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Scanning Electron Microscopic Study of Capillary Change in Bleomycin-Induced Pulmonary Fibrosis

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The architectural changes which occur in the capillaries are difficult to illustrate without a three-dimensional tool, such as scanning electron microscopy. Therefore, a scanning electron microscopic study was occasionally undertaken to show the capillary changes of lung fibrosis. Fibrosis was induced in twenty rats by an intratracheal injection of bleomycin. After 30 days the rats were sacrificed, and light microscopy and scanning electron microscopy were performed. The vascular trees of both lungs were cast with methacrylate. Light microscopically, the pulmonary fibrosis was patchy and inflammatory cell infiltration was rather sparse. Scanning electron microscopically, the intercapillary spaces became wider; and some capillaries revealed large irregular dilatation. The pleural and alveolar capillaries were variably dilated. The pleural capillary diameter was increased (P = 0.06), and the capillary plexus diameter was decreased (P = 0.00). Distance between the capillary branches of the pleural surface was increased (P = 0.06). The appearance of irregularly shaped capillaries, an increase in diameter with variable dilatation of alveolar capillary rings and a decrease in branching between the capillaries, resulting in a loss of surface area are the main scanning electron microscopic findings of the remodeling which occurs pulmonary capillaries in bleomycin-induced pulmonary fibrosis.

Key Words : *Scanning electron microscopy, Bleomycin, Pulmonary fibrosis, Vascular casting*

INTRODUCTION

Pulmonary fibrosis is usually initiated by some form of acute lung injury and inflammation, and that can progress to fibrosis. The etiologic factors which cause pulmonary fibrosis are greatly variable and a considerable residue of cases must be classified as idiopathic (Thrall et al., 1979 ; Snider et al., 1978 ; Carrington, 1968). Several substances such as paraquat (Vijeyaratnam and Corrin, 1971), busulfan (Oliner et al., 1961), and bleomycin (Adamson and Bowden, 1974) have been shown consistently to induce pulmonary fibrosis in humans and animals. Bleomycin is an antineoplastic agent which is a well-known cause of pulmonary fibrosis in humans (Delena et al., 1972). In the bleomycin model of interstitial lung disease, an initial phase of injury and alveolitis eventually progress to a chronic inflammatory process characterized by fibrosis (Snider et al.,

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1978 ; Adamson and Bowden, 1974 ; Chandler et al., 1983). The pathologic changes produced by a single endotracheal dose of bleomycin in hamsters result in an animal model having many of the morphologic characteristics of interstitial lung fibrosis (Snider et al., 1977). Gracey et al. (1968) found a decrease in the number of capillaries within alveolar septa and asymmetric interposition of collagen and cells between the surface of the capillaries and alveolar lining cells in areas of fibrosis at autopsy in human lungs. Bignon et al. (1975) found that the volume of vessels less than 1 mm in diameter was increased and the number of branches was reduced, with diffuse narrowing of distal arteries in areas of fibrosis from a study of the biopsy specimens of 37 patients who underwent open lung biopsy for fibrosing alveolitis. Coalson (1982) found a decrease in the luminal size of vessels within remodeled alveolar walls. Turner-Warwick (1963)demonstrated the presence of many bronchial vessels communicating with branches of the pulmonary arteries in areas of abnormal lung, in patients dying with pulmonary fibrosis. These vessels were thin-walled, about 50 μ in diameter, and found most often beneath the pleura and at the level of the fourth generation bronchi.

We postulated that major architectural changes occurred in capillaries in pulmonary fibrosis and undertook this scanning electron microscopic study using vascular cast because the corrosion vascular casting is an important tool for the study of microvascular structure (Schraufnagel and Schmid, 1988 ; Kwon et al., 1990). We chose the bleomycin model, which has been employed in many studies, to produce the lung fibrosis (Thrall et al., 1979 ; Snider et al., 1978).

MATERIALS AND METHODS

Thirty, Sprague-Dawley rats weighing between 200 and 250 g were intra-peritoneally anesthetized with 0.16 mg of pentobarbital sodium (nembutol). Bleomycin (Blenoxane, Bristol), 1.5 units, in 0.3 ml of saline was injected endotracheally into twenty animals, and an equal volume of normal saline alone was injected into the rest. After 30 days, the animals were again anesthetized. The thorax and abdomen were opened, and heparin, 0.3 ml, was promptly infused into the left ventricle. The inferior vena cava was cannulated and perfused using normal saline through the vascular channels. The thoracic aorta was also cut to release the blood content. After complete removal of the blood components, buffered fixative solution (0.5% glutaraldehyde and 0.5% paraformalde-hyde) was perfused into the vascular channels to fix the lung tissue. The lungs were processed for scanning electron microscopy and light microscopy. The paraffin sections were stained with hematoxylin and eosin (H & E), trichrome and elastic-van 'Gieson's stains. For scanning electron microscopy using the vascular casting method in twenty animals, milliliters of methacrylate monomer (Mercox CL-2B, Dainippon Ink and Chemicals, Tokyo, Japan) mixed with a catalyst, 0.3 gm, was then slowly injected into the inferior vena cava. Polymerization of the casts progressed. The casting lung tissues were removed from the animal and placed in room temperature for 2 hours to allow completion of the polymerization. The polymerized specimens were placed in concentrated NaOH solution until all tissue was digested.

The vascular casts were rinsed in normal saline, inspected with a stereoscopic microscope, and serially sectioned. Sections, about 3 mm thick, were fastened to the aluminum stubs with silver conducting cement, sputter-coated with a layer of platinum palladium (Pt-Pd) about 20 nm thick, and viewed with a Hitachi S-520 scanning electron microscope. The objective aperture was 3 ; the accelerating voltage was 15 KV ; and the working distance was 15 mm.

