Oxygen Toxicity in the Newborn

The Effect of Chronic Continuous 100 Percent Oxygen Exposure on the Lungs of Newborn Mice

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Continuous exposure of newborn mice of a single, highly inbred strain to 100% oxygen at normal atmospheric pressure for up to 6 weeks resulted in a progressive evolution of pulmonary changes which consisted of dense fibrous tissue deposition, chronic bronchitis and bronchiolitis, and emphysema. Survival of the experimental animals decreased with the duration of exposure, and it was 18% after the sixth week. While the pulmonary changes were evolving, lung growth was markedly inhibited in the experimental animals, whereas lung weight increased significantly. The present study indicates that in contrast to the adult mouse, survival of a substantial percentage of newborn mice for at least 6 weeks is possible, but it is associated with severe changes in pulmonary structure that doubtlessly lead to serious derangement of cardiopulmonary functions. (Am J Pathol 85:623–650, 1976)

CHRONIC RESPIRATORY INSUFFICIENCY following ventilation and supplemental oxygen therapy of newborn infants with severe respiratory distress syndrome (RDS) is a persistent problem.[1-5] The clinicopathologic changes that were initially seen in this syndrome—bronchopulmonary dysplasia [1]—suggested a profound disruption of lung growth and development. In order to elucidate the pathogenetic features of pulmonary oxygen toxicity in the newborn, we developed a paired littermate model utilizing the newborn mouse. We have previously reported inhibition of lung growth [6] and ultrastructural changes in the cellular components of the bronchi and bronchioli [7] as well as the alveoli [8] of newborn mice continuously exposed to 100% oxygen for up to 1 week. While there have been several light and electron microscopic studies on the effect of high concentrations of normobaric oxygen on the lungs of newborn [7-11] and adult [12-19] experimental animals, most of the exposure periods were of short duration, primarily because of poor survival beyond the fourth day. In our earlier experiments the survival rate was 95% through the first 4 days and 75% through the seventh day. This high survival rate allowed us to study what we have defined as the chronic injury and repair phase of

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pulmonary oxygen toxicity in the newborn mouse. The present study indicates that, in contrast to the adult mouse, survival of the newborn mouse for the entire 6-week period is possible and that there are associated progressive diffuse pulmonary pathologic changes which culminate in dense fibrous tissue deposition, chronic bronchitis and bronchiolitis, and emphysema.

Materials and Methods

In each of 30 experiments, four litters of naturally born C57BL/Ka mice were weighed and their sex determined within 18 hours of birth. Systematic littermate pairing of experimental and control newborn animals was used to minimize the influence of the natural mother and the natural litter. "Natural" litter size was reduced to four by selecting the 4 animals with the highest birth weights. After the systematic litter mixing, the average initial weight for control animals was 1.40 g and for experimental animals 1.39 g. The newborn animals were then assigned to a "standard" litter so that each litter would contain only one newborn mouse naturally born to the mother who was chosen to nurse the standard litter. Each standard litter, with a foster mother, was placed in a plastic mouse cage in a controlled environment chamber. The chamber received either humidified 100% oxygen at normal atmospheric pressure or humidified air at a rate of 6 liters/min.

Nursing mothers were exchanged between the control and O_2-exposed litters every 12 hours to reduce possible differences in nursing ability, as well as to avoid oxygen intoxication of the mothers. The littermate mixing and standard conditions of the animals have been described in detail.[6]

The controlled environmental chambers, which have been previously described,[6] incorporate lock and glove ports enabling the exchange of the mothers and the handling of newborn animals within the chambers without altering the environment. Monitoring of the experimental and control atmospheres demonstrated them to be stable for the entire 6-week period. The oxygen concentration was determined by a Beckman oxygen analyzer (model D-2), the CO_2 concentration by a Beckman medical Gas analyzer for CO_2 (model LB-1; Beckman Instruments, Inc., Palo Alto, Calif., the humidity by a Serdex relative humidity meter, and the temperature by mercury incubator thermometers. The oxygen concentration varied during the experiments from 96 to 100% in the experimental atmosphere and 21 to 22% in the control. The CO_2 concentration was less than 0.5% in both. The relative humidity varied from 40 to 70% in both. The temperature varied from 21.5 to 24 C.

In 30 separate experiments, 496 newborn mice were continuously exposed either to the experimental environment or to the control environment for 2, 3, 4, and 6 weeks. A minimum of five experiments with 8 littermate-paired experimental animals and 8 control animals for each experiment was performed at each interval. Lung tissue from a total of 46 control and experimental littermate-paired newborn mice was studied electron microscopically. For these studies, 12 animals were sacrificed at 2 and 3 weeks, 14 at 4 weeks, and 8 at 6 weeks. In each of these groups each pair consisted of 1 control and 1 experimental animal.

Each pair of control and experimental animls examined was from an original natural litter. After sacrifice, the separated lobes of the lung were immersed in buffered 3% glutaraldehyde and immediately minced into 2 to 3 cu mm pieces. Fixation in aldehyde was continued for several days, after which the tissue was rinsed in buffered sucrose solution, postfixed in osmium tetroxide, dehydrated, and embedded in Maraglas. Multiple blocks were taken from each one of the five lobes of the control and experimental lungs and sectioned for light microscopy. At least five blocks of each lobe of the control lungs and 10 blocks of each lobe of the experimental lungs were examined electron micro-

scopically. The sections prepared for electron microscopy were mounted on copper grids, either mesh or single hole, stained with uranyl acetate and lead citrate, and examined with a Siemens 101 electron microscope. The morphologic changes observed in the electron microscope were documented in over 2000 electron micrographs.

The data given in Text-figure 1 are based on the wet weight of the lungs of the oxygen exposed and control animals; they are expressed as means (± 1 standard error). Statistical analysis was by Student's *t* test for littermate-paired differences.

Results

Continuous prolonged exposure of newborn mice to 100% oxygen for up to 6 weeks resulted in pulmonary damage which evolved progressively and affected the various components of the bronchi and bronchioli and the alveoli. These structural alterations advanced in proportion to the duration of exposure to 100% oxygen. Survival of the experimental animals decreased as the exposure period lengthened. Eighteen percent of the experimental animals survived the entire 6-week exposure period (Table 1).

After *two weeks* of oxygen exposure, loss of cilia of the cells lining large bronchi and also those of the terminal and respiratory bronchioles was a prominent finding. Often the ciliary damage was associated with more severe cellular injury including patchy necrosis of the bronchial and bronchiolar lining. The changes at the alveolar level consisted predominately of a striking proliferation of the granular pneumocytes, a process that, to a lesser extent, had been previously observed (Figure 1). These granular pneumocytes, which constituted the dominant cell type lining the alveoli, were mature, morphologically normal, and showed no evidence of cellular damage. Alveolar atelectasis that was focal, of moderate severity, and not present in all animals was also noted during this period of time. The alveolar capillaries showed no significant alterations. Occasionally the alveolar septa appeared to be moderately thickened and contained an increased amount of elastic fibers (Figure 2).

After *three weeks* of continuous exposure to 100% oxygen in some

Table 1—Percent Survival

	Survival	
Duration (weeks)	Air	100% O_2
1	98%	60%
2	97%	57%
3	88%*	59%
4	95%	48%
6	96%	18%

* Mortality of air-exposed controls was enhanced in one experiment by failure of maternal nursing. All deaths of controls in this experiment occurred during the first 2 days.

bronchi and bronchioli, the lining epithelium showed severe degenerative changes. In others, there was a noticeable disturbance of the polarity of the bronchiolar mucosal cells which, in addition, displayed a marked discrepancy in size and shape (Figure 3). At the alveolar level, predominance of the granular pneumocytes lining often severely distorted alveoli was still a distinct feature. This distortion of the alveolar architecture was primarily caused by a marked thickening of the alveolar septa brought about by an increase in the number of undifferentiated mononuclear septal cells and scattered infiltrates of polymorphonuclear leukocytes (Figure 4).

After *four weeks* of exposure, focal necrosis of the bronchial and bronchiolar mucosa with denudation of the underlying basement membranes was now a frequent occurrence (Figure 5). The epithelial cells adjacent to these denuded foci showed a wide spectrum of nonspecific cytoplasmic changes, including the formation of cytoplasmic vacuoles and the presence of myelin figures. Some of these cells demonstrated an increase in the number of mitochondria. Changes involving the submucosal fibromuscular tissues were also pronounced. These consisted of fragmentation of the elastic tissue and damage of the adjacent smooth muscle cells, portions of which were frequently replaced by irregular bands of collagen fibers (Figure 6). Scattered infiltrates of inflammatory cells, lymphocytes, plasma cells, and mainly polymorphonuclear leukocytes were still encountered in the lumina and the walls of bronchi and bronchioli.

The most severe changes were, however, seen at the alveolar level; these consisted of large poorly circumscribed areas showing a marked, irregular widening of the alveolar septa caused by deposition of a large amount of collagen (Figure 7). There were also areas of massive alveolar architectural distortion in which alveoli were replaced by an irregular tissue mass consisting of undifferentiated cells, polymorphonuclear leukocytes, bundles of collagen, and remnants of alveolar capillaries. These heterogeneous cellular elements were tightly held together and they occasionally formed large, round cellular conglomerates which were protruding into the surrounding alveolar spaces (Figure 8). These bodies, the formation of which appears to have resulted from the organization of an exudate in one or a group of neighboring alveoli, were partially or completely covered by granular pneumocytes, as depicted in Figure 8. Granular pneumocytes remained the predominant type of the alveolar lining cells though their number was significantly reduced in comparison to that seen in animals exposed to oxygen for 2 or 3 weeks. A floccular precipitate

intermingled with membranous-like material, lamellar whorls, and myelin figures often filled several adjacent alveolar spaces.

