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Pathological changes of the lungs after prolonged inhalation of high concentrations of oxygen

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Summary. The pathological changes in the lungs of six patients who were treated by an artificial ventilation with a Bird or Bennett type respirator for three days to six months with oxygen concentrations of 24–100% were examined by light microscopy, studies on thick-sections, reconstruction models and vascular casts, and morphometric methods. After prolonged inhalation of high concentrations of oxygen the lungs showed thickening of the alveolar wall, marked deposition of reticulin fibers and fibroblastic proliferation in the alveolar wall, reduction in the number of capillaries, an abnormal configuration of the capillary network and hyperplasia of alveolar lining cells. These lesions are not specific to this condition, and seemed to be less marked than similar lesions in cases of chronic forms of fibrosing alveolitis, chronic interstitial pneumonia, usual interstitial pneumonia and so-called pulmonary fibrosis. Morphometric results confirm these histological observations and show not only the concentrations of oxygen but also the duration of high and pure oxygen inhalation have important roles for these pulmonary lesions. The main reasons for these lesions seemed to be repeated damage to the capillaries and loss of the normal configuration of the capillary network, accompanied by rearrangement and reconstruction of the two types of reticulin fibers.

Key words: Pulmonary fibrosis – Oxygen toxicity – Respirator – Reticulin fiber

Introduction

Pulmonary lesions attributable to high concentrations of inspired oxygen were first noted by Pratt in 1958. Subsequent reports and investigations showed that these pathological changes occurred frequently (Barter et al.

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1968; Kapanci et al. 1972; Pratt 1974; Sevetti 1974; Cederberg et al. 1975). Most previous studies have been concentrated on the appearance of hyaline membranes in acute oxygen toxicity and the only reports on pathological changes of the lung after prolonged inhalation of high concentrations of oxygen are those of Nash et al. (1967), Barber et al. (1970) and Singer et al. (1970). We examined pulmonary changes in patients after prolonged inhalation of high concentrations of oxygen and prolonged artificial ventilation. This paper describes the architectural changes of the alveolus of these patients and discusses the characteristic lesions in this condition.

Materials and methods

Samples of the lungs were obtained postmortem from six patients who had been exposed to high concentrations of oxygen of 24–100% at a total pressure of 760 mmHg from a Bird or Bennet respirator. The periods of exposure varied from three days to six months. None of the patients had any previous pulmonary disease. Pertinent clinical data are summarized in Table 1. All patients had severe brain damage and required artificial ventilation until death. They did not receive oncostatics or drugs that are known to cause lung injury.

The lungs were fixed by trans-bronchial injection of 10% formalin at a pressure of 25 cmH_2O. When the outer surface of the visceral pleura became smooth each hilus was ligated and the lungs were kept in a fixative box for about a week. Gross examination was done by slicing the lungs at 1 cm thickness. At least two samples from each lobe were examined by light microscopy. The sections were stained with haematoxylin and eosin, Masson's trichrome, elastic/van Gieson, PAS, a modification of Gomori's method for silver impregnation, and a modification of Jone's method for basement membrane. In addition to ordinary microscopy, various methods were used for investigating architectural changes of the alveolar sac and septum:

1. Tissue which was not too dense was examined in sections of 30–200 μ thickness stained with Azan-Mallory, aldehyde fuchsin, PAS and silver impregnation and mounted in Bioleit resin. The special configuration of the terminal bronchioles, the alveolar duct, the alveolar sac and the stromal framework of the alveolar septum was examined by focusing up and

Table 1. Clinical data on the patients

Case No.	Age (y)	Sex	Concentration of oxygen (%)	Exposure period		Type of the respirator	Principle disease
				High oxygen (>40%)	Pure oxygen (100%)		
1	7	m	40	3 days	0	Bird	Cerebellar medulloblastoma
2	32	f	40	3 days	0	Bird	Falx meningioma
3	27	f	40–100	42 days	24 days	Bird	Multiple angioma of the brain
4	65	m	24–100	58 days	24 days	Bennett	Cerebral astrocytoma
5	52	f	40–100	137 days	68 days	Bird	Pick's disease
6	38	m	40–100	182 days	122 days	Bird	Cerebral astrocytoma

down in the block. The configuration of the capillary network was examined by staining the same blocks by the benzidine reaction, but these preparations cold not be photographed clearly because they were too thick. More than 50 sketches of collagen, elastic and reticulin fibers in the serial sections were drawn from projections with a profile projector model V-16A (Nikon, Tokyo).

2. Three dimensional reconstruction models of the regional alveolar sac and septum were made in each case. Photographs at a magnification of $\times 200$ were taken of more than 100 serial sections of 4 μ thickness stained both by a modification of Jone's method and Masson's trichrome method and then projected onto transparent acryl plates of 2 mm thickness at a magnification of $\times 5$. These images were then cut out, small acryl pieces of 2 mm thickness were inserted between them, and then they were piled up. In this way a $\times 1{,}000$ magnified reconstruction model was made.

3. For more precise observation of the three-dimensional structure of the capillary network, in Cases 2 and 5 and control lungs silicone rubber (Microfil MV-112, Canton Bio-Medical Products, IN., USA) was injected into the pulmonary artery in S-8 of the right lower lobe after perfusion with heparinized saline. The preparations were then vulcanized at room temperature, dehydrated by passage through a graded alcohol series and cleared in methyl salicylate. Stereoscopy and a profile projector were used for observation and photography.