Measurments and photographs for the capillary diameter and distance between the capillaries were taken at X 1000 magnification (Goldstein et al., 1981). The areas on the specimen to be studied were selected at random in forty casting specimens, but photographs were taken only if the regions were adequately preserved. The diameters of the center of capillary rings were recorded.

RESULTS

Five lungs appeared on gross examination to have diffuse mottling and two lungs appeared to show abscess formation. All of these were in the bleomycin group. The light microscopy showed fibrosis and chronic inflammation in the bleomycin group, although the changes were patchy, and varied in extent. The fibrosis and chronic inflammation occured mainly around the bronchi, bronchioles and near the pleura (Fig. 1,2). A few lungs had extensive diffuse damage, but honeycombing was not found.

On scanning electron microscopy in normal lungs the pleural and alveolar capillaries were easily distinguished in low magnification. The casts of capillaries revealed uniform size, without tapering or change in diameter with branching. Branching usually occurs at about 90 degrees in T, Y or other shapes. The pleural capillaries were round or oval and loosely arranged in two-dimensional sheets. The alveolar capillaries originating from pulmonary arteries often flattened as they approached the alveoli and were molded to the alveolar spaces, forming basket-like structures (Fig. 3). The peribronchial and periarterial capillaries had a sparser pattern similar to the pleural capillaries, but were arranged in a single layer around the larger structures.

One capillary often served more than one alveolus. The alveolar capillary bed arborized more than the pleural. Casts frequently ended blindly in the subpleural and pleural areas. Occasionally small capillaries, less than 5 μ in diameter, were observed in the parenchymas of the normal and bleomycin groups.

The bronchial arteries usually traveled parallel to the bronchial tree and formed a coarse capillary plexus extending as far as the terminal bronchioles. Connections between alveolar capillaries and capillary plexuses were frequently observed (Fig. 4). In the bleomycin-induced rats with pulmonary fibrosis some distinctive features occurred in the alveolar capillaries, but not readily distinguished from the normal, especially at low magnification (Fig. 5). However, the intercapillary spaces appeared greater and the capillary rings were less regular and variably dilated (Fig. 6). Irregular dilatations of the capillaries occurred and occasionally giant capillaries, up to 20 μ in diameter, were noted (Fig. 7). The casting surfaces showed irregular elevation or depressed grooves at high magnification. Some casting globules are observed within the alveolar spaces (Fig. 8).

The capillaries were regularly distributed in both the pleural and alveolar bed of the sa-

Table 1. Diameters of capillaries, alveolar capillary rings and capillary plexuses between saline and bleomycin groups (μ)*

	Saline (n=50)**	Bleomycin (n=50)	P
Alveolar cap.	5.8−12.0	4.5−17.7	0.13
	(7.9±1.4)	(8.9±2.5)	
Alveolar cap. rings	21.0−45.5	10.0−85.2)	0.45
	(32.0±6.1)	(33.5±12.9)	
Pleural cap.	4.0−117	4.2−10.2	0.06
	(7.1±1.4)	(7.6±1.5)	
Capillary plexus	4.4±9.0	2.1−8.0	0.00
	(6.6±1.1)	(5.6±1.4)	

* The mean value and standard deviation are in parentheses.

** The number of random selections in vascular casting specimens.

line group and in the pleural, but not the alveolar, capillary bed of the bleomycin group. In both the pleural and alveolar surfaces, the diameters of the capillaries of the bleomycin group were slightly larger than in the saline group. The pleural capillaries revealed irregularity in diameter and an indistinct two dimensional pattern (Fig. 9). Frequently bronchial vessels communicated with branches of pulmonary arteries in areas of subpleural and peribronchial fibrosis. The capillary plexus revealed more irregularity but the mean diameters were smaller than in the saline group ($P = 0.00$) (Fig. 10 and Table 1). The diameters of the center of the alveolar capillary rings were more variably dilated in the bleomycin group but the mean diameters were not significantly different between the bleomycin and saline groups (Table 1). Distances between the capillary branches in the pleural surface were increased in the bleomycin group ($P = 0.03$), but in the alveolar surface the distances were not definitely changed in either the bleomycin or saline groups ($P = 0.38$) (Table 2).

Legends For Figures

Key for abbreviations

AC : Alveolar capillary
BA: Bronchial arteriole
CG: Cast globule
CP: Capillary plexus
PA : Pulmonary artery
PC : Pleural capillary
PV : Pulmonary vein

Fig. 1. A : Inflammatory cell infiltration and fibrosis are patchy, around the dilated bronchioles. B : The alveoli are clear and irregularly distended bronchioles contain some inflammatory cells.

Fig. 2. A : Subpleural fibrosis with patchy infiltration of inflammatory cells are seen. B : The inflammatory cells are composed mostly of lymphocytes, plasma cells and some neutrophilic leukocytes.

Fig. 3. Alveolar capillaries from saline treated rats appear basket-like structure. Bar = 50 μ (X 410).

Fig. 4. Vascular connections between bronchial and pulmonary capillaries (arrow heads) are seen. Small capillary (*) less than 5 μ in diameter is noted. Bar = 24 μ (X 830).

Fig. 5. Alveolar capillaries from bleomycin treated rats appear almost normal at low magnification. Bar = 120 μ (X 170).

Fig. 6. At high magnification, alveolar capillary rings are less regular and the central holes are larger than normal animals. Bar = 110 μ (X 550).