After *six weeks* of continuous exposure to 100% oxygen, the morphologic alterations were similar to those encountered in animals exposed for 4 weeks to oxygen, except that they were more advanced in severity and more extensive. Necrosis of brochial and bronchiolar epithelium was a prominent finding while the surviving mucosal cells continued to demonstrate a pronounced ciliary damage (Figure 9). In some of the cells lining the airways, an increased number of mitochondria was noted. The previously observed injury of the submucosal fibromuscular tissues was still present during this period of time in most of the major bronchial segments. At the alveolar level, the damage was now even more pronounced. In relatively less severely injured alveoli, granular pneumocytes were still present in increased numbers; some of these cells were occasionally seen undergoing mitosis (Figure 10). At this time, thrombi in various stages of organization were often observed within the alveolar capillary lumina (Figure 11). In areas where the alveolar damage had been more severe, irregular strands of dense fibrous tissue were replacing large areas of alveoli, the normal architecture of which was almost completely demolished (Figure 12). In these areas, a few granular pneumocytes could occasionally be identified randomly embedded within a predominately collagenous stroma; present within some of the remaining distorted alveolar lumina were large numbers of myelin figures often intermingled with a finely fibrillar material of distinct periodicity, while other alveolar spaces were filled with alveolar macrophages, the cytoplasm of which contained numerous longitudinal electron-dense rods and many lysosomal bodies (Figure 10).

While the pulmonary changes were evolving, lung growth, as judged by an increase in size, was significantly inhibited in the experimental animals. This inhibition was apparent by 7 days and persisted throughout the 6-week exposure period (Figure 13). Emphysematous areas could readily be discerned on gross examination of the experimental lungs by 3 weeks, yet the lungs of the experimental animals were usually smaller in size than those of the controls. Microscopically, the alveolar wall architecture was relatively unaltered in many of the emphysematous areas which often appeared well circumscribed; however, similar changes which were frequently observed in and adjacent to areas of alveolar fibrosis were more irregular and ill defined (Figure 14).

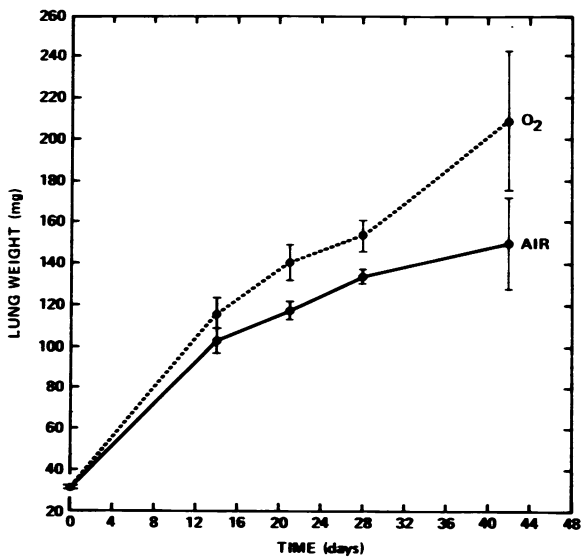
In contrast to the lung growth, total lung weight increased significantly in the experimental animals during the same period. This increase in

lung weight initially correlated electron microscopically with pulmonary edema and, at the end of the 6-week exposure period, with marked fibrous tissue deposition. The inflammatory cell infiltration and exudation which played a role initially was relatively less important in the late increase in lung weight.

Control lungs displayed no gross histologic or ultrastructural abnormalities during the entire time period of our experiment (Figure 13).

Discussion

Our findings indicate that newborn mice exposed continuously to 100% oxygen for 6 weeks can survive but develop progressive diffuse pulmonary pathologic changes which culminate in dense fibrous tissue deposition, chronic bronchitis, broncholitis, and emphysema. The appearance of these changes correlated with the increasing mortality rate in our experimental animals illustrated in Table 1. These severe morphologic pulmonary alterations must have led to serious derangements of cardiopulmonary function. The pathologic changes found in our experimental animals after 4 and 6 weeks of exposure are also reflected in the size and weight of the lungs (Figure 13 and Text-figure 1). These lungs were smaller, yet heavier, than those of their normal counterparts. The massive overgrowth of fibroconnective tissue in these lungs appears to account for this phenomenon because pulmonary edema, a striking finding in animals exposed up to 1 week to O_2, was not encountered in the animals exposed beyond 2



TEXT-FIGURE 1—Wet weight of lungs of newborn mice exposed to air, or 96 to 100% oxygen. (Each mean and its single standard error represents a minimum of 12 animals, with the exception of 6 weeks which represents 7 animals.) Values for air- and 96 to 100% oxygen-exposed mice differ significantly at 2 ($P < 0.01$), 3 ($P < 0.01$), 4 ($P < 0.025$), and 6 weeks ($P < 0.025$).

weeks. In spite of these alterations, a substantial number of animals (18%) survived for 6 weeks in 100% oxygen. This contrasts markedly with the 4-day survival of adult C57BL/Ka mice similarly exposed.[20] At this point, it is not clear which specific adaptive mechanisms account for survival of the newborn mice.

Although marked alterations were observed in the bronchi and bronchioli, the alveoli appear to be structurally more severely affected than the conductive airways. Ciliary damage and mucosal necrosis and ulceration were the major findings of the airways in our study. The toxic effect of high concentrations of oxygen on the mucociliary apparatus has been established,[7,21-23] yet oxygen is a prerequisite for sustained ciliary activity.[24] The observed bronchial and bronchiolar ulceration and necrosis also reported to have followed prolonged exposure to high concentrations of oxygen in humans [2-4,25] and experimental animals,[7,11,22] as well as in organ cultures *in vitro*,[26] may affect the subsequent growth of the lung of neonates. It has been recently demonstrated in the rat that postnatal alveolar growth may also take place from outpouchings in the walls of terminal bronchioles,[27] as well as through septation of primary channels and saccules corresponding to the prospective alveolar ducts and sacs. Thus, pulmonary injury of the degree observed in our study can seriously compromise further lung development; this has already been demonstrated experimentally.[6,28,29] Furthermore, failure of repair of the severely injured bronchial and bronchiolar epithelium with resulting scar formation may lead to permanent pulmonary damage, such as emphysema. In keeping with this hypothesis is the clinical observation that bronchiolitis and infectious bronchitis were found to be among the most common causes of obstructive emphysema in preschool children.[30]

One of the most prominent findings in our study was the marked diffuse proliferation of granular pneumocytes with a concomitant reduction of their membranous counterparts. It was only at the late stage of advanced pulmonary injury, after 6 weeks of continuous exposure to oxygen, that granular pneumocytes were found in relatively smaller numbers. But, even at this point these cells demonstrated a considerable degree of mitotic activity and their total number was greater than that of the controls. This proliferation of granular pneumocytes has been previously reported in pulmonary injury following prolonged exposure to high concentrations of oxygen in humans [25,31,32] as well as experimental animals.[8,33] It has also been shown that various other agents can induce the same reaction [34-36] which is considered to be a nonspecific, common pathway of epithelial repair in the lung periphery.[37] It is of interest that during exposure of rats to sublethal doses of oxygen, granular pneumocytes have

been seen to proliferate at the time when tolerance develops and superoxide dismutase (the enzyme protecting against the toxic effects of oxygen) activity increases, an observation consistent with the hypothesis that this adaptive response to oxygen may be specifically located in the granular pneumocytes.[38] Recent autoradiographic studies have demonstrated that the proliferating granular pneumocytes are eventually transformed into membranous pneumocytes.[33,39] The presence of granular pneumocytes in large numbers during the entire time period of our experiment, paralleled by a striking reduction of their membranous counterparts, indicates that such a transformation probably does not take place before the noxious agent responsible for the pulmonary injury is removed. Whether this excessive proliferation of granular pneumocytes interferes with the gas exchange across the alveolocapillary membrane is not clear, although it may result in a change of the lung's compliance.

The presence of microaggregates consisting mainly of platelets and fibrin within alveolar capillary lumina was a frequent finding in our study. This type of aggregate in the alveolar capillaries is not considered to be specific for oxygen toxicity because it can also be seen following endothelial damage by irradiation [40] or in other conditions; [41] nevertheless, it was a common finding of our study. We have also observed this after short-term exposure of experimental animals to 100% oxygen [8] and in babies who received high concentrations of oxygen for prolonged periods of time.[25] Similar observations have been made by Büsing and Bleyl in a study in which rabbits were exposed to normobaric hyperoxia for 30 to 85 hours. These authors postulated that intravascular fibrin thrombi in the pulmonary flow tract represent a local manifestation of a generalized intravascular activation of coagulation which involved also the liver, spleen, and especially, the kidneys. However, no explanation was put forth for the cause of this intravascular coagulation activation.[42] But, it is reasonable to assume that formation of capillary thrombi is mainly the result of alterations of the physical properties of the cell membranes of the oxygen-damaged endothelial cells.[8,12,13,43] Furthermore, it has been shown, experimentally, that intravascular aggregation and pulmonary microbolization of platelet aggregates can occur rapidly after injury, particularly trauma.[44] The consequences of this multifocal alveolar capillary thrombosis can be very severe, since apart from a serious mechanical compromise of the pulmonary microcirculation, it can cause pulmonary insufficiency and it may also lead to alveolar capillary necrosis, alveolar wall collapse, and fibrosis.

