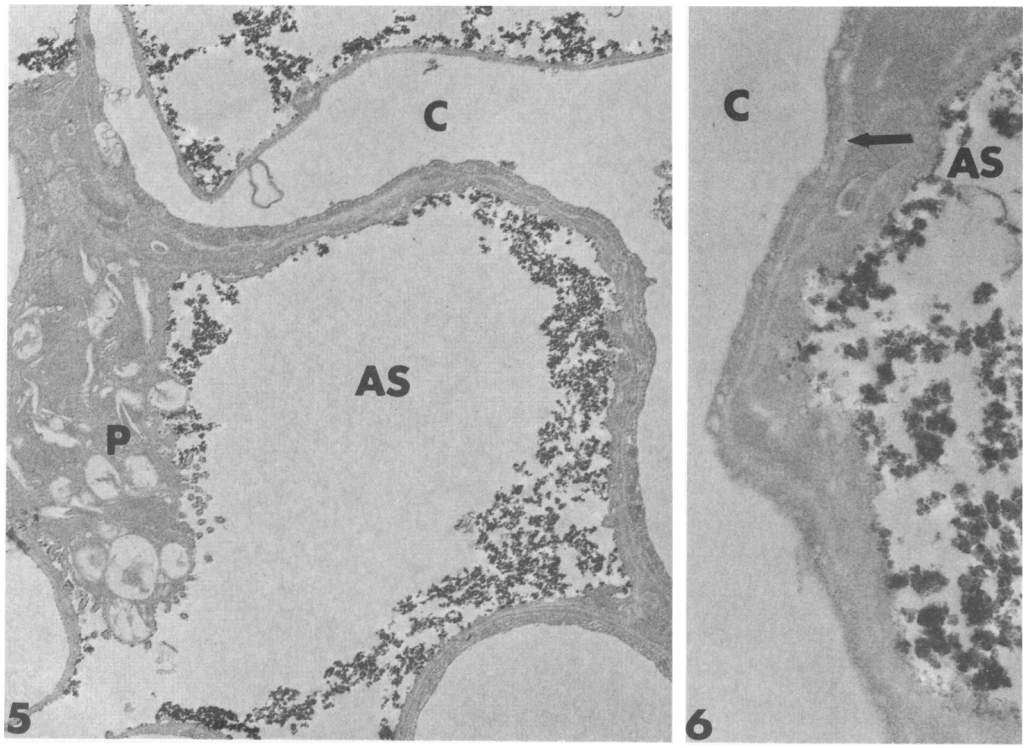
No reaction was present in controls for the ultracytochemical reaction in which substrate or DAB was omitted; in areas of the lung without tracer incubated in complete medium, red blood cells, when present, and granules within neutrophils and eosinophils, except the central crystalline structure, showed a positive reaction for endogenous peroxidase activity. In order to rule out the possibility that some of the electron-opaque reaction product represented nonspecific binding of oxidized DAB sections of lung containing no cytochrome C were incubated with the medium containing DAB oxidized by sunlight.[10,12] No nonspecific binding of electron-opaque reaction product to the sections was observed.

Rabbits Breathing Air

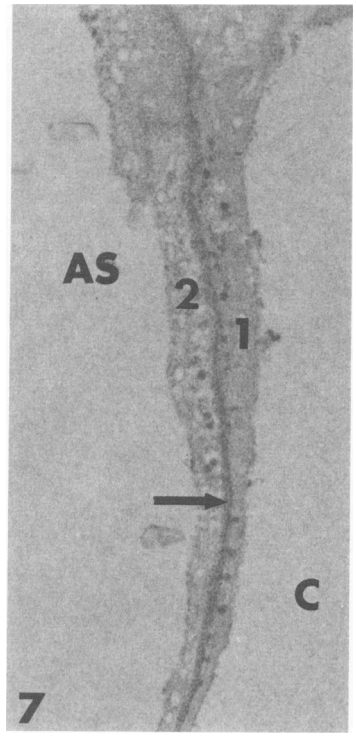
In control animals breathing air, electron-opaque reaction product was present in the alveolar space and in focal deposits located on the external surface of Type 1 alveolar cells (Figure 3). No reaction product was localized to the basal lamina (Figure 4), capillary endothelium, interstitium of the lung, or Type 2 alveolar cells.