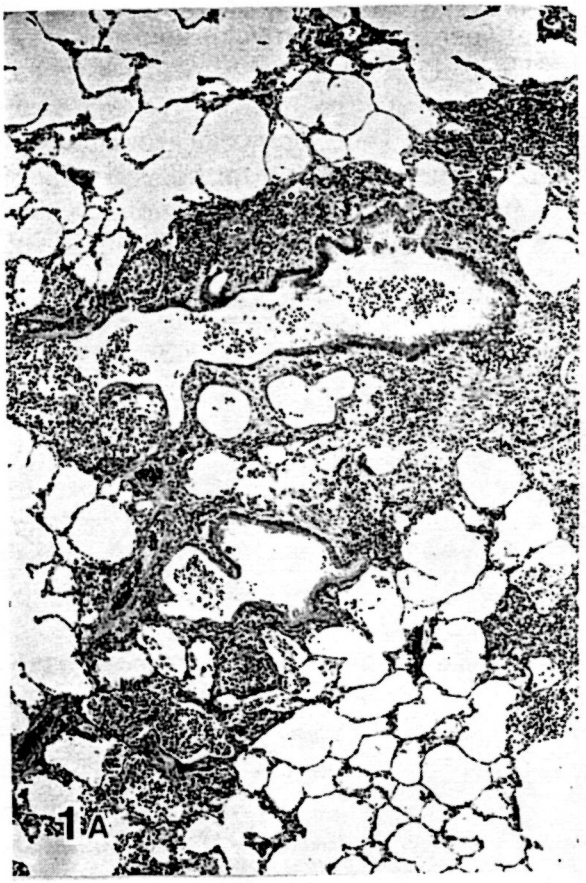
Fig. 7. Alveolar capillary casts of bleomycin treated rats with very large dilated capillaries (*). Bar = 14 μ (X 1400).

Fig. 8. Occasionally some casting globules are observed within the alveolar spaces. Arrow head : leak point of cast material. Bar = 60 μ (X 300).

Fig. 9. The pleural capillaries reveal irregular dilatation and indistinct two dimensional pattern. Bar = 24 μ (X 830).

Fig. 10. The capillary plexus in bleomycin group shows more irregular diameter and arrangement than in saline group. Bar=50 μ (X 410).