The most striking finding in our study was the tremendous pulmonary fibrosis that was more severe at the alveolar level. This fibrosis was already

prominent and extensive after 4 weeks, but after the sixth week of continuous exposure it had resulted in a marked distortion of the alveolar architecture. Large numbers of collagen fibrils, but also elastic fibrils, were present with mesenchymal cellular elements and other connective tissue components in the greatly thickened alveolar interstitium and within alveolar lumina. This pulmonary fibrosis is a well-recognized sequela of pulmonary oxygen toxicity [13,19,25,32,45,46] which can also be induced by thoracic radiation,[40,47] the administration of certain antineoplastic drugs,[48,49] and other chemical agents.[50,51] It is well known that this pulmonary fibrosis, in turn, leads to an obliteration of the alveolar capillary bed. The pathogenesis of this fibrosis appears to be primarily the organization of intraalveolar or interstitial exudate. But, it has also been thought that alveolar fibrosis may follow incorporation of cellular debris of damaged alveolar ephithelial cells into septal walls with resulting proliferation of fibroblastic septal cells.[32] Interestingly, it has been shown, however, that interestitial cells, other than fibroblasts, are capable of synthesizing some types of collagen under tissue culture conditions.[52] In this respect, the role in the formation of alveolar fibrosis of the recently described septal "contractile interstitial cells," [53] sharing features of both fibroblasts and smooth muscle elements, should also be considered. The participation of this type of cell in some reparative and proliferative processes is well documented.[49,54-56] There are even claims that alveolar fibrosis could be secondary to transformation of regenerating and undifferentiated epithelial alveolar cells into fibroblast-like cells during the late stage of repair of damaged alveolar lining; [57] there have been recent indications that certain cultured lung cells of supposed epithelial origin are capable of synthesizing both Type I and Type II collagen.[58] Also, the role of the alveolar macrophage in this desmoplastic process should not be ignored; it has been demonstrated, experimentally, that intact alveolar macrophages are capable of stimulating collagen synthesis in fibroblasts.[59,60] Finally, it is difficult to assess the extent to which the bronchial and bronchiolar damage contributed to the alveolar fibrosis. It is likely that the continuing bronchial and bronchiolar injury and repair process affected the lymphatic drainage; lymphostasis is known to play a role in desmoplasia.

Slight thickening of the interstitium does not necessarily lengthen the path of gas diffusion,[61] but advanced and widespread fibrosis of the alveolar septa might have severe, frequently indirect consequences on gas diffusion and ventilation. For instance, distention of alveoli following scar retraction may cause rupture of alveolar septa and coalescence of the fibrotic foci. It has also been proposed that diffuse fibrosis, in particular, hinders the movement of tissue fluid through the pulmonary lymphatics

by impairing the efficiency of the so-called pulmonary sump.[62] This stagnation of metabolic waste products and other irritating substances in the alveolar interstitium can probably lead to progressive atrophy and eventual dissolution of the alveolar framework. In fact, in a study on the pathogenesis of pulmonary emphysema, it has been postulated that metabolic disturbances might interfere with the cohesive strength of the ground substance of the lung parenchyma because of alterations of its proteoglycans.[63] It is difficult to determine whether these pathogenetic mechanisms can, in part or fully, account for the emphysematous changes observed in the lungs of our experimental animals. However, the marked and extensive fibrosis observed after the fourth week of exposure could be seriously implicated in the formation of these foci of emphysema. The appearance of emphysema coincided with the spread of desmoplastic activity and its severity paralleled the severity of the emphysema which was most pronounced at the end of the sixth week. It is of interest that we have observed a similar pattern of focal, irregular emphysema in the fibrotic lungs of infants with bronchopulmonary dysplasia who had been treated with respirator and oxygen therapy for prolonged periods of time.[25]

From the foregoing, it is apparent that the sequelae of prolonged administration of high concentrations of oxygen can lead to profound disruption of lung growth and development. The significance of this becomes apparent if it is realized that, frequently, high concentrations of oxygen have to be administered to prolong the life of newborn human infants. The survival of newborn mice for 6 weeks in a 100% oxygen environment which kills adult mice of the same species in 4 days is remarkable. Study of the adaptation or selection process which lead to this difference in survival could suggest means of ameliorating or preventing pulmonary oxygen toxicity. In closing, it should also be emphasized that the study of the sequential evolution of the severe pulmonary pathologic alterations resulting from prolonged administration of toxic oxygen concentrations provides a model which may lead to better understanding of the pulmonary fibrogenic response and some forms of emphysema.

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[*Illustrations follow*]

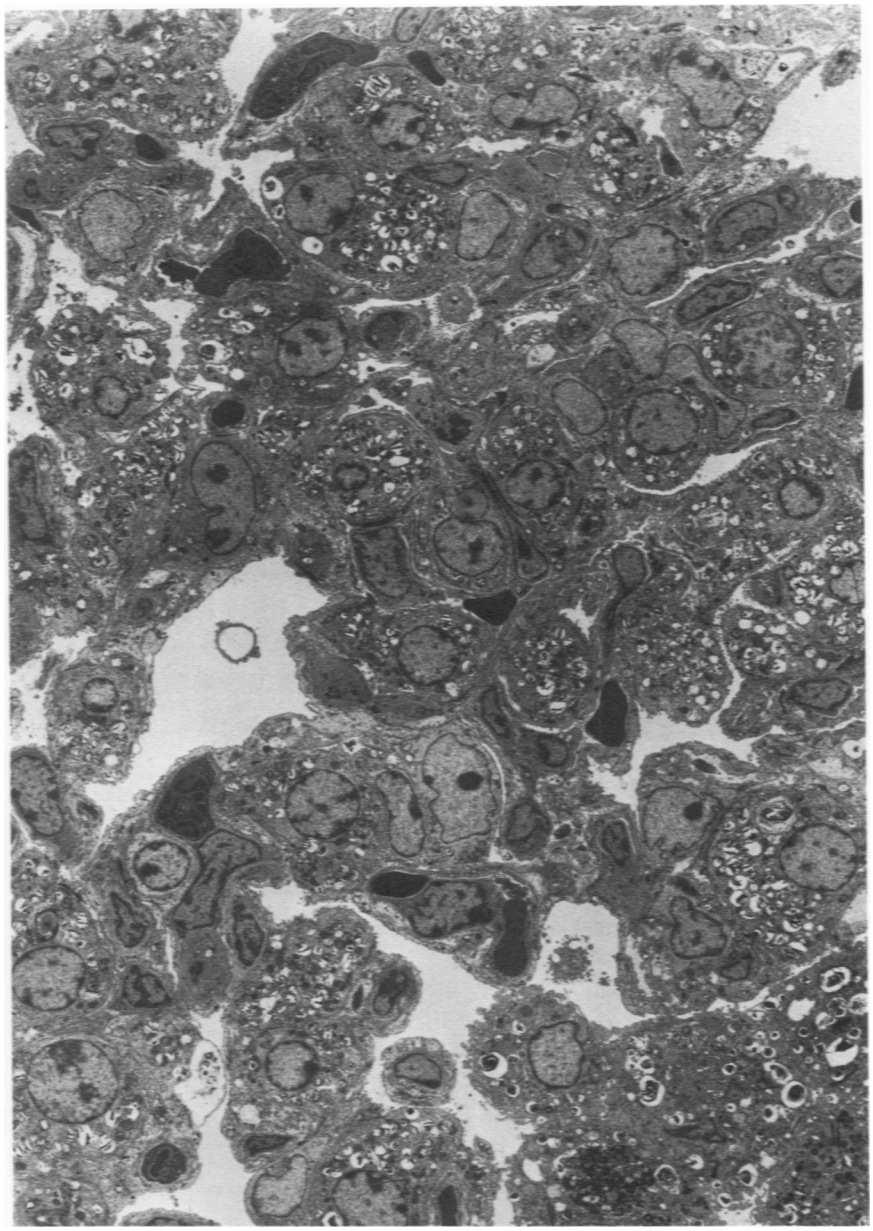


Figure 1—Mouse lung after 2 weeks of oxygen exposure. Notice the extensive proliferation of granular pneumocytes and partial alveolar collapse. (× 1750)