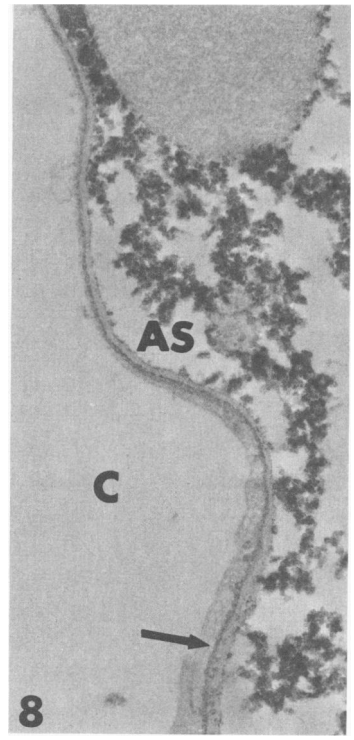
Rabbits Breathing 100% Oxygen

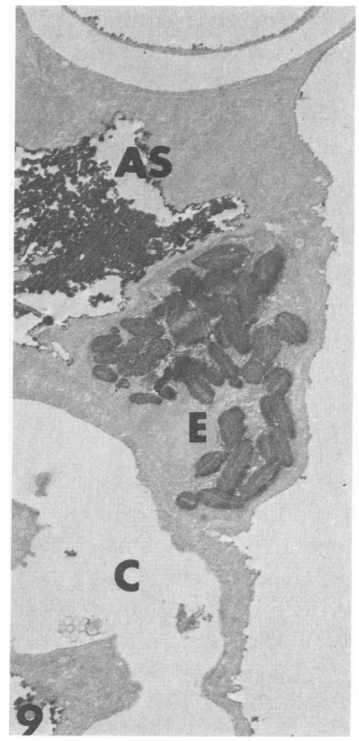
As in controls, reaction product in the lung of rabbits breathing 100% oxygen for 24 hours was confined to the alveolar space, and no electron-opaque product was seen in the basal lamina or in vesicles within Type 1 cells (Figures 5 and 6). After 48 hours of breathing 100% oxygen, electron-opaque reaction product was localized to the basal lamina outside the alveolar space of capillary loops in focal areas (Figures 7 and 8). Vesicles in Type 1 pneumocytes and in the endothelial cell of the capillary contained electron-opaque reaction product. Other adjacent vesicles in capillary endothelial cells were nonreactive for peroxidase activity. Also at 48 hours in other alveolar capillary loops, no reaction product was seen in vesicles of Type 1 cells, in endothelial cells, or within the basal lamina (Figures 9 and 10). By 66 hours, reaction product was also observed (in a linear distribution) along the basal lamina of virtually all capillary loops throughout the lung (Figure 11). Reaction product was observed in vesicles within Type 1 cells and in capillary endothelial cells (Figures 12–14). Electron-opaque reaction product was seen penetrating part of the way into junctions between