Inset : Saline group. Regular arrangement of capillary plexus (arrow heads) is noted. Bar = 65 μ (X 150).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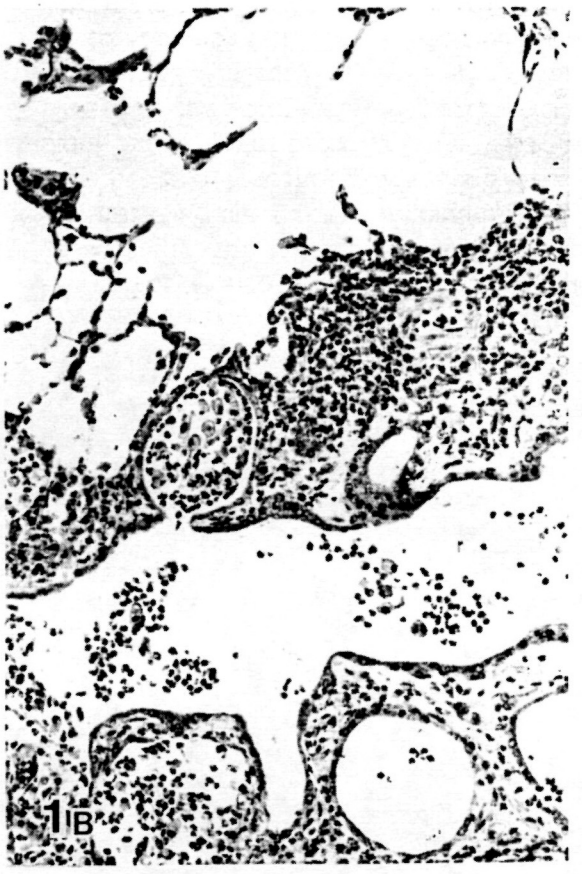
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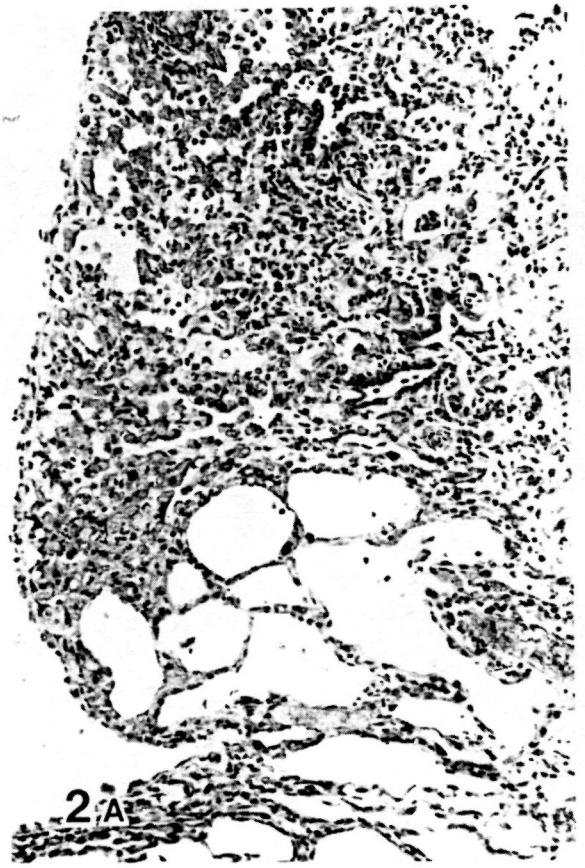