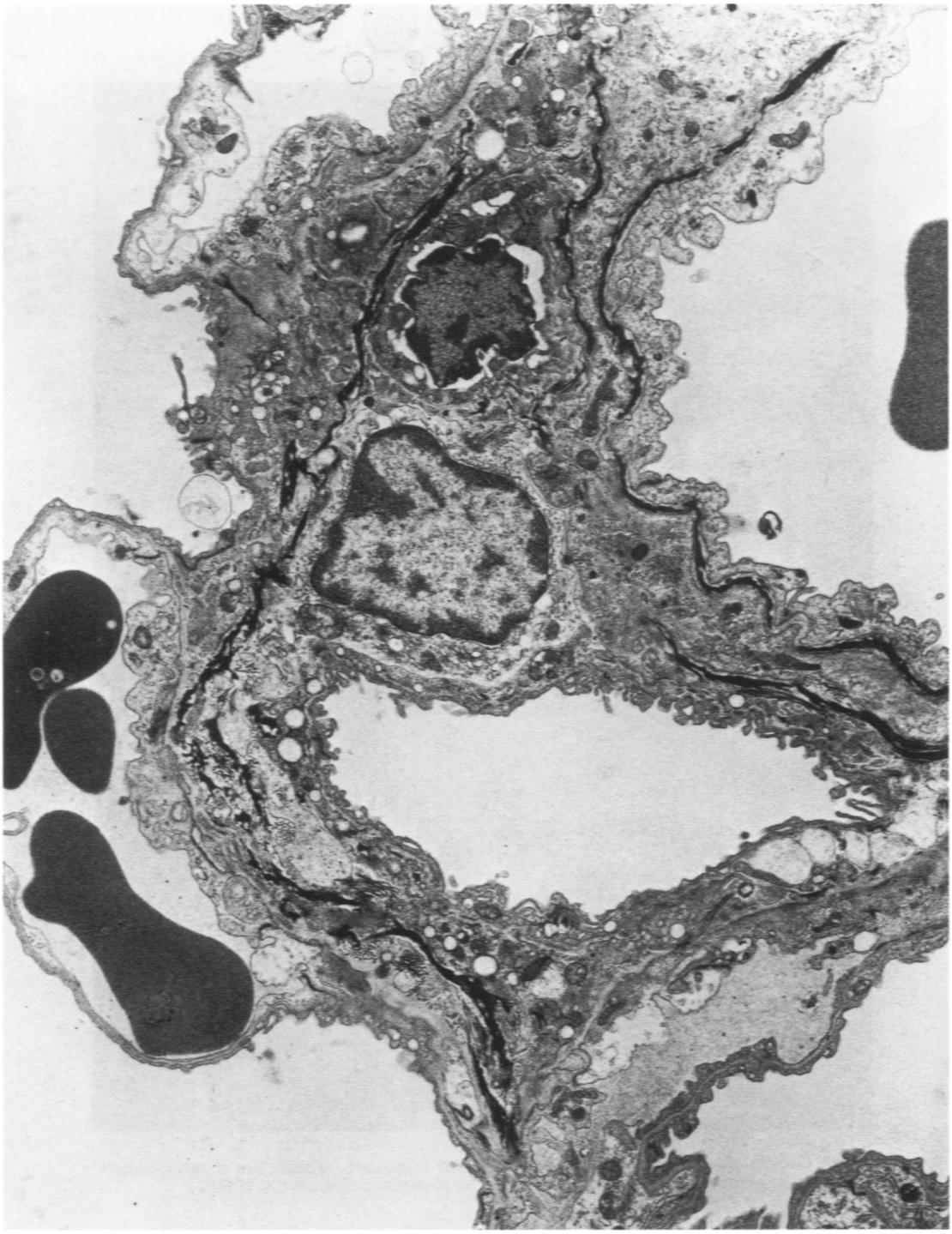


Figure 2—Mouse lung after 2 weeks of exposure showing moderately thickened alveolar septa which contain an increased amount of elastic fibers (× 7480).

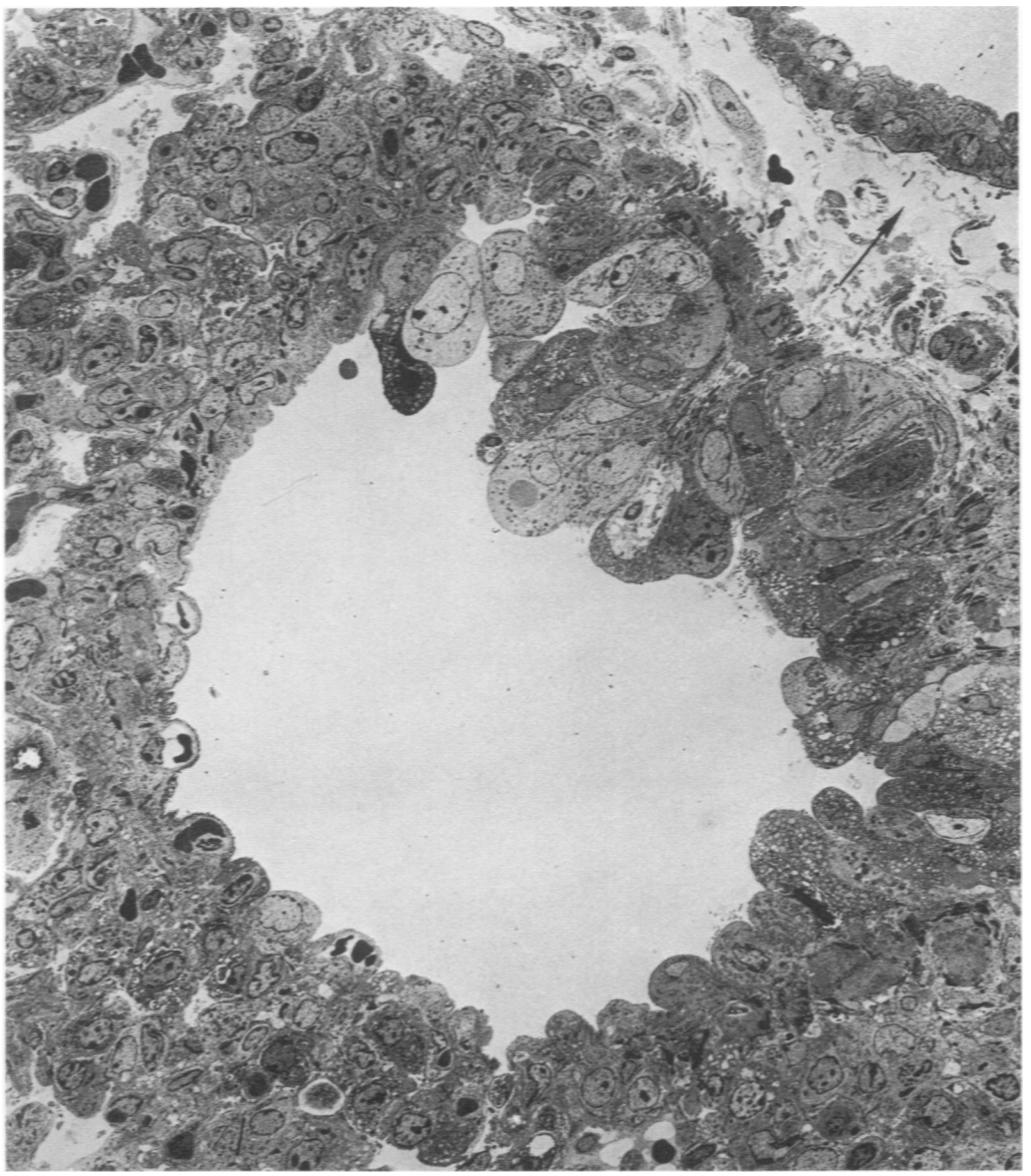


Figure 3—Bronchiolo-alveolar junction in mouse lung after 3 weeks of exposure. Note the haphazard arrangement of the bronchiolar mucosal cells, most of which display a marked discrepancy in size and shape, as well as loss of cilia. Edema of the perivascular space is seen in the right upper corner of the picture (*arrow*). (× 850)