4. Morphometric analyses were made by Weibel's point counting method (1966). In each case, ten sections, two per lobe, were examined. The sections, stained both by a modification of Jone's method and Masson's trichrome method, were projected onto the screen of a profile projector and measured using a standard multipurpose test system with 42 points and 21 lines. The initial magnification was $\times 500$. In each lobe, 50 randomly selected fields were analysed, and over 200 alveolar septa and over 400 capillary lumina were measured. The relative volume fraction of the pulmonary parenchyma, the thickness of the alveolar septum, the surface to volume ratio of the alveolar septum, and the mean diameter and the mean cross-sectional area of the alveolar capillary lumen were recorded. As control lungs, five (mean age 50 ± 4 years) were chosen from a number which showed minimal changes macroscopically and microscopically. The chi-square test was used to evaluate correlations between data, Student's two-tailed test was used to assess significance of differences, and Welchi's test was used to compare data in the exposed and control groups. A p value of less than 0.05 was accepted as the limit of significance.

Results

Macroscopical changes of the lungs were diffuse and fairly uniform. Oedema and congestion were the main changes in Cases 1 and 2. Many mucous plugs were seen in small bronchial lumina in Cases 1, 2 and 3. The bronchial mucosa appeared hyperaemic in all cases, and the wall showed slight infiltration of small lymphocytes and neutrophils. However, there were no macroscopical bronchopneumonic foci. The weights of the lungs (left/right) were 250/370 g (Case 1), 240/310 g (Case 2), 440/660 g (Case 3), 580/540 g (Case 4), 510/690 g (Case 5) and 590/550 (Case 6). Slices of the lung showed marked congestion, and the tissue was elastic, non-crepitant and hardened in Cases 4, 5 and 6. The common microscopical findings in Cases 1 and 2 were marked congestion of alveolar capillaries, oedema of the alveolar septum, sparse macrophages in the alveolar space, scattered hyaline membranes along the alveolar duct and scattered fibrin thrombi in the capillaries. In Cases 4 and 6, some peribronchial alveoli contained haemosiderin-laden macrophages. In Case 3 marked desquamation of alveolar lining cells was observed (Figs. 1 and 2), which seemed to be partly due to a postmortem change. The main changes in Cases 3–6 were thickening of the alveolar

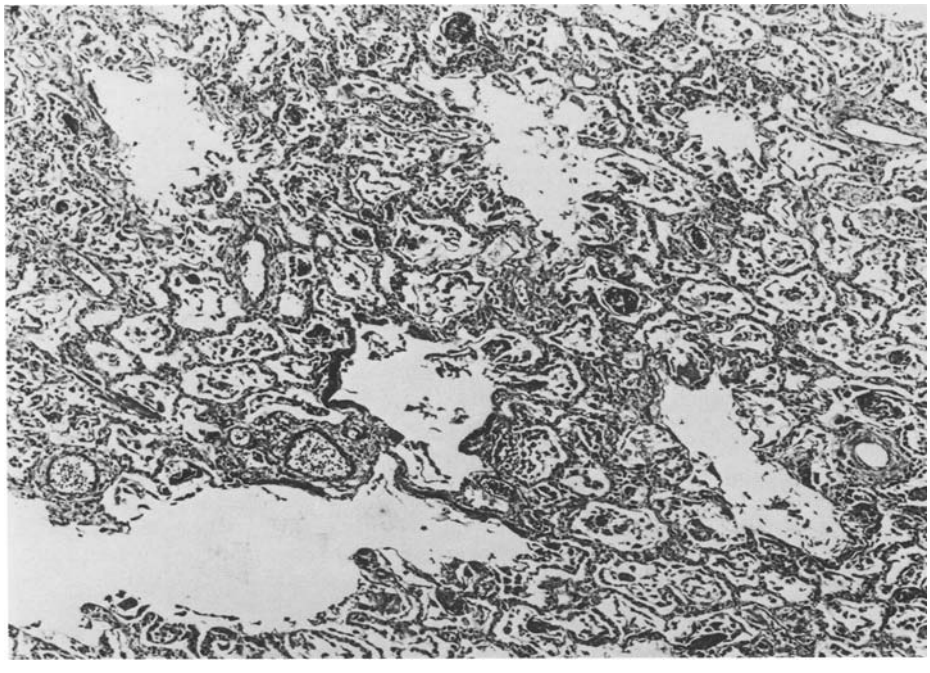


Fig. 1. Apperance of alveoli at low magnification showing consolidation with thickened alveolar septa. Case 3. Haematoxylin and eosin. ×20