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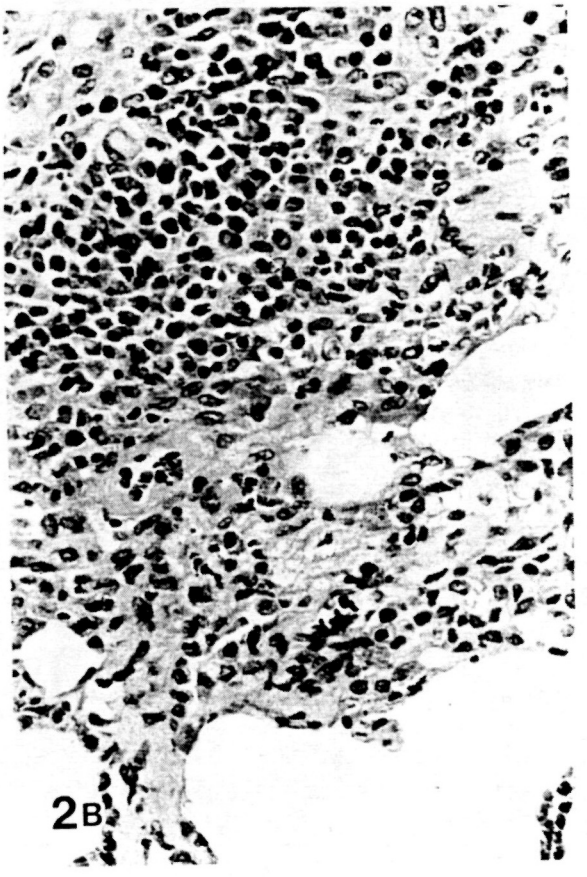
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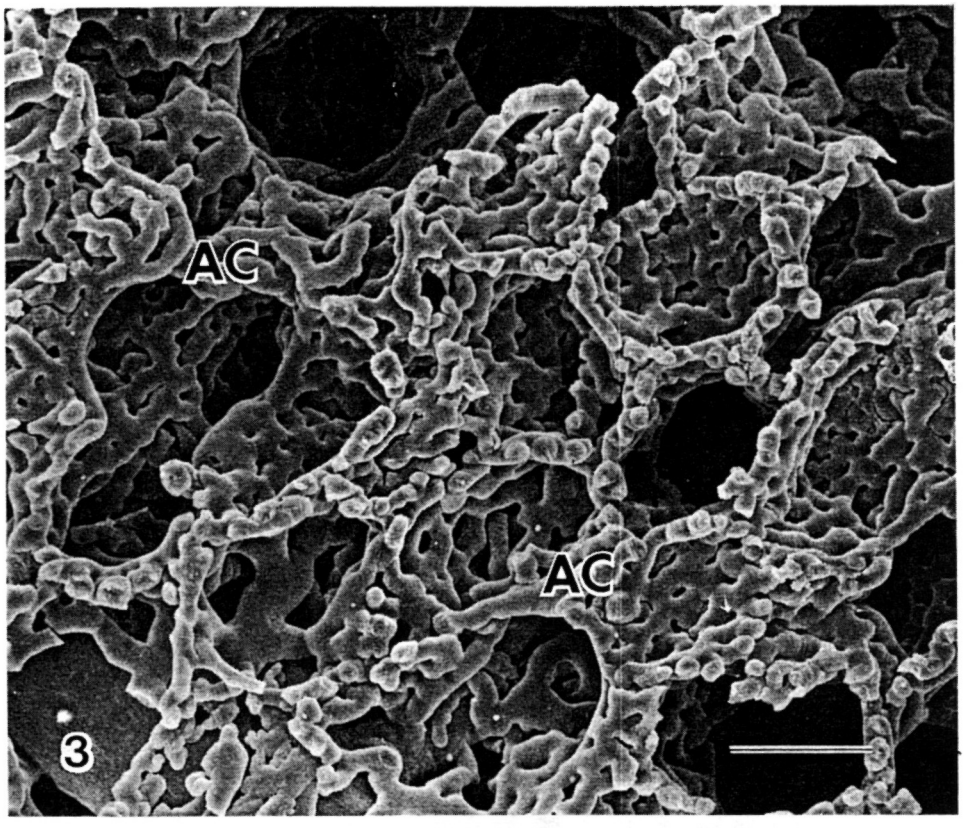
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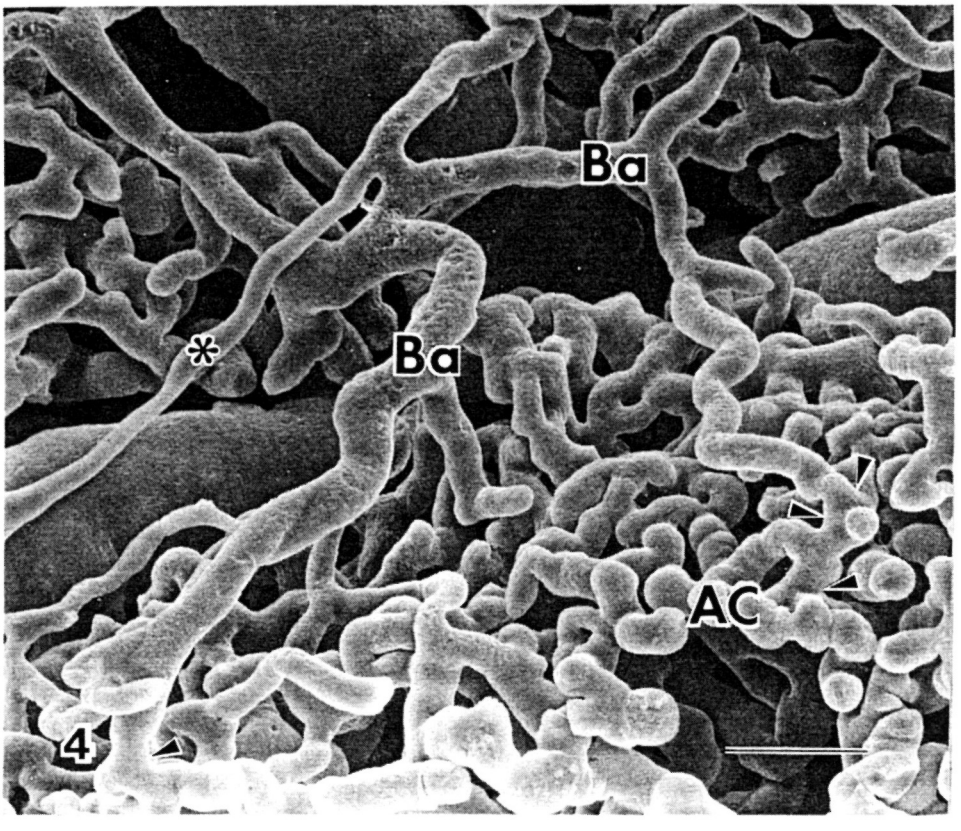
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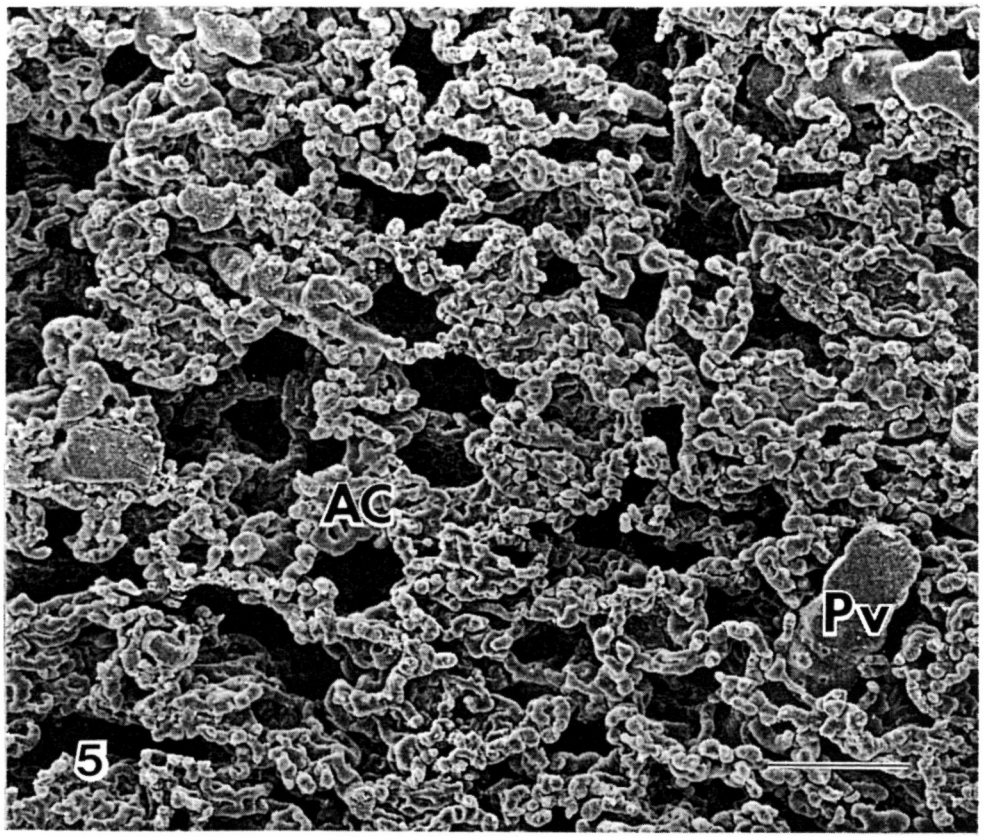