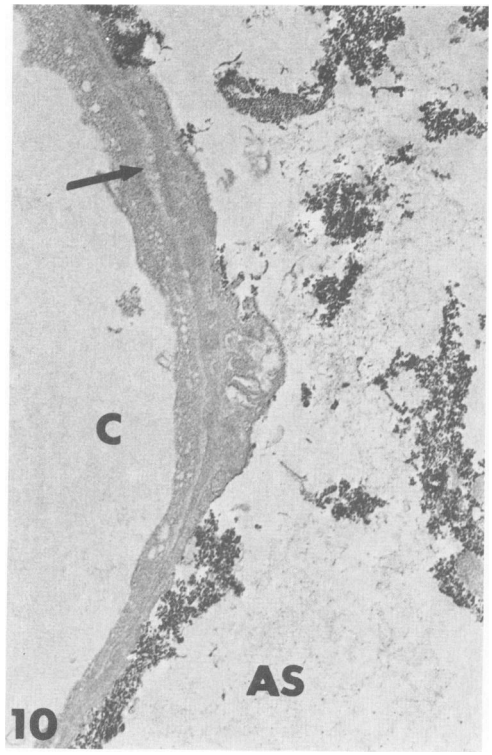


Figures 5 and 6—Lung of a rabbit exposed to 100% oxygen for 24 hours. Electron-opaque reaction product is confined to the alveolar space (*AS*) as seen at low (**Figure 5**) and high (**Figure 6**) magnification. Basal lamina (*arrow*); Type 2 pneumocytes (*P*); capillary lumen (*C*). (Unstained sections reacted for peroxidase activity of cytochrome C.) (**Figure 5**, ×8400; **Figure 6**, ×2400)









Figures 7-10—Lung of a rabbit that breathed oxygen for 48 hours. In **Figures 7** and **8**, electron-opaque reaction product is present in the basal lamina (*arrows*) and within vesicles of Type 1 pneumocytes (*1*) and capillary endothelial cells (*2*) (**Figure 7**). In **Figure 8**, electron-opaque reaction product is observed within the alveolar space (*AS*). In **Figures 9** and **10**, although reaction product is located in the alveolar space (*AS*), no reaction product is present in basal lamina. An eosinophil (*E*) whose crystalline core has no reactivity for peroxidase under the conditions used in our study is observed in the interstitial space (**Figure 9**). Capillary lumen (*C*). (Unstained sections reacted for peroxidase activity of cytochrome C.) (**Figure 7**, ×26,400; **Figure 8**, ×22,400; **Figure 9**, ×6480; **Figure 10**, ×9600)

endothelial cells, but it did not extend to the luminal side of the capillary (Figure 13).

Discussion

Our control studies indicate normal lung structure both by light- and electron-microscopic examination and the confinement of the intraalveolarly instilled cytochrome C in the alveolar space. These results are in agreement with a number of morphologic and physiologic studies. Schneeberger[8] has shown that the Type 1 alveolar cells are impermeable to intravenously injected cytochrome C. Apparently this molecule is too large (mol wt = 12,523 daltons, $r \sim 17$ Å) to cross the intercellular junctions. Tracer, however, was observed in the basal lamina and capillary junctions, indicating that the normal capillary endothelium is permeable to this tracer. Taylor et al,[17] utilizing the technique of the osmotic transient, demonstrated that the equivalent pore radius of the alveolar epithelium was $\sim$ 10 Å, while that of the capillary endothelium was $\sim$ 80 Å. The "pores" are the pathways through which lipid-insoluble molecules move and are thought to be located in the intracellular junctions. Finally, in a recent study, Matalon et al[6] instilled ^{125}I-cytochrome C in the alveolar space of rabbits, using the same technique, and showed that the concentration of this tracer when corrected for the absorption of fluid remained unchanged for a period of 60 minutes. Furthermore, the area of the lung containing the fluid looked normal (ie, no perivascular edema and no interstitial inflammatory cells) confirming previous observations of Gorin et al[18] that the introduction of small amount of isoosmotic saline in the alveolar space does not alter the permeability characteristics of the blood-gas barrier.

After 48 hours of oxygen breathing, cytochrome C gains access to the basal lamina beneath Type 1 cells, suggesting that these cells became permeable to the tracer as a result of the exposure. These results agree well with the findings of Matalone et al,[6] who were able to detect in rabbits exposed to 100% oxygen for 48 hours radioactively labeled cytochrome C in the arterial blood $\sim$ 20 minutes after its placement in the alveolar space. Furthermore, they clearly demonstrate that the tracer crosses the alveolar barrier instead of entering the blood via an alternate pathway, ie, the bronchial circulation.