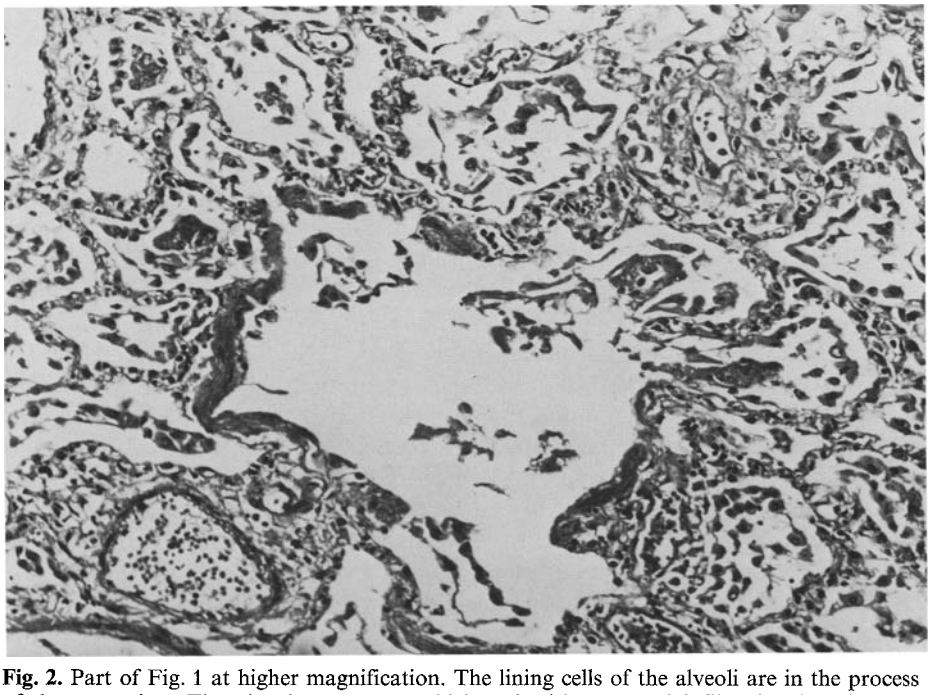


Fig. 2. Part of Fig. 1 at higher magnification. The lining cells of the alveoli are in the process of desquamation. The alveolar septa are thickened with scattered infiltrating lymphocytes and plasma cells. Case 3. HE. ×50

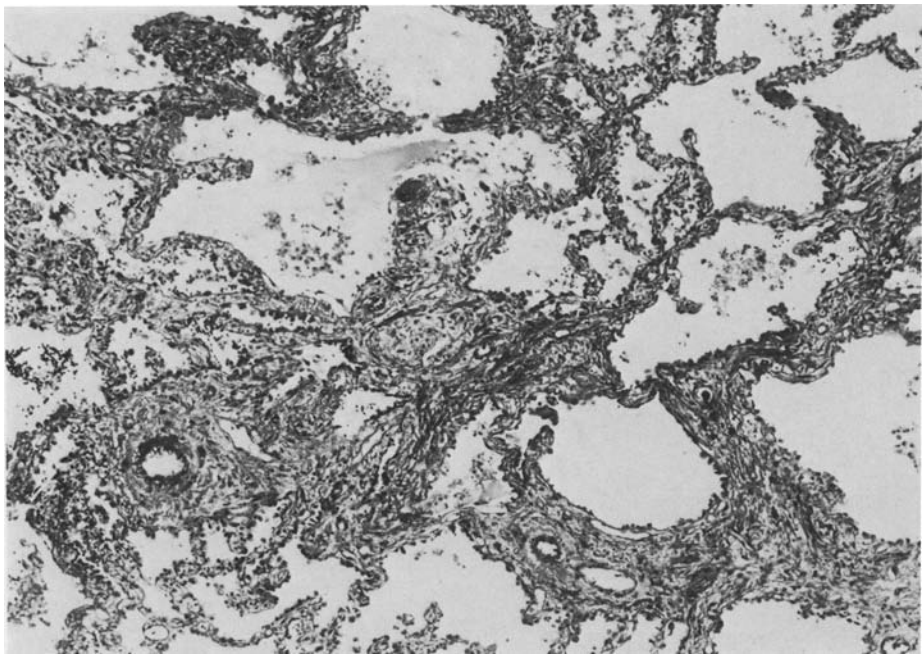


Fig. 3. Extensive interstitial thickening of the alveolar septa and interlobular connective tissue. Case 6. Azan-Mallory stain. ×30

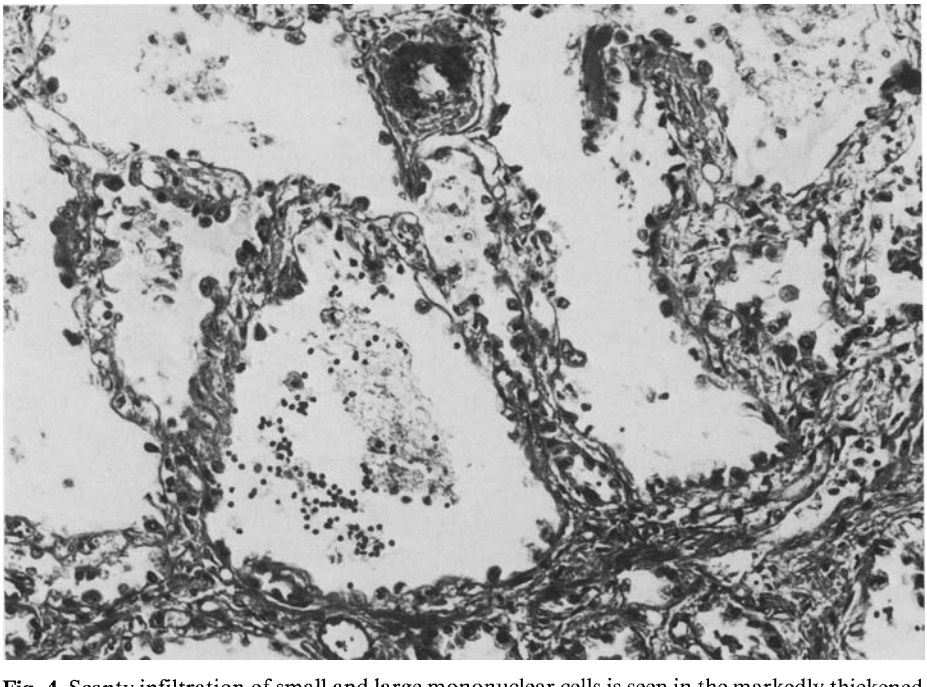


Fig. 4. Scanty infiltration of small and large mononuclear cells is seen in the markedly thickened alveolar septa. Case 6. HE. ×80