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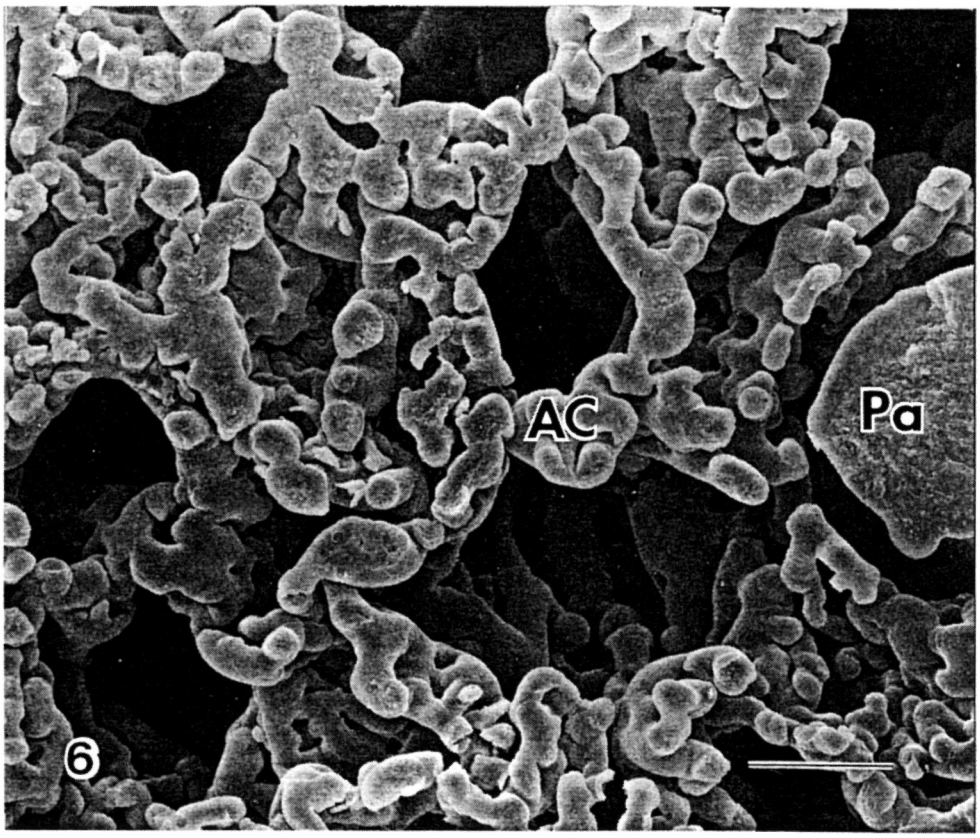
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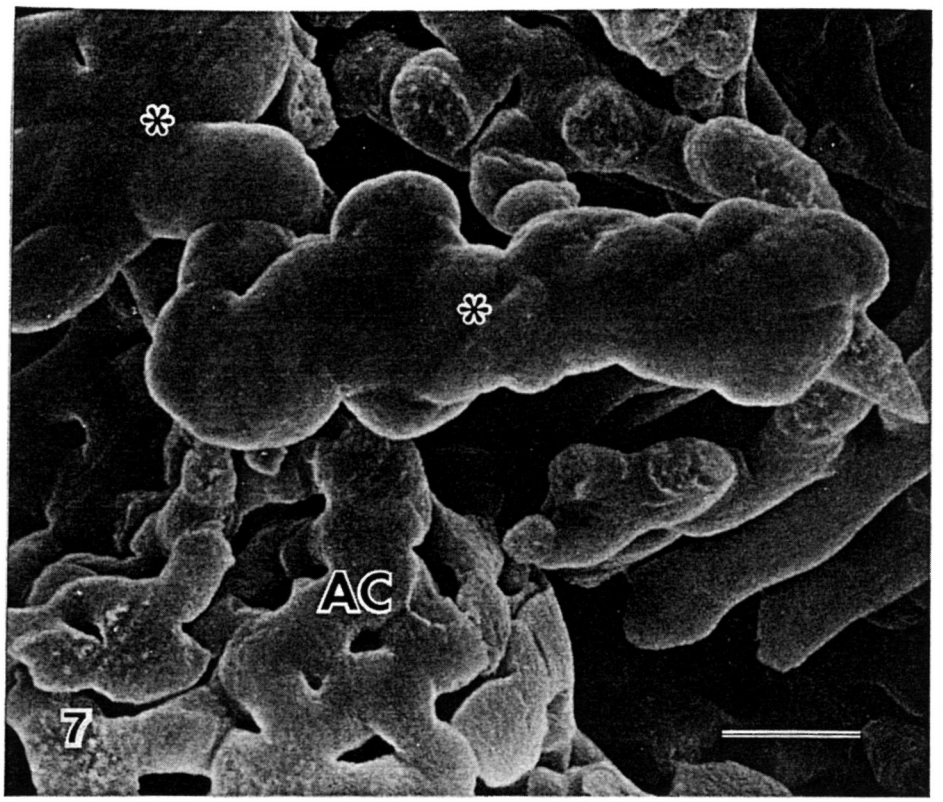
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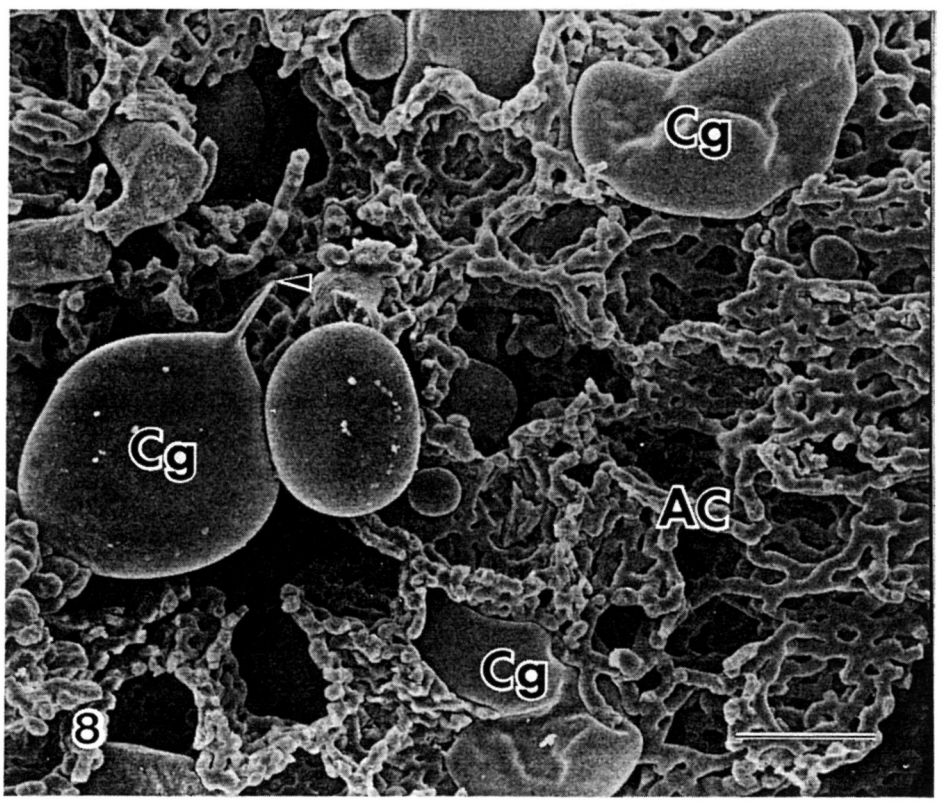