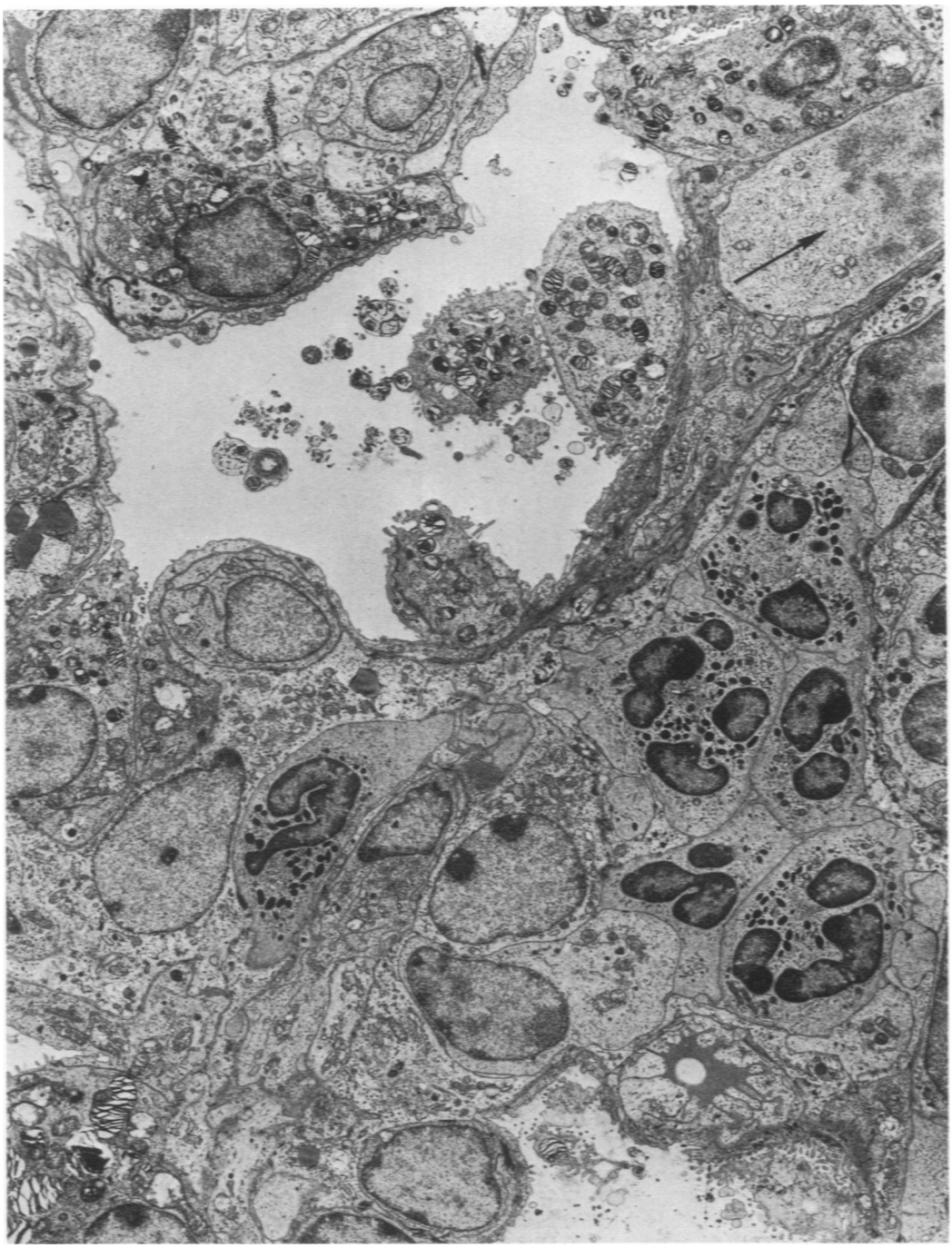


Figure 4—The lining of the alveolus in the upper half of the illustration consists mainly of granular pneumocytes. Note the markedly thickened alveolar septum due to the presence of large numbers of polymorphonuclear leukocytes and an increase of undifferentiated mononuclear septal cells, one of which is in mitosis (*arrow*). *A* = alveolar lumen. Three weeks of exposure. (× 4160)

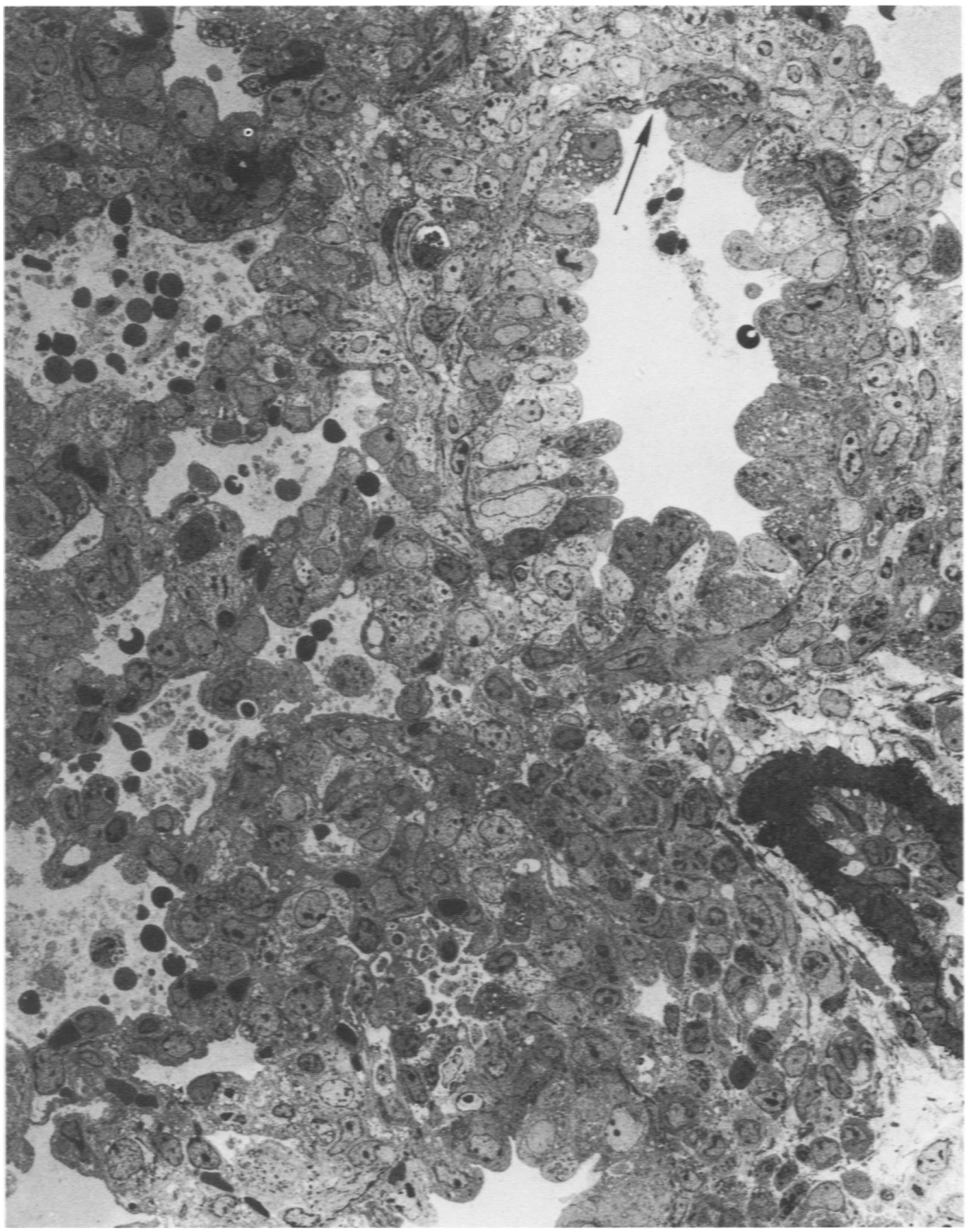
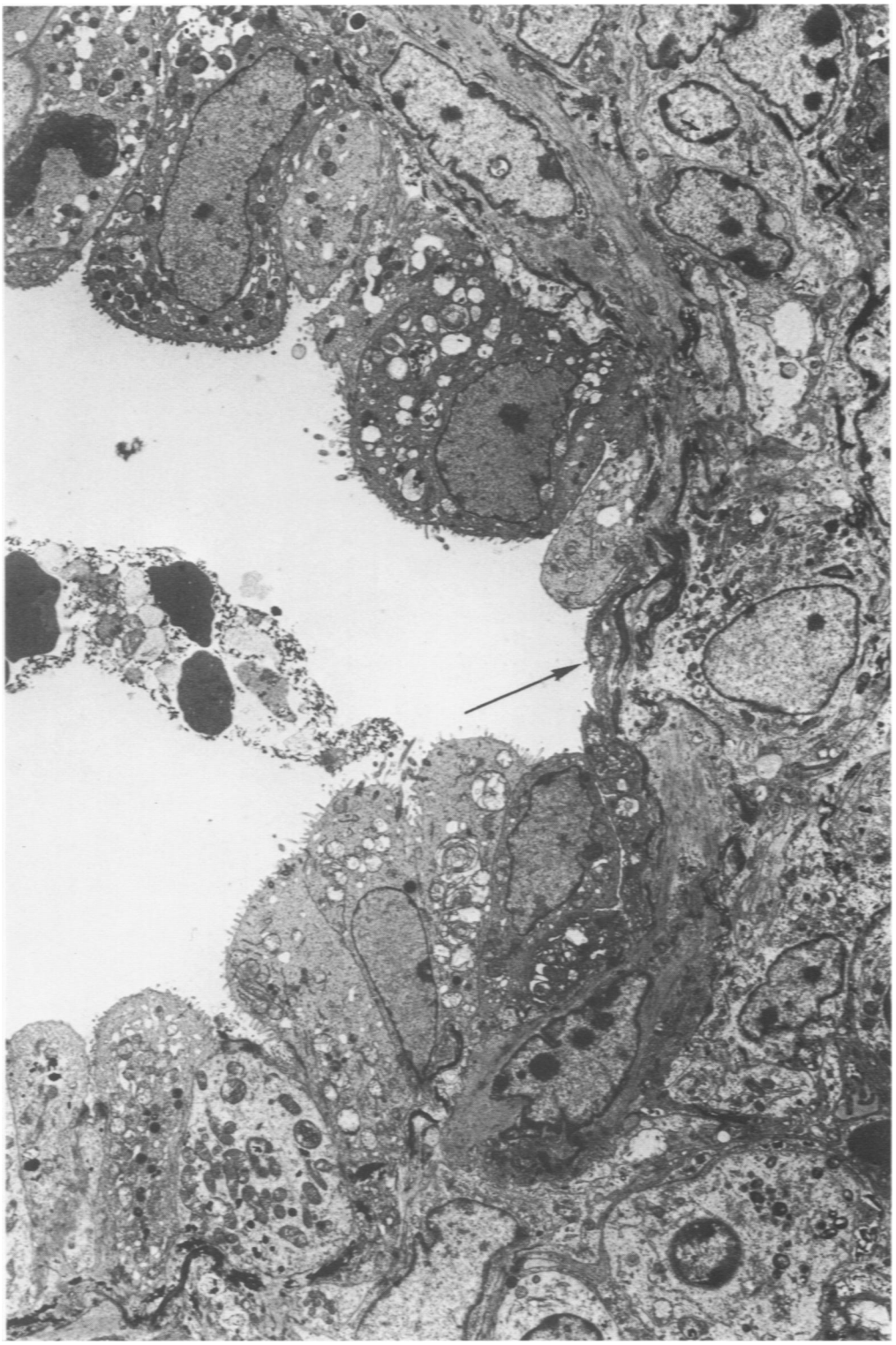


Figure 5—Mouse lung after 3 weeks of oxygen exposure. Note focal necrosis of bronchiolar mucosa (*arrow*), as well as thickening of the septa of surrounding alveoli. A granular precipitate intermingled with cellular debris is seen within the alveolar lumina. (× 800)

