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Interstitial Associations of Cells Lining Air Spaces in Human Pulmonary Fibrosis*

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Summary. An ultrastructural study of the cells that line air spaces in human pulmonary fibrosis is reported. Intimate associations between these cells and cellular elements in the interstitium were consistently found in biopsies from 25 cases. Cytoplasmic extensions of cuboidal pneumocytes protruded through discontinuities in the subjacent basement membrane. Attenuated cells having structural properties of fibroblasts were situated on connective tissue that formed the walls of numerous air spaces. In this situation, a basement membrane was not demonstrable. These heretofore undescribed features suggest a dynamic interaction between certain mesenchymal and epithelial elements in the fibrotic lung.

Key words: Pulmonary fibrosis – Alveolar lining cells – Pulmonary interstitial cells.

Introduction

The prominent histopathologic features of pulmonary fibrosis in man have been recorded in numerous publications (Anderson and Foraker, 1960; Livingstone et al., 1964; Spencer, 1967; Carrington, 1968; Gaensler et al., 1972; Watanabe et al., 1972; Fraire et al., 1973). Although these reports characterize the structural alterations in the parenchyma of the lung, they provide limited insight into the pathogenesis of the lesions. The observations reported here were made during a systematic ultrastructural study of idiopathic diffuse pulmonary fibrosis in man. This paper is concerned with certain features of the cells that line air spaces and their association with cells in the interstitium of the fibrotic lung.

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Methods and Materials

Light and electron microscopic studies were carried out on biopsies of lung tissue from 25 adults with diffuse pulmonary fibrosis. This group was biopsied after a thorough clinical and epidemiologic investigation had indicated that the process was progressive and of unknown etiology. One or, in a few cases, two areas of the parenchyma from extensively involved portions of the lung were selected by the surgeon.

This study was conducted as part of a comprehensive collaborative investigation concerned with the pathogenesis and pathology of pulmonary fibrosis. The findings reported herein represent a synthesis of our observations on tissues obtained from this group of 25 patients, but do not completely characterize the features of the lesion in the lungs of any one patient. Subsequent reports from this laboratory will be concerned with other aspects of the pathology of pulmonary fibrosis.

Portions of lung were minced in 4% glutaraldehyde within 10 to 15 min after surgical excision. The tissue was post-fixed in 1% osmium tetroxide and embedded in Epon after dehydration in alcohol and propylene oxide. Both thick sections (1–2 μ) and thin sections (ca. 600 Å) were prepared from the plastic-embedded tissue. Thick sections were stained with methyl blue and mounted in mineral oil. They were used to select areas for more detailed study by electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope. Serial sections from several of the cases were studied to confirm the association of surface and interstitial elements in the tissue.

Lung from 10 patients served as "normal" controls. Eight specimens from cases of bronchogenic carcinoma were obtained at the time of thoracotomy but before lobectomy or pneumonectomy. Diagnostic biopsies from two additional patients were included. These tissues exhibited little or no interstitial fibrosis.

Lung biopsies from eight of the cases (three control, five with fibrosis) were expanded by perfusion with glutaraldehyde according to procedures described elsewhere (Brody and Craighead, 1975). The artifacts of collapse were avoided in these tissues, although ultrastructural findings were identical to those in non-perfused material.

Control tissue was examined routinely by the senior author. After the completion of this work, the lesions described herein were demonstrated to another experienced electron microscopist who had not participated in the study. This individual then systematically reviewed control and diseased tissue in a "blind" fashion to confirm or refute the presence of the anatomic features described herein.

Results

The lung tissue in the cases selected for this study was distorted by varying degrees of interstitial and intra-alveolar fibrosis. In most biopsies, bands and nodular masses of fibrous tissue were scattered in the parenchyma (Fig. 1a). Although anomalous air spaces often comprised much of the tissue, relatively normal appearing alveoli often were present.

In many of the cases, lumina of air spaces contained cells having the fine structural appearances of macrophages and type II pneumocytes (Fig. 1a). Cuboidal and attenuated cells lined these spaces (Fig. 1b). The cuboidal cells either had the characteristic cytologic features of type II epithelial cells (Fig. 2), or were of a similar configuration but lacked myelin figures (Fig. 3). Many of the attenuated cells exhibited fibrils in the cytoplasm (Figs. 4 and 5).