Morphologic ultracytochemical localization to focal alveolar capillary loops after 48 hours of breathing 100% oxygen suggests that not all alveoli develop altered permeability simultaneously. The precise explanation for a variability in susceptibility in oxygen is not known, although the absence of

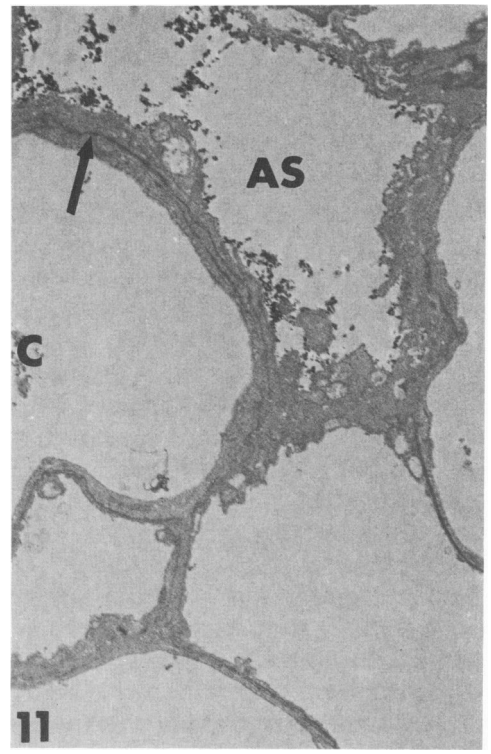
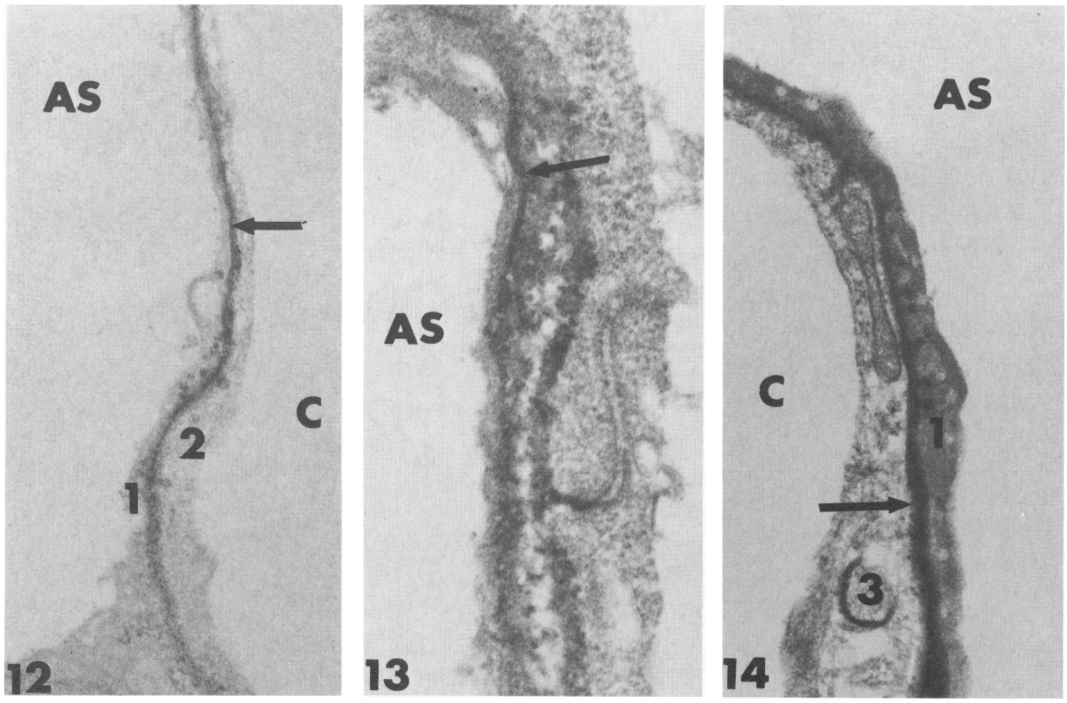