Figure 6—Higher magnification of Figure 5 showing the area of necrosis of the bronchiolar mucosa with denudation of underlying basement membrane (*arrow*). The epithelial cells adjacent to this denuded area show loss of cilia, as well as nonspecific cytoplasmic alterations, such as cytoplasmic vacuoles and myelin figures. A dying cell with a pyknotic nucleus is noted in the left upper part of the picture. The bronchiolar lumen contains cellular debris. Fragmentation of the submucosal elastic tissue and disruption of the smooth muscle cells are seen in the submucosal layer. (× 4000)



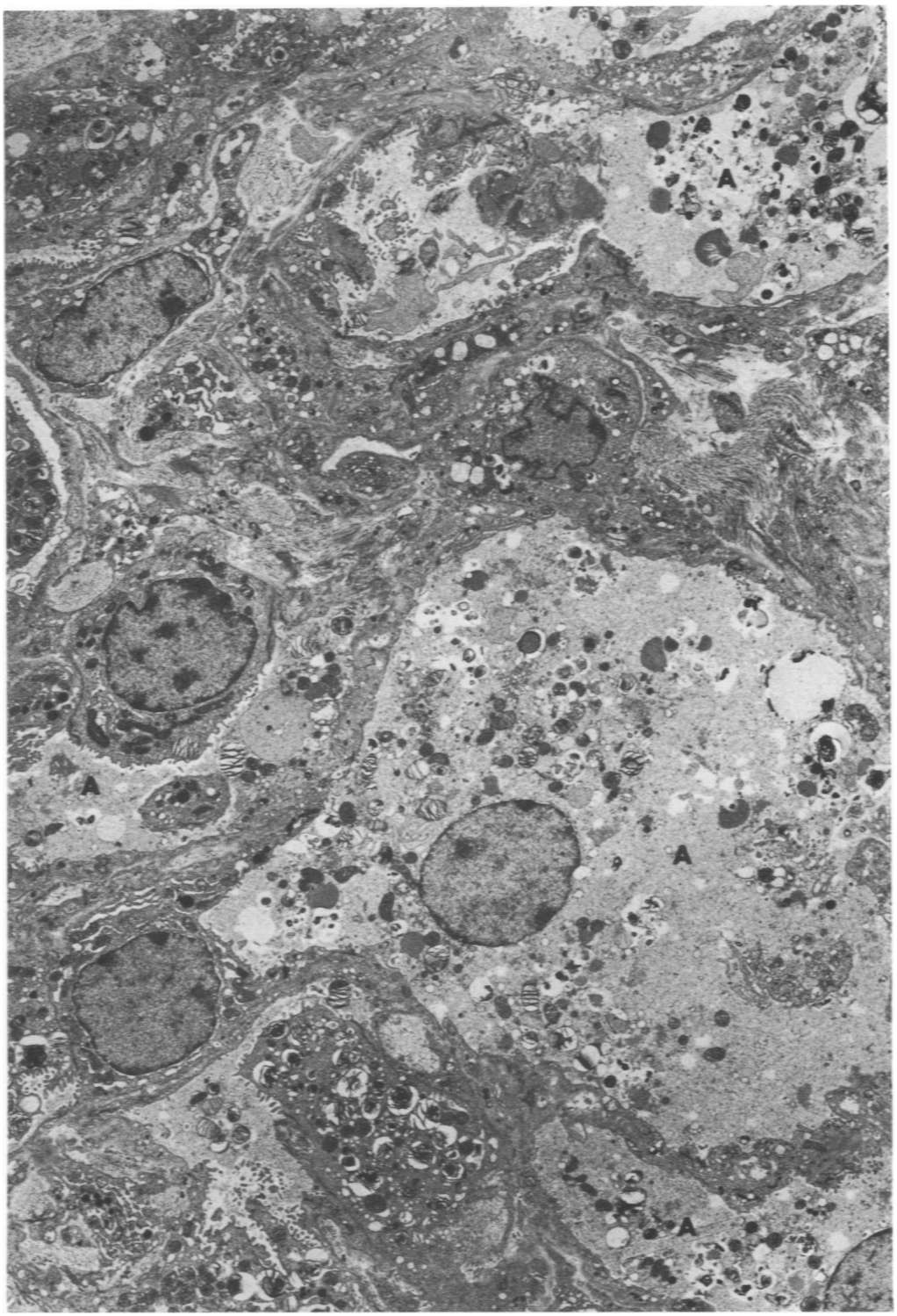


Figure 7—Severe distortion of the alveolar architecture with greatly thickened septa displaying a marked increase of collagen. Granular pneumocytes, as well as interstitial and undifferentiated cells, are the predominant cellular elements; some of the granular pneumocytes are in various stages of degeneration. A floccular precipitate intermingled with membranous-like material, lamellar whorls, and myelin figures fills the alveolar lumina (*A*). Four weeks of exposure. (× 3840)

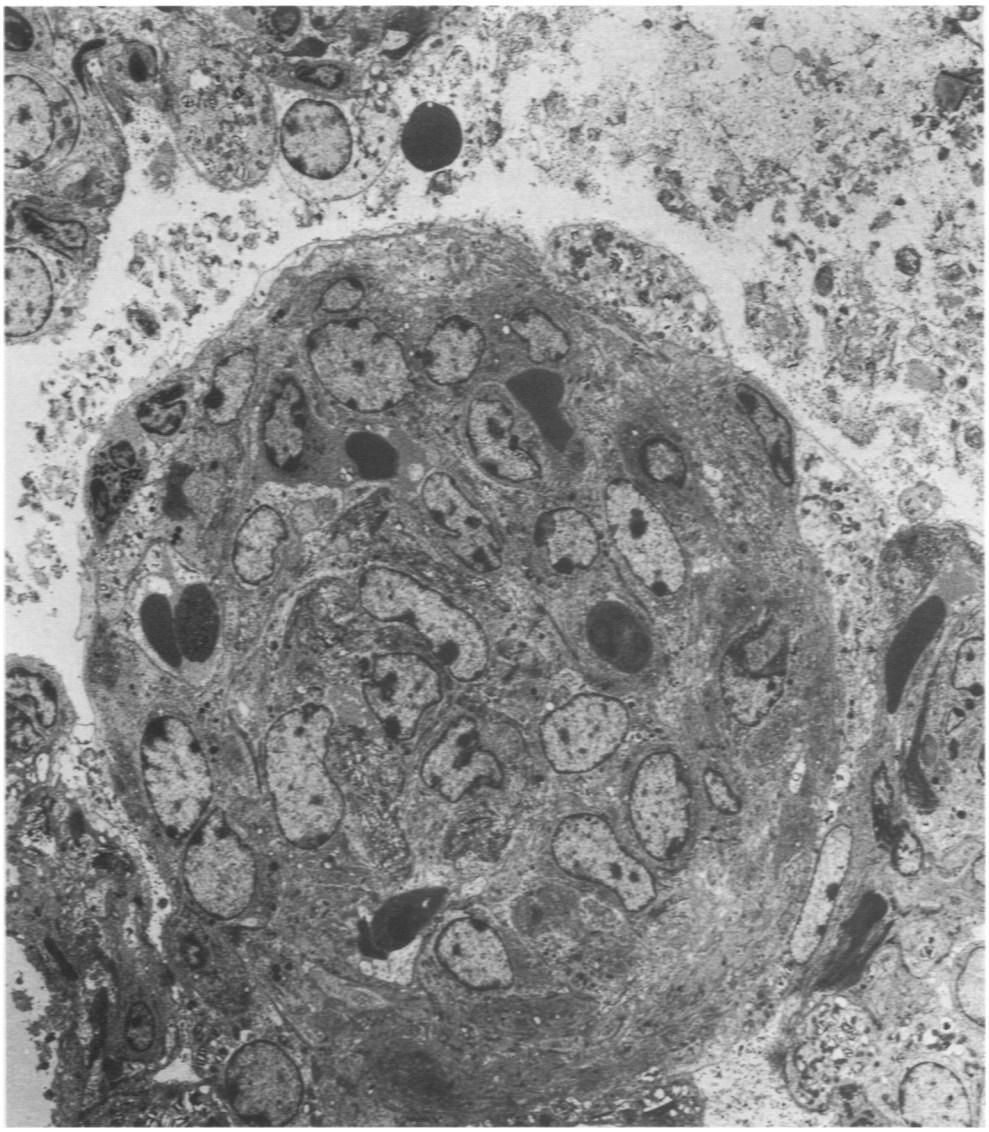


Figure 8—Alveolar space, protruding into which there is a heterogeneous cellular conglomerate consisting of tightly held together undifferentiated cells, polymorphonuclear leukocytes, bundles of collagen, and remnants of alveolar capillaries. Four weeks of exposure. (× 1960)