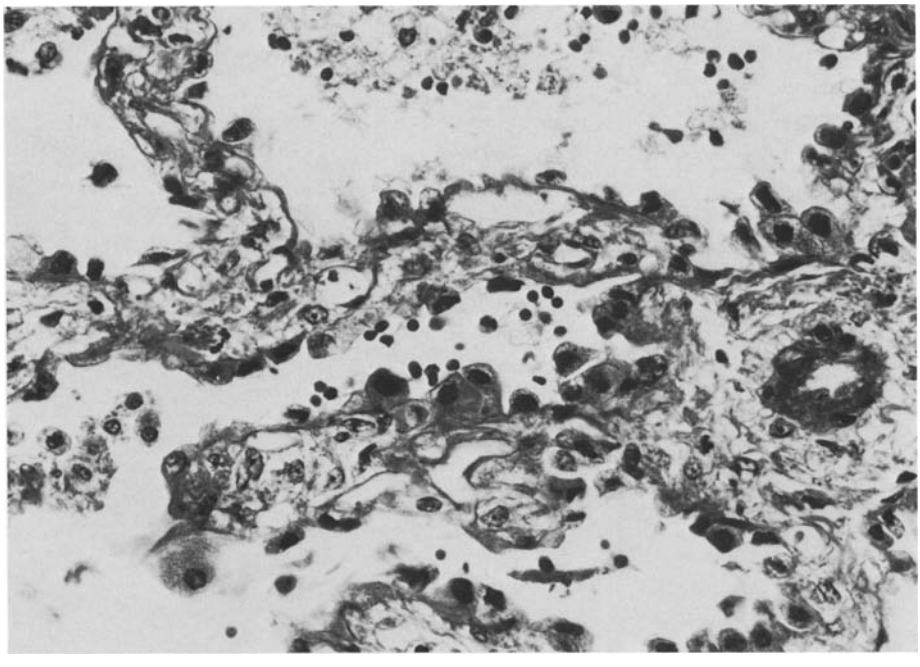


Fig. 5. Part of Fig. 4 at higher magnification. Striking hyperplasia of the alveolar lining cells is seen. Newly proliferated epithelial cells with basophilic cytoplasm and hyperchromatic nuclei of variable size are noted. HE. ×120

septum, marked deposition of reticulin fibers and fibroblastic proliferation in the alveolar septum, reduction in number of capillaries, and hyperplasia of the alveolar lining cells, which sometimes showed cuboidal metaplasia (Figs. 3–5). A few foci of proliferation of smooth muscle cells were seen in the interstitium of the lungs in Case 5. Slight thickening of some small pulmonary arteries was observed in Cases 5 and 6. No lymphoid nodules, granulomas or arteritis were seen in any case. A striking finding was that neither collagenization nor significant change of the elastic fibers was observed in the alveolar septa, but deposition of reticulin fibers, namely, fibrillosis had occurred in Cases 3–6. Although mild dilatation of the alveolar duct was observed in Cases 5 and 6, no case showed a honey-comb appearance or bronchioloectasis with shrinkage of the regional alveoli.

The reconstruction models of Cases 1 and 6 are shown in Figs. 6a and b. Marked filling of the capillaries and similar thickness of the alveolar wall, even of the alveolar septum and sac, were observed in Cases 1 and 2. Rarefaction of the capillary network, irregular capillary figures, marked and irregular thickening of the alveolar wall, especially in the side of the alveolar sac, and irregular distances between the capillaries and the alveolar surface were observed in Case 6. Similar changes, though less marked, were seen in Cases 3, 4 and 5.

Morphometric results are shown in Table 2. The relative volume fraction of pulmonary parenchyma, the mean thickness of alveolar septum ($P<$