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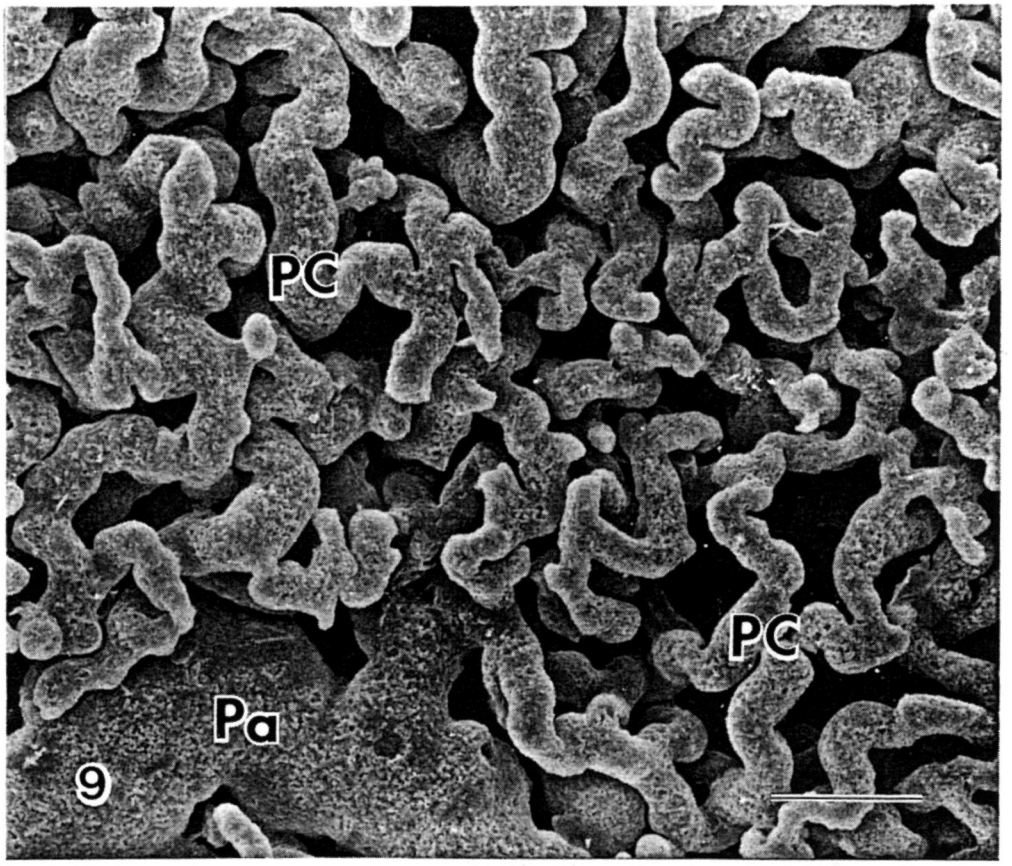
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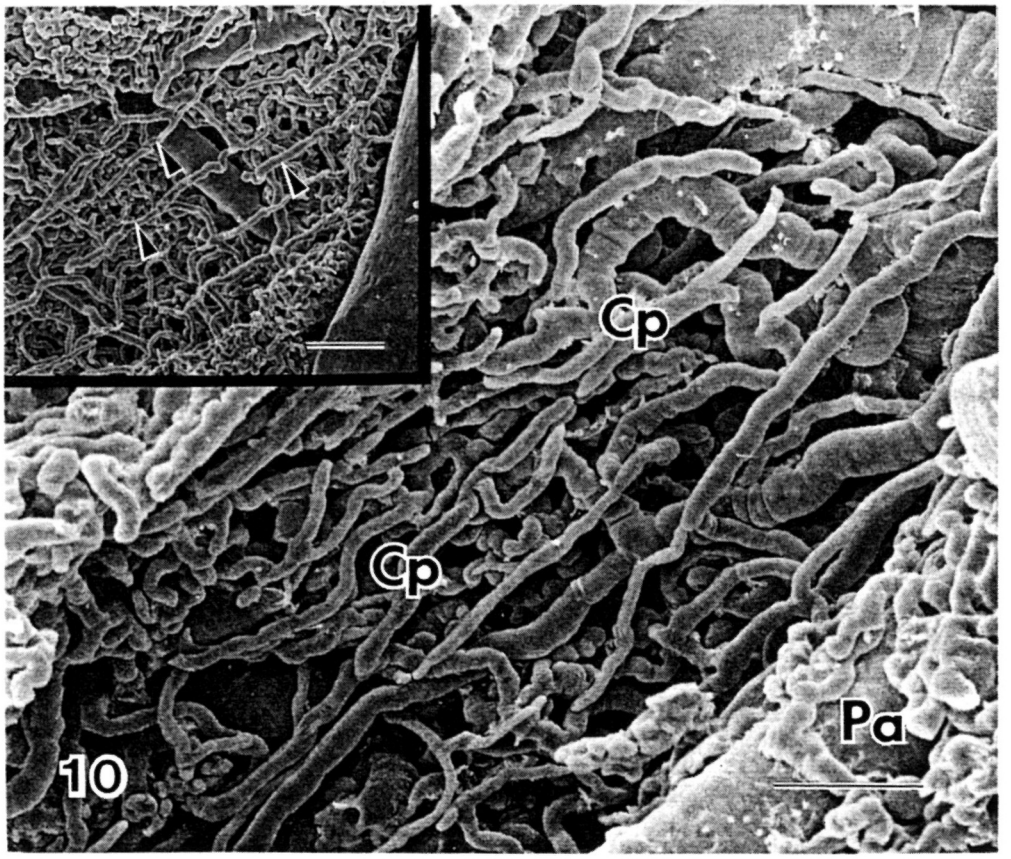
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Table 2. Distance between the capillary branches (μ)*