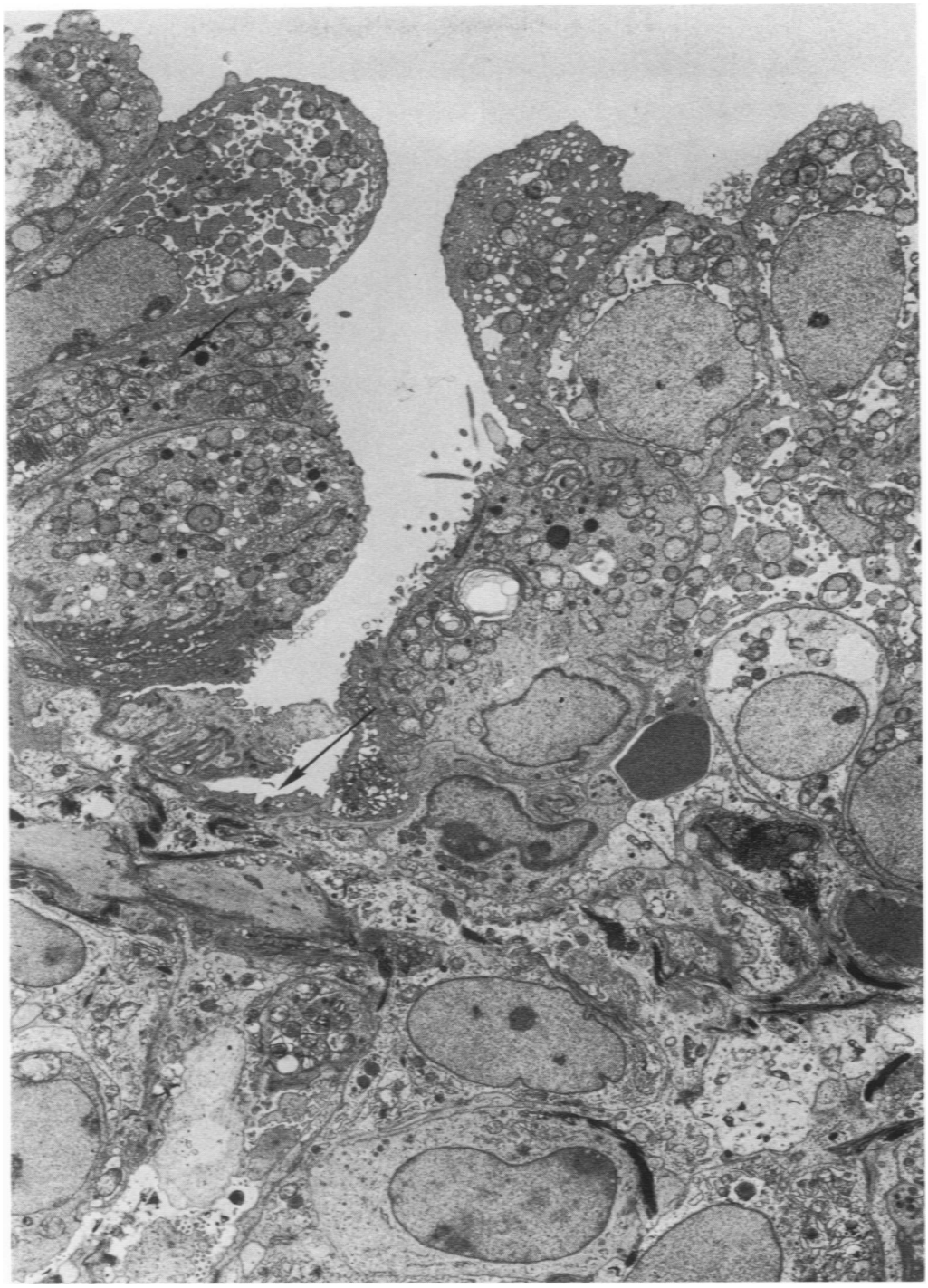


Figure 9—Mouse lung after 6 weeks' exposure showing marked bronchial ciliary damage and focal necrosis of the bronchial mucosa (*arrow*). Some of the cells of the lining epithelium demonstrate an increase in the number of mitochondria (*short arrow*). Nonspecific cytoplasmic changes are seen in the rest of the epithelial cells. Notice interruption of submucosal smooth muscle ring and the haphazardly scattered bundles of collagen fibrils. (× 3840)

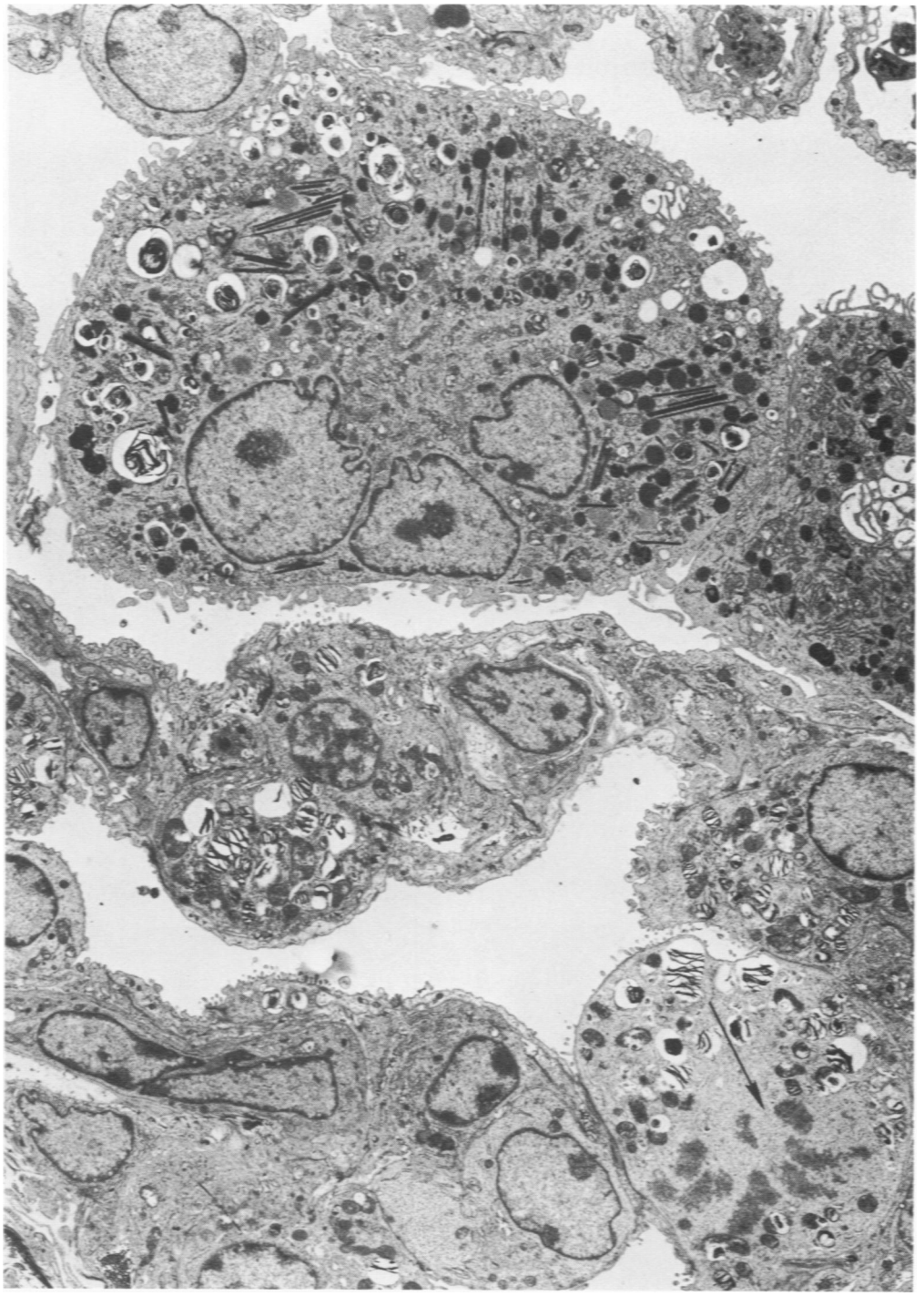


Figure 10—Alveolar space lined predominantely by granular pneumocytes, one of which appears to be in metaphase (*arrow*). One of the two alveolar macrophages in one of the alveolar lumina contains a large compliment of lysosomal bodies, as well as numerous longitudinal electron-dense rods of unknown nature. Six weeks of exposure. (× 3840).

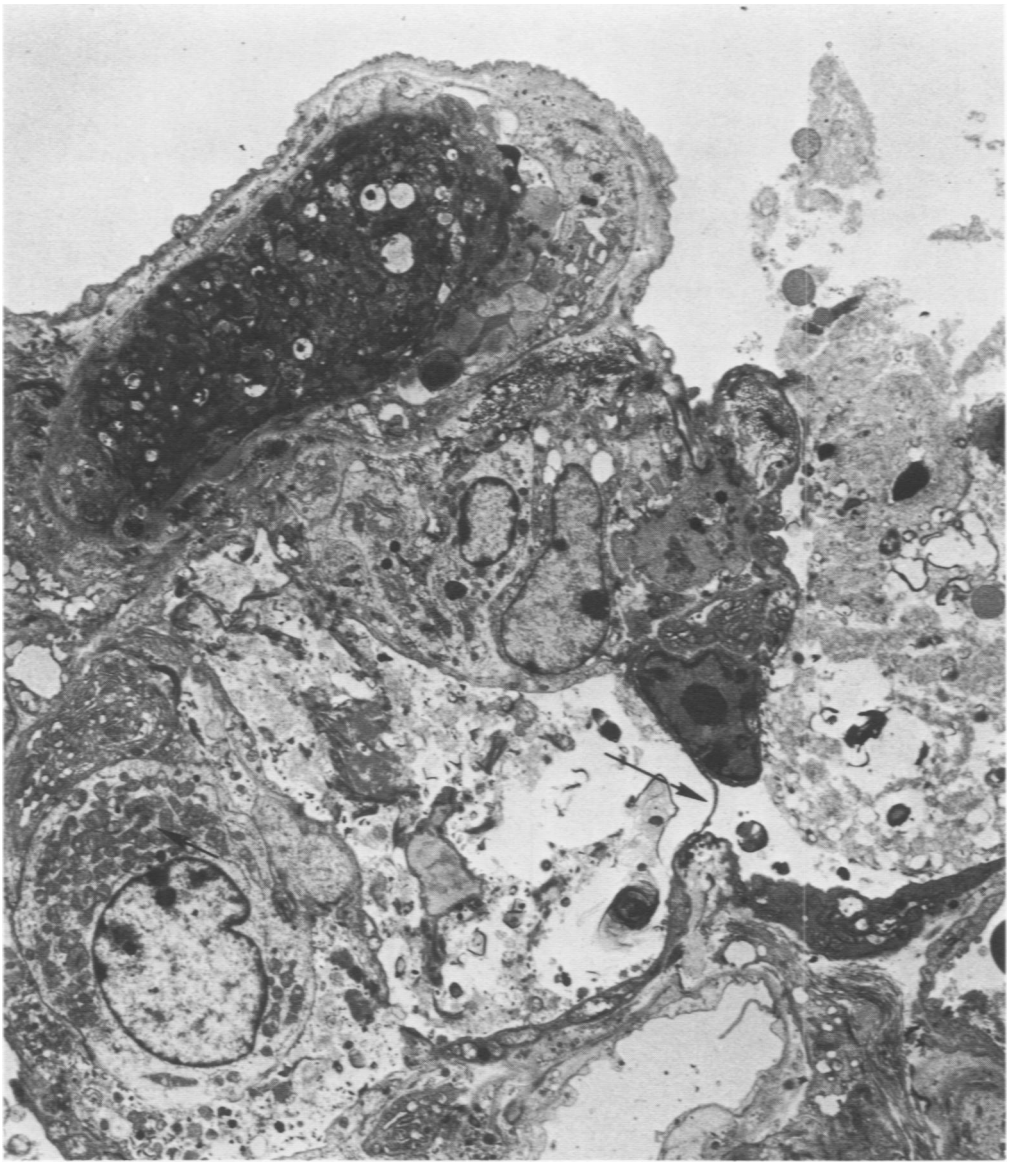


Figure 11—A thrombus is seen totally occluding the lumen of an alveolar capillary. A thin cytoplasmic strand (*arrow*), extending from one part of the alveolus to the other, seems to divide the alveolar space which contains an abundance of cellular debris and exudative material. Notice again the increased numbers of mitochondria in one of the cells abutting on the debris-filled alveolar lumen (*short arrow*). Six weeks of exposure. (× 4000).

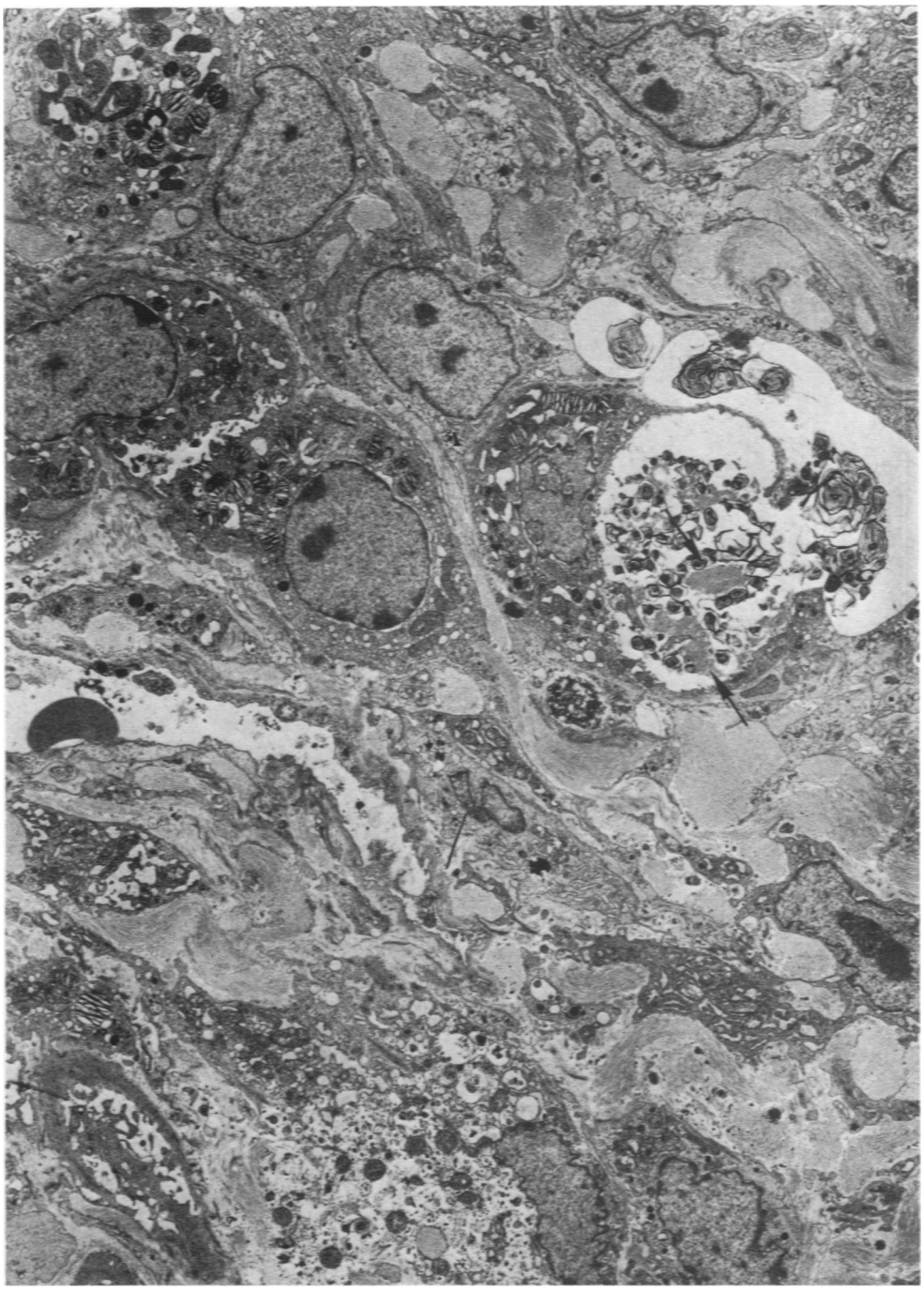


Figure 12—Total destruction of the alveolar architecture by a tremendous overgrowth of dense fibrous tissue. Occasional granular pneumocytes are identified randomly embedded within this collagenous stroma. An abundance of lamellar whorls intermingled with a finely fibrillar material displaying a distinct periodicity, and occasionally a latticework pattern (*arrows*) is noted within an alveolar space (*A*). Six weeks of exposure. (× 3840).

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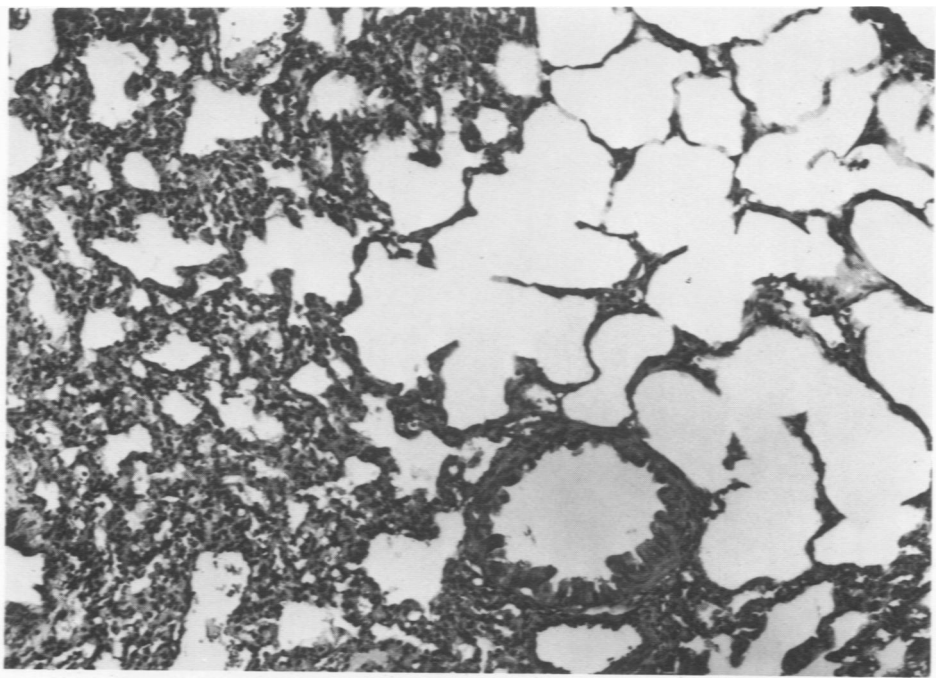

Figure 13—The lungs of a 6 weeks control (*left*) and of an experimental animal (*right*) 6 weeks after continuous oxygen exposure are shown in this picture. The lungs of the experimental animal are strikingly smaller and their pleural surfaces demonstrate scattered hemorrhagic spots (*long arrows*), as well as light areas probably representing foci of fibrosis (*short arrow*). Compare, also, the size of the air-filled spaces of the experimental animal with that of the control (*arrowheads*). **Figure 14**—This light micrograph of the lungs of a 4-week experimental animal shows a well-circumscribed emphysematous area adjacent to alveoli demonstrating considerable variation in size and shape, as well as thickened walls (H & E. × 300).