Extensions of the cytoplasm of both attenuated and cuboidal cells appeared to be associated with subjacent cells in the interstitium through discontinuities in the basement membrane (Figs. 2, 3, and 4). Often these interstitial cells

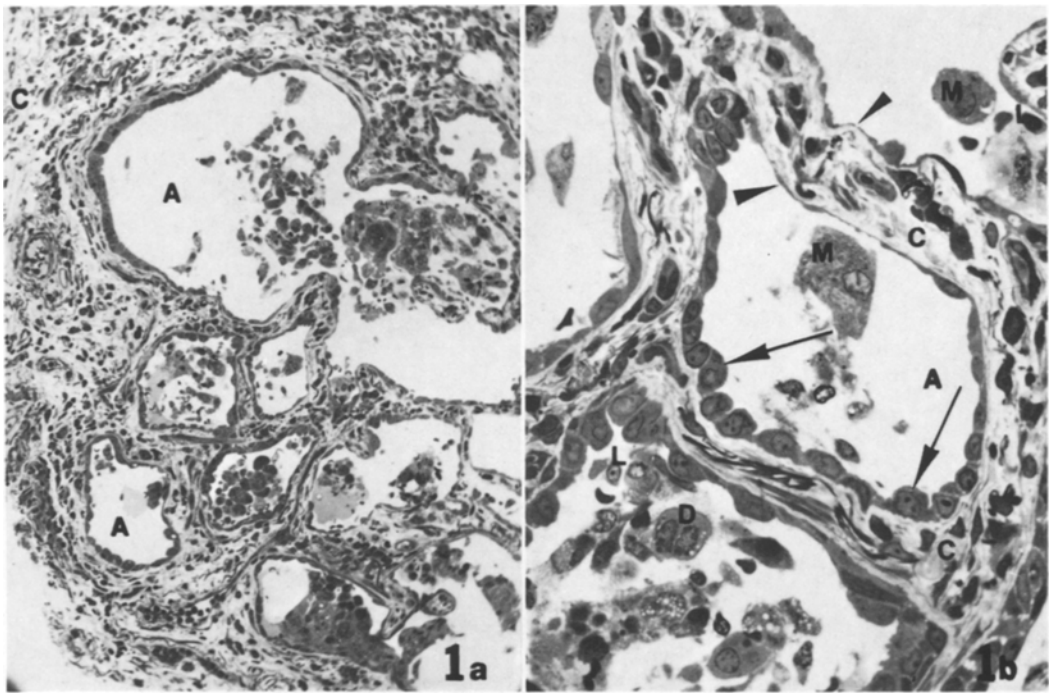
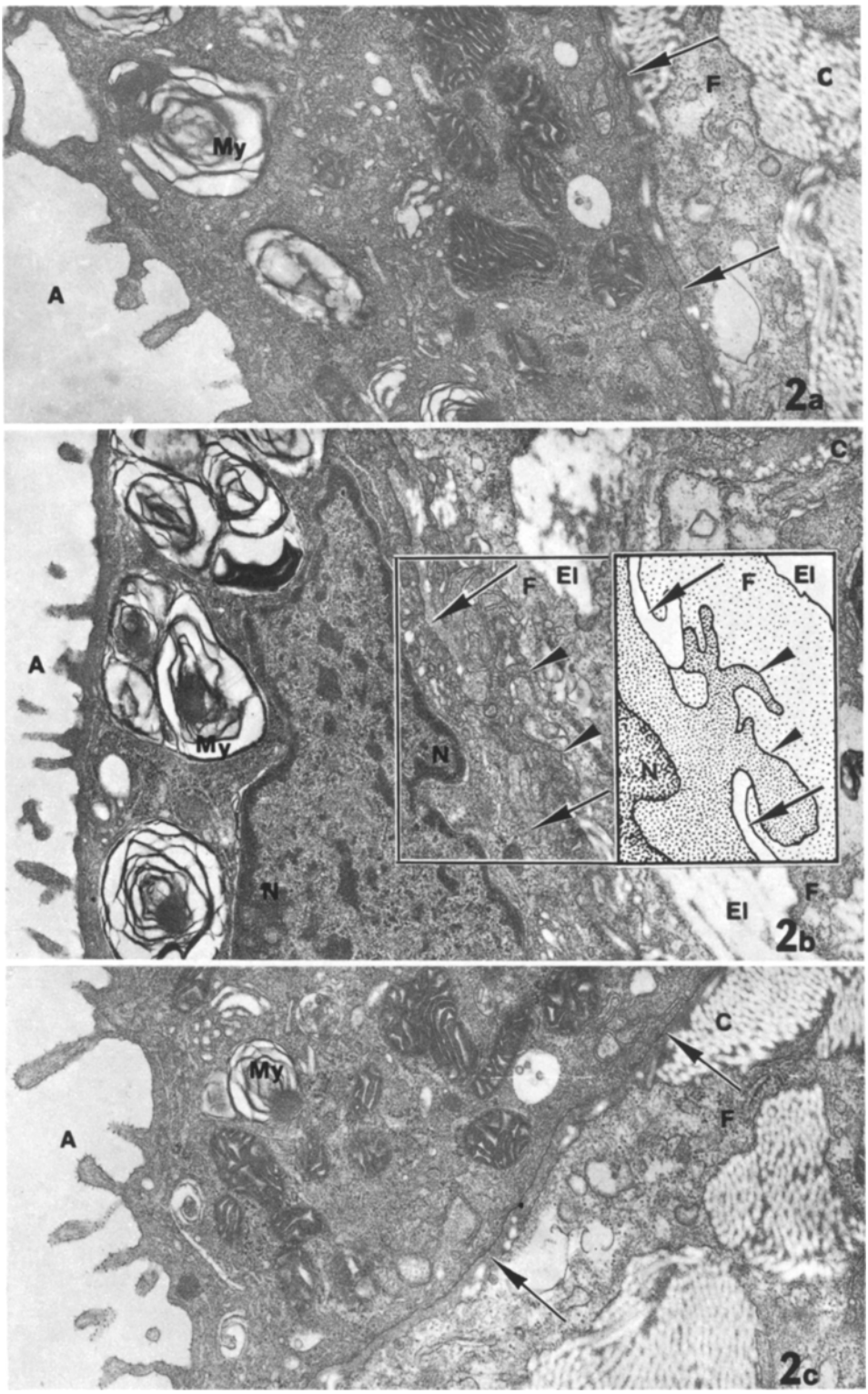


Fig. 1. a Photomicrograph demonstrating the parenchymal architecture of the lung from a 57-year-old woman with idiopathic pulmonary fibrosis. There are masses of connective tissue (*C*) and thickened interalveolar septa. The air spaces (*A*) are occupied by a variety of cells. ×65. **b** Air spaces (*A*) in this tissue are lined by both cuboidal (arrows) and attenuated cells (arrowheads). Cells in the air spaces appear to be desquamated lining cells (*D*), macrophages (*M*), and lymphocytes (*L*). The septa are thickened by connective tissue (*C*). ×250

Fig. 2a–c. Electron micrographs selected from a series of contiguous sections prepared from the wall of an air space in the lung of a 31-year-old man with idiopathic interstitial fibrosis and non-caseating granulomata. ×18,500. **a** A type II pneumocyte with several myelin figures (*My*) is situated adjacent to an a space (*A*) on a continuous basement membrane (arrows). A fibroblast (*F*) and collagenous tissue (*C*) are observed in the interstitium. **b** A deeper section reveals the nucleus (*N*) of the cell. At this level the basement membrane (arrows) is interrupted and cytoplasmic processes (arrowheads) are protruding into the interstitium where elastic (*El*) and collagenous (*C*) connective tissue and fibroblasts (*F*) are found. The inset reproduces this region diagrammatically. **c** A deeper section demonstrates a continuous basement membrane (arrows) beneath the cell

Fig. 3. A cuboidal, undifferentiated cell lining an air space (*A*) in the lung of a 52-year-old woman with diffuse interstitial fibrosis. Cytoplasmic processes (arrowheads) protrude through discontinuities in the basement membrane (arrows) and are closely associated with fibroblast-like interstitial cells (*F*) and connective tissue fibrils (*Fi*). ×13,600

Fig. 4. Electron micrograph from the same lung illustrated in Figure 1. An air space (*A*) is lined by attenuated epithelial cells (*Ep*). The basement membrane (arrows) is interrupted at a point where a lining cell and an interstitial cell (*IC*) are in contact (arrowheads). The interstitial cell resembles a fibroblast for it exhibits numerous organelles, fibrils, and processes which are closely associated with surrounding collagen (*C*). A capillary (*Ca*) and a type II pneumocyte (*Pn*) are observed. ×7600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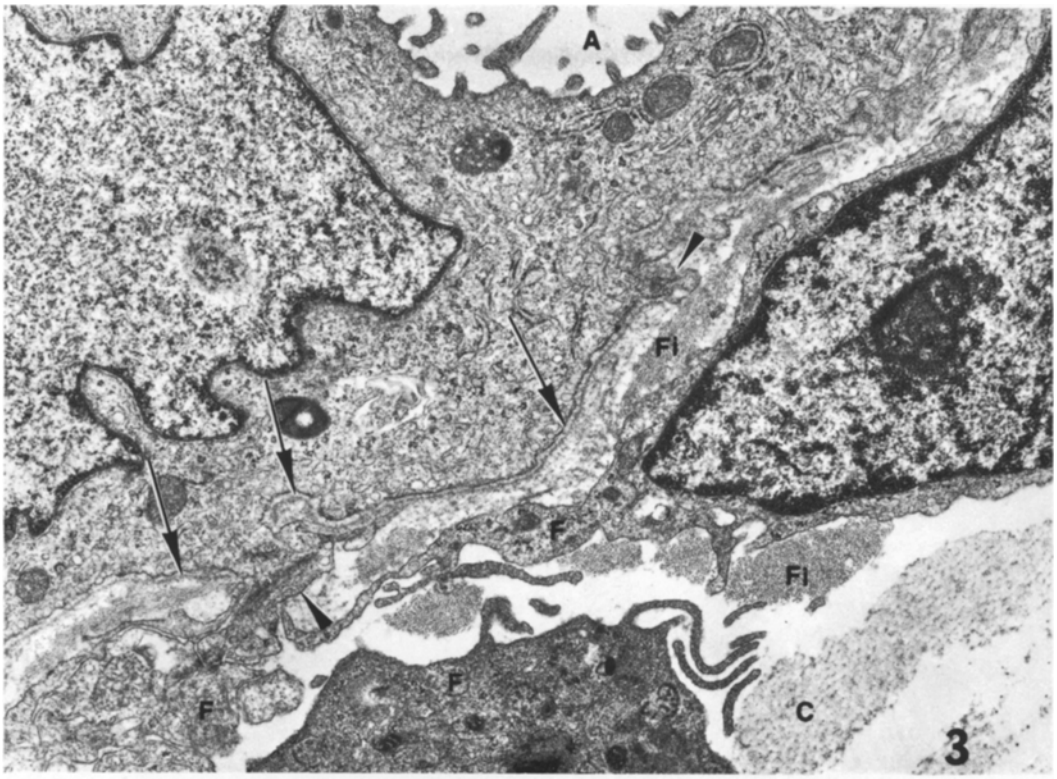
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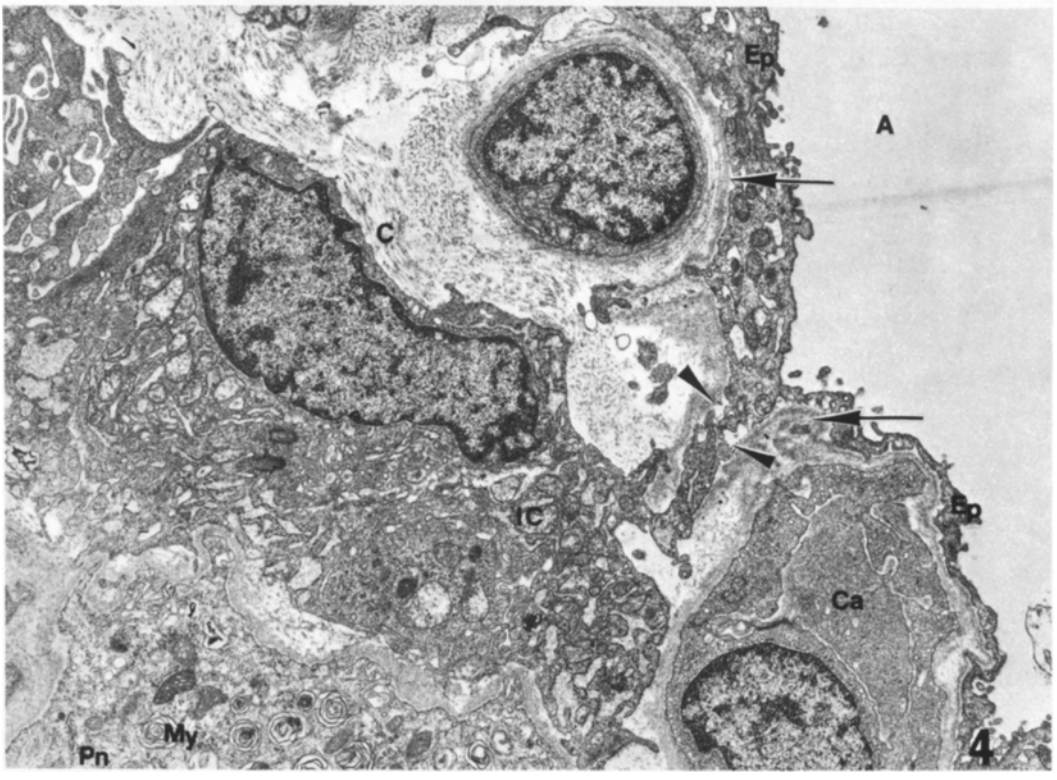
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