Figure 11—Lung from a rabbit that breathed 100% oxygen for 66 hours. Electron-opaque reaction product is present within the alveolar space (*AS*) and in the basal lamina (*arrows*). Capillary lumen (*L*). (Unstained section reacted for peroxidase activity of cytochrome C.) (×7500)

reaction product in the basal lamina of some alveolar capillary loops is undoubtedly not attributable to an artifact of lack of DAB penetration inasmuch as cytochrome C was demonstrable in the alveolar space bordering the capillaries; furthermore, the tracer gained access to the basal lamina of adjacent capillary loops. After 66 hours of oxygen exposure, electron-opaque reaction product penetrated Type 1 alveolar cells uniformly throughout the lung.

Despite the obvious increases in permeability, the structure of the alveolar epithelium remained intact. The appearance of vesicles containing electron-opaque reaction product in the Type 1 cells of oxygen-treated rabbits and their absence in rabbits breathing air strongly suggests that these vesicles play an important role and are perhaps primarily responsible for the increased permeability. Vesicular transport has been advocated to explain the transport of large proteins like IgG, IgA, and perhaps albumin, which have been demonstrated in secretions of the lower respiratory tract.[19,20] An increase in the number of vesicles within Type 1 pneumocytes and endothelial cells of alveolar capillaries occurs in edema associated with increased hydrostatic micro-



Figures 12-14—Lung from a rabbit exposed to 100% oxygen for 66 hours. In all 3 sections, electron-opaque reaction product is present in the basal lamina (*arrows*). In **Figure 12**, electron-opaque reaction product is present within vesicles of a Type 1 pneumocyte (*1*) and in the capillary endothelium (*2*). In **Figure 13**, electron-opaque reaction product extends part of the way through the junction between 2 capillary endothelial cells. In **Figure 14**, electron-opaque reaction product is observed in Type 1 cells (*1*) and in a portion of cellular junction (*3*). Capillary lumen (*L*). (**Figures 12-14** reacted for peroxidase activity of cytochrome C.) (**Figure 12**, unstained, ×24,000; **Figure 13**, uranyl acetate and lead citrate, ×60,000; **Figure 14**, uranyl acetate and lead citrate, ×35,000)

vascular pressure or with decreased albumin concentration.[21]

Comparison of our results with those of others is complicated by the different response of species to oxygen. Kistler et al[5] reported that the thickness of the pulmonary interstitial space of rats increased by a factor of 2 after 48-hour exposure to oxygen and by a factor of 4 after ~ 72 hours. It is interesting to note that even after 72 hours in oxygen the alveolar epithelium appeared normal. The apparent discrepancy between our results and theirs may be explained by the shorter survival time of rats exposed to 100% oxygen.[1] It could be that their rats were at a terminal phase.

The pathogenesis of the altered permeability is unclear, although breathing 100% oxygen exerts toxic effects on the lung by several mechanisms.[1,22,23] The presence of inflammatory cells in an extravascular location as well as increased numbers of these cells in capillary loops may well play some role in the pathogenesis of the toxicity and pathologic changes through release of permeability mediators.[24-26] In fact, Sacks et al[24] have attributed endothelial cell injury in the lung to release of oxygen radicals by activated intravascular granulocytes.