Surface	Saline	Bleomycin	P
Alveolar	5.6−13.0	2.6−15.6	0.38
	(7.5±1.4)	(8.0±3.2)	
Pleural	5.0−12.0	5.7−17.0	0.03
	(7.9±1.9)	(9.9±3.0)	

* The mean value and standard deviation are in parentheses.

DISCUSSION

There have been a number of studies of the effects of bleomycin on the lungs of animals (Thrall et al., 1979 ; Adamson and Bowden, 1974 ; Kwon et al., 1990). These studies were done mainly with light and transitional electron microscopic methods. Fleischman et al. (1971) and Schaeppi et al. (1974) studied beagle dogs, and Schaeppi et al. (1973) studied rhesus monkeys, all treated with repeated intravenous injections of bleomycin.

The interstitial lung fibrosis produced was characterized by a delayed onset and a tendency to peribronchial and subpleural localization (Snider et al., 1978). The histopathologic findings in these studies were similar to those of our study. But only a few vascular casting studies have been done on lungs that have been experimentally injured (Lametschwandtner et al., 1984 ; Schraufnagel et al., 1986).

In lung fibrosis, inflammation and collagen deposition in the interstitium disturb the capillary-alveolar relationship.

Hijiya (1978) noted strictures in pulmonary arteries. Schraufnagel et al.(1986) frequently found enlarged capillaries with irregular dilatations and decreased branching. In both normal and fibrotic animals, we found capillaries less than 5 μ in diameter. This is unlikely to be solely the result of shrinkage from fixation (Lum and Mitzner, 1985), because these small capillaries were localized.

Similar structures can be seen on a published micrograph of Hijiya, who cast the lungs of rats after giving them intraperitoneal bleomycin sulfate (Hijiya, 1978). In his model, endothelial swelling occurred and could have caused decreased capillary diameters. Endothelial swelling is an early occurrence in lung injury caused by intraperitoneal bleomycin but is less noted in the intratracheal model (Schraufnagel et al., 1986) and our study. Assimacopoulos et al. (1976) noted folding and decrease in capillary size at low lung volumes. It is possible that these localized small capillaries resulted from the low lung volume in which casting was carried out. However, the mean capillary diameter in our normal animals was larger than or about the same as what others have reported (Wang and Wei, 1976 ; Guntheroth et al., 1982). In our study, diameters of the alveolar capillaries were larger than pleural capillaries.

The increased interstitial tissue that grows between alveoli in fibrosis causes the capillaries to lose their sheetlike compactness and decreases the parenchymal capillary density (Schraufnagel et al., 1986).

Although the increased diameter of both the pleural and alveolar capillaries in fibrosis could be related to chronic increased blood flow or tethering of capillaries by retracting interstitial tissue, the configuration of the dilated capillary segments suggests that disruption and remodeling occurred (Schraufnagel, 1987). The abnormal enlargements may be similar to the dilated capillary, cirsoid nests which Tomashefski and colleagues described in humans, who died after a prolonged course of adult respiratory distress syndrome (Tomashefski et al., 1983). It is also possible that the capillary bulged from the casting material, because the walls of capillaries in fibrotic areas may be weak and might leak with the pressure of the methac-

rylate being infused. Filling pressures cannot be accurately monitored during casting, because the viscosity of the methacrylate constantly changes, and the pressure away from the syringe rapidly decreases with intravascular branching and distance.

Even though there was an increase in the size of capillaries in the fibrotic animals, the area occupied by the alveolar capillaries was not increased, which means that there must be fewer capillaries (Schraufnagel et al., 1986).

This agrees with the findings of Gracey et al. (1968), who noted a decrease in the number of capillaries in fibrosis. The decrease of capillary branches in fibrosis also suggests a loss of the capillary bed. There are fewer and larger capillaries, some disappeared and others increased in size. If the volume of the total capillary bed remained constant but the diameter of individual capillaries increased, then there would be a decrease in pulmonary, capillary surface area (Schraufnagel et al., 1986).

We found the central hole size of alveolar capillary rings to be increased in bleomycin-induced fibrosis. The centers of these rings were originally thought to be the sites of Type II cells by Alexander et al. (1973). Hijiya and Okada (1978) identified Type II cells and brush cells in the center of these rings.

They also noted the ring was square when the lung was fixed at low lung volumes. More recently, Assimacopoulos et al. (1976) have identified them as sites of contractile interestitial cells. They have shown that the cells contract under hypoxic conditions (Kapanci et al., 1974) which alter the capillary configuration and, therefore, blood flow. Callahan et al. (1986) have recently shown that these contractile interestitial cells are markedly increased in the lungs of rats with bleomycin-induced fibrosis. Schraufnagel et al. (1986) explained that the central holes may be sites of interstitial contractile cells, and these cells are increased in fibrosis, then the size of the holes are increased.

In summary, the appearance of irregularly shaped capillaries, an increase in diameter with variable dilatation of alveolar capillary rings and a decrease in branching, resulting in a loss of surface area are the main architectural changes of pulmonary capillaries in bleomycin-induced pulmonary fibrosis.

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