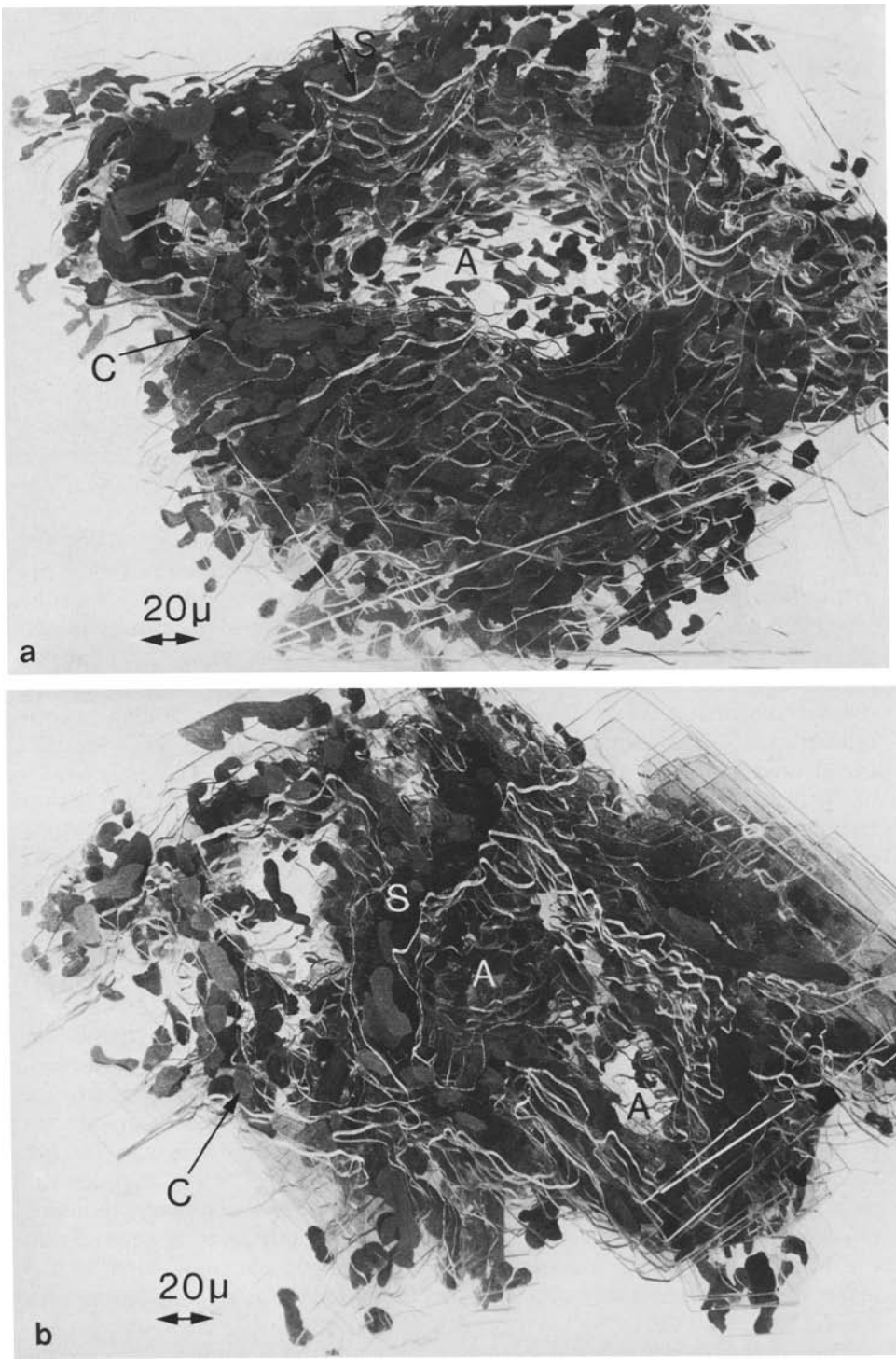


Fig. 6a, b. Reconstruction models of alveolar sacs of Case 1 **a** and Case 6 **b**. In **a**, the basic pattern of the alveolar septum and sac is the same as in the control, but the caliber of capillaries is uniformly larger than that of the control. The alveolar septum is slightly larger than that of the control and of similar thickness everywhere. In **b**, rarefaction of the capillary network, irregular thickening of the alveolar septum, and irregular distances between the capillaries and the alveolar surface are observed. Alveolar space (*A*), alveolar septum (*S*) and capillaries (*C*). Original magnification of reconstruction models is $\times 1{,}000$

Table 2. Morphometric data

Case No.	Relative volume fraction of pulmonary parenchyma (%)	Mean thickness of alveolar septum (μ)	Ratio of surface to volume of alveolar septum (μ^2/μ^3)	Mean diameter of alveolar capillaries (μ)	Mean cross-sectional area of alveolar capillaries (μ^2)
Controls	1.86	6.83±1.98	0.3155	5.50±1.75	97.3±22.5
1	2.37	7.82±2.31	0.1938	6.36±1.81	129.6±38.0
2	2.35	7.63±2.24	0.2000	6.16±1.61	120.8±34.4
3	4.76	17.10±5.67	0.2257	6.35±2.24	122.8±44.6
4	6.03	24.85±9.88	0.1406	6.29±2.76	115.4±40.3
5	5.24	28.64±12.7	0.0808	6.96±3.82	129.7±46.1
6	8.94	33.07±13.2	0.0686	6.77±3.78	122.9±44.0

0.0001), the mean diameter of alveolar capillaries ($P<0.025$, except for Case 4), and the mean cross-sectional area of alveolar capillaries ($P<0.01$) in the four cases with longer exposure were larger than those of controls. However, the ratios of surface to volume of the alveolar septum in the six cases were smaller than those of controls. To examine the relations of the duration of high or pure oxygen inhalation to various variables, there were statistically significant correlations of the duration of high oxygen inhalation to the mean thickness of alveolar septum ($r=0.9042$, $P<0.02$), the mean diameter of alveolar capillaries ($r=0.7886$, $P<0.04$), and the ratio of surface to volume ($r=-0.8527$, $P<0.05$). There were also significant correlations of the duration of pure oxygen inhalation to the relative volume fraction of pulmonary parenchyma ($r=0.9068$, $P<0.02$), the mean thickness of alveolar septum ($r=0.8999$, $P<0.02$), and the ratio of surface to volume ($r=-0.7183$, $P<0.04$). There were no statistically significant correlations between the duration of high oxygen inhalation and the mean cross-sectional area of alveolar capillaries ($r=0.3801$, $P>0.3$), nor between the duration of pure oxygen inhalation and the mean diameter ($r=0.7307$, $P>0.05$), or the mean cross-sectional area ($r=0.3376$, $P>0.5$) of alveolar capillary. And so, when the duration of high and pure oxygen inhalation is longer, the mean thickness of alveolar septum increases and the ratio of surface to volume decreases. In addition, there were statistically significant correlations between the relative volume fraction of pulmonary parenchyma and the mean thickness of alveolar septum ($r=-0.9457$, $P<0.01$), between the ratio of surface to volume and the mean diameter of alveolar capillaries ($r=-0.9219$, $P<0.01$), between the mean thickness of alveolar septum and the ratio of surface to volume ($r=-0.8587$, $P<0.05$), and between the mean diameter and the mean cross-sectional area of alveolar capillaries ($r=0.8558$, $P<0.05$).

The capillary network was examined by making vascular casts in controls, Case 2 (Fig. 7a) and Case 5 (Fig. 7b). In the controls and Case 2, the capillary vessel formed closely set and complex systems of rectangular,

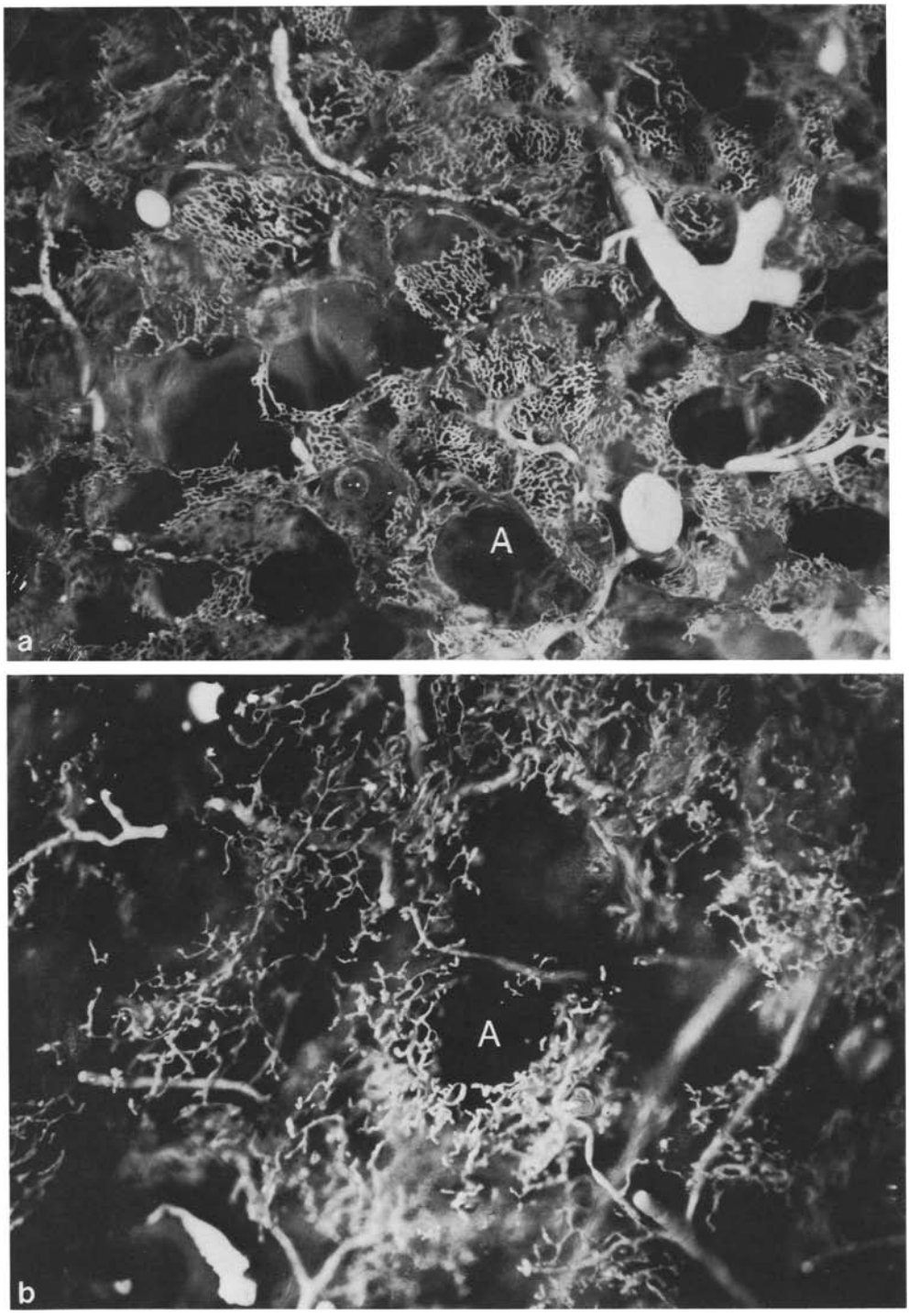
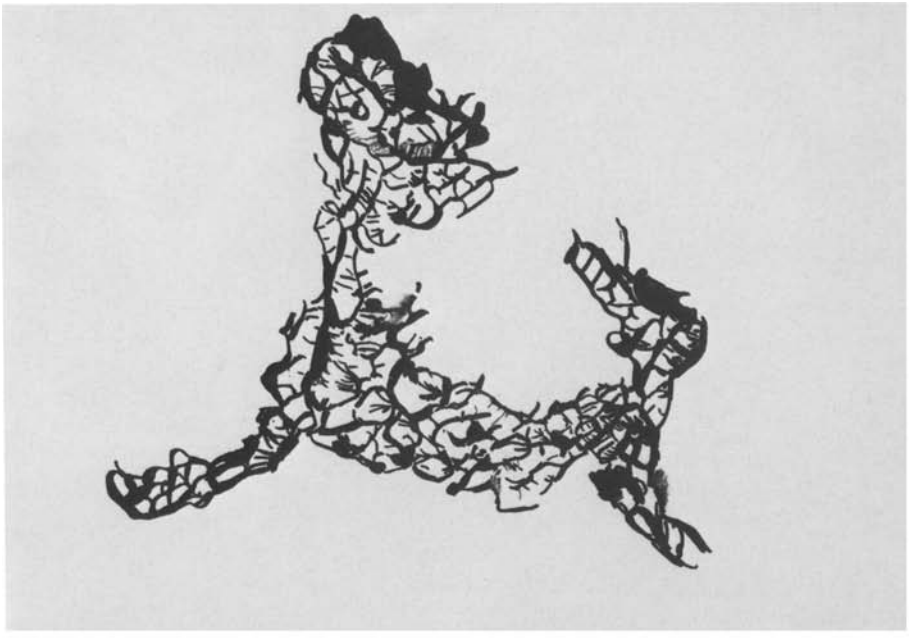


Fig. 7a, b. Vascular casts of Case 2 **a** and Case 5 **b**. In **a**, the capillary network is formed by a complex of regular rectangular, pentagonal and hexagonal ringlets, and crossing of independent capillaries can be seen. **b**, rarefaction of the capillary network, irregular shaped capillaries showing sinusoidal dilatation and strand-like obstruction, and the absence of crossing of independent capillaries are observed. Alveolar duct (*A*). × 50



a

b

Fig. 8a–c. Sketches of the reticulin fiber framework of the alveolar septum from thick sections, stained by a modification of Gomori's method for silver impregnation, of a normal control **a**, Case 1 **b** and Case 6 **c**. In **a**, thicker axial fiber bundles and finer perpendicular fibers can be distinguished. In **b**, the axial fiber bundles are slightly thickened, but their basic configuration is the same as that of **a**. In **c**, the axial fiber bundles tend to be irregular in contour, fragmented and condensed irregularly, and the perpendicular fibers tend to be reduced in number and coarser. × 1,000

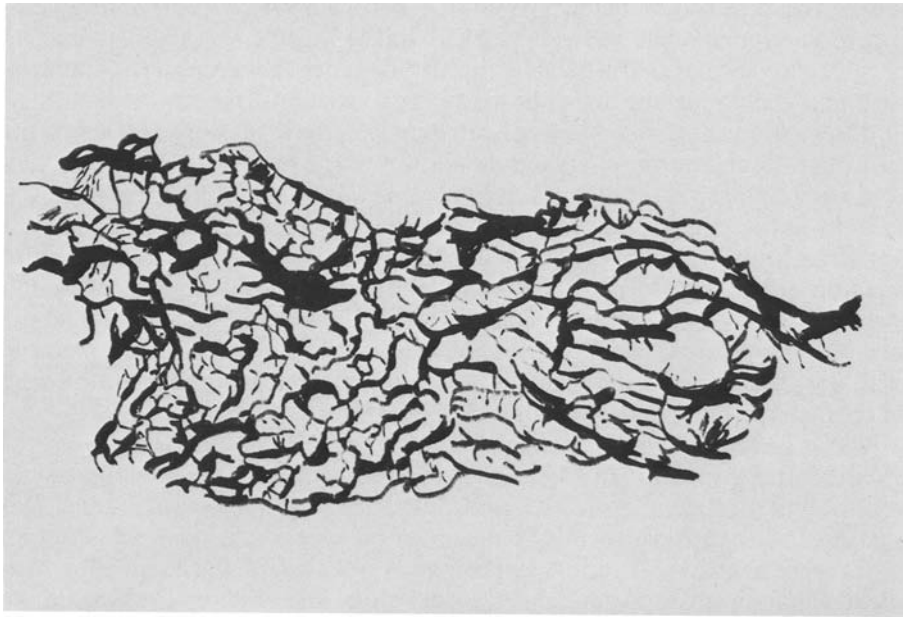


Fig. 8c

pentagonal and hexagonal ringlets, and crossing of independent capillaries, which must have been on opposite sides of central sheets, could often be seen clearly. In Case 5 the harmonic configuration was completely lost: vessels of irregular thickness varying in profile from sinusoids to strands were irregularly distributed, and scarsely any crossing of independent capillaries could be observed. Small bud-like sinusoids were also seen, but these could be artifacts due to insufficient filling of the vascular cast.

Two types of reticulin fibers were distinguishable in the normal control (Fig. 8a). Axial fiber bundles were of thicker reticulin which ran along the capillaries and crossed them, while perpendicular fibers were finer with a circular or hemicircular arrangement around capillary walls. In Cases 1 and 2, slightly thickened axial fiber bundles were observed (Fig. 8b). In Cases 3–6, the axial fiber bundles tended to be irregular in contour, very variable in thickness, and fragmented and condensed irregularly. The perpendicular fibers tended to be fewer in number and coarser (Fig. 8c). After prolonged inhalation of oxygen, the fibrillosis was characterized by rearrangement and reconstruction of the two types of reticulin fibers.

Discussion

In this study, using a variety of methods, we examined the histological changes in the lungs of patients who had received high concentrations of oxygen during artificial ventilation over long periods. The histological changes consisted of thickening of the alveolar wall, marked deposition of reticulin fibers and fibrocytic proliferation in the alveolar septum, reduc-

tion in the number of capillaries and hyperplasia of alveolar lining cells. Reconstruction models showed marked thickening of the alveolar wall in the alveolar sac, and irregularity in the distance between the capillaries and the alveolar surface. Morphometric analyses confirmed these histological observations and also showed statistically significant correlations of the duration of high and pure oxygen inhalation to the mean thickness of alveolar septum and the ratio of surface to volume of the alveolar septum. Vascular casts showed complete loss of the harmonic configuration of alveolar capillaries after prolonged inhalation of oxygen. Rearrangement and reconstruction of the two types of reticulin fibers were also detected by examination of thick tissue sections. From these findings it is concluded that the harmonic configuration of the alveolar lining cells – stromal framework – capillary network in the lungs is lost after prolonged inhalation of high concentrations of oxygen by artificial ventilation.

There have been no previous reports of details of pulmonary lesions in cases after exposure to high oxygen concentrations for as long as six months, but Barber et al. (1970) and Singer et al. (1970) reported changes in cases after exposure to high concentrations for seven days. Nash et al. (1967) were the first to report pathological changes in the lung after prolonged inhalation of oxygen, and they classified the changes into exudative and proliferative stages. They reported that earlier exudative phase was characterized by congestion, alveolar oedema, intra-alveolar haemorrhage and hyaline membranes, and that the later proliferative phase was characterized by marked alveolar and interlobular septal oedema and fibroblastic proliferation, with early fibrosis and prominent hyperplasia of the alveolar lining cell. In our study, Case 1 and 2 could be classified as in the early exudative phase and Cases 3–6 as in the late proliferative phase.

No specific histological findings in the lung in this condition have been detected. We were unable to find any pathognomonic lesions in the present cases. However, apparently associated characteristics included the lesser degree of injury of the pulmonary parenchyma, since the lesions did not include a honey-comb appearance, bronchioloectasis with shrinkage of the regional alveoli, or bronchiolar adenomatosis, all of which are common changes in patients with chronic forms of diffuse fibrosing alveolitis, chronic interstitial pneumonia, usual interstitial pneumonia, or so-called pulmonary fibrosis (Scadding and Hinson 1967; Liebow 1975; Crystal et al. 1976; Spencer 1977; Dunnill 1982; Katzenstein and Askin 1982). With regard to reticulin fibers, Spencer mentioned that they were laid down in relation to intramural histiocytic cells, and that further maturation of the reticulin into collagen leads to a stage of chronic interstitial fibrosis of the lung. We could not find any relation between proliferation of reticulin fibers and histiocytes in our cases. Several points, such as proliferation of reticulin fibers instead of fibrosis, marked thickening of the alveolar wall without shrinkage of the alveolar space, and hyperplasia of the alveolar lining cells without bronchiolar adenomatosis, seem to be characteristic of prolonged inhalation of oxygen. Repeated damage of the capillary wall and loss of the harmonic configuration of the capillary network, accompanied by rearrangement and

reconstruction of the two types of reticulin fibers, seem to be most important causes of this condition.

The problem of whether artificial ventilation or a high concentration of oxygen was more closely related with these pulmonary lesions could not be determined from this work. Studies in experimental animals (Aikawa and Bruns 1956; Kistler et al. 1967; Katzenstein et al. 1976; Frank and Massaro 1980) have shown the direct toxic effect of prolonged inhalation of oxygen. In our experience, patients with amyotrophic lateral sclerosis who were treated by artificial ventilation with an adequate concentration of oxygen showed little fibrotic change of the lungs, except an emphysematous change. Thus the lesions seem to be more closely related to a high concentration of oxygen than to prolonged artificial ventilation. The exact concentration at which oxygen is toxic to human lungs is unknown, but in general inspired oxygen at a concentration of less than 40–60% seems to be safe (Gould et al. 1972; Frank and Massaro 1979; Deneke and Fanburg 1980). In addition to the concentrations, duration of inhalation of high concentration and pure oxygen seems also to be important for preventing pulmonary injury. There are few reports of pulmonary lesions in relation to different types of artificial ventilator. The patients in our study were all treated with pressure-limited ventilators of the Bird or Bennett type. This type of ventilator may be less injurious to the pulmonary parenchyma than volume-limited ventilators.

In general pulmonary alveolar damage must be frequent in patients receiving mechanical ventilation and high concentrations of oxygen, but these patients have usually suffered from previous lung injury or some disease that necessitates such therapy. In these patients it is therefore difficult to judge whether the pathological changes of the lung are the result of the oxygen inhalation and artificial ventilation or the progressive lesions of the previous lung injury. The patients in our study had no previous history of a lung disease but had severe brain damage that necessitated artificial ventilation. Thus the pathological changes could be regarded as mainly caused by prolonged inhalation of high concentrations of oxygen and/or prolonged artificial ventilation.

In managing of patients who require artificial ventilation and high concentrations of oxygen, considerable attention must be paid to prevent of such changes.

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