References

1. Clark JM, Lambertsen CJ: Pulmonary oxygen toxicity: A review. Pharmacol Rev 1971, 23:37-133
2. Katzenstein A-LA, Bloor CM, Leibow AA: Diffuse alveolar damage: The role of oxygen, shock, and related factors. Am J Pathol 1976, 85:210-228
3. Balentine JD: Experimental pathology of oxygen toxicity, Oxygen and Physiological Function. Edited by FF Jöbsis. Dallas, Texas, Professional Information Library, 1977, pp 311-378
4. Matalon S, Farhi LE: Respiratory acidosis in conscious sheep breathing 100% O_2 at 1 ATA. Physiologist 1979, 22(4):82
5. Kistler GS, Caldwell PRB, Weibel ER: Development of fine structural damage to alveolar and capillary lining cells in oxygen-poisoned rat lungs. J Cell Biol 1967, 32:605-628
6. Matalon S, McIntyre BR, Egan EA: O_2 exposure and permeability of the alveolar epithelium. Fed Proc 1979, 38:1264
7. Nickerson PA, Matalon S: The effects of breathing 100% O_2 at 1 ATA on the movement of cytochrome C across the alveolar membrane: An ultrastructural study. Fed Proc 1980, 39:765
8. Schneeberger EE: Ultrastructural basis for alveolar-capillary permeability to protein Lung Liquids. Amsterdam, Elsevier, Ciba Foundation Symposium, 1976, 38: 3-29
9. Karnovsky MJ, Rice DF: Exogenous cytochrome C as an ultrastructural tracer. J Histochem Cytochem 1969, 17:751-753
10. Essner E: Hemoproteins, Electron Microscopy of En-

zymes. Vol 2. Edited by MA Hayat. New York, Van Nostrand Reinhold, 1974, pp 1-33
11. Mollenhauer HH: Plastic embedding mixtures for use in electron microscopy. Stain Technol 1964, 39:111-114
12. Hirai K-I: Comparison between 3,3'-diaminobenzidine and auto-oxidized 3,3'-diaminobenzidine in the cytochemical demonstration of oxidative enzymes. J Histochem Cytochem 1971, 19:434-442
13. Stempak JG, Ward RT: An improved staining method for electron microscopy. J Cell Biol 1964, 22:697-701
14. Reynolds ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 1963, 17:208-212
15. Weibel ER: Airways and respiratory surface, The Lung, International Academy of Pathology, monograph 8. Edited by AA Liebow, DE Smith. Baltimore, Maryland, Williams and Wilkins, 1968, pp 1-18
16. Gil J: Ultrastructure of lung fixed under physiologically defined conditions. Arch Intern Med 1971, 127: 896-902
17. Taylor AE, Gaar AK: Estimation of equivalent pore radii of pulmonary, capillary and alveolar membranes. Am J Physiol 1970, 218:1133-1140
18. Gorin AB, Stewart PA: Differential permeability of endothelial and epithelial barriers to albumin flux. J Appl Physiol 1979, 47:1315-1324
19. Hand WL, Cantey JR, Hughes CG: Antibacterial mechanisms of the lower respiratory tract. J Clin Invest 1974, 53:354-362
20. Scarpelli EM, Wolfson DR, Colacicco G: Protein and lipid-protein fractions of lung washings: Immunological characterization. J Appl Physiol 1973, 34:750-753
21. Chinard FP: The alveolar-capillary barrier: Some data and speculations. Microvascular Res 1980, 19:1-17
22. Haugaard N: Cellular mechanisms of oxygen toxicity. Physiol Rev 1968, 48:311-373
23. Swartz HM: Toxic oxygen effects. Int Rev Cytol 1973, 35:321-343
24. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS: Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes: An in vitro model of immune vascular damage. J Clin Invest 1978, 61:1161-1167
25. Fox RB, Hoidal JR, Brown DM, Repine JE: Hyperoxia causes a preterminal influx of polymorphonuclear leukocytes (PMN) into the lungs and is associated with increased lung lavage chemotaxin (CTX) from PMN and death of alveolar macrophages (AM). Am Rev Respir Dis 1980, 121:340
26. Harada RN, Fox RB, Bowman MD, Shasby DM, Repine JE: Inflammatory mechanisms contributing to pulmonary oxygen: Hyperoxia causes alveolar macrophages (AM) in culture to release chemotaxins (CTX) for neutrophils (PMN). Am Rev Respir Dis 1980, 121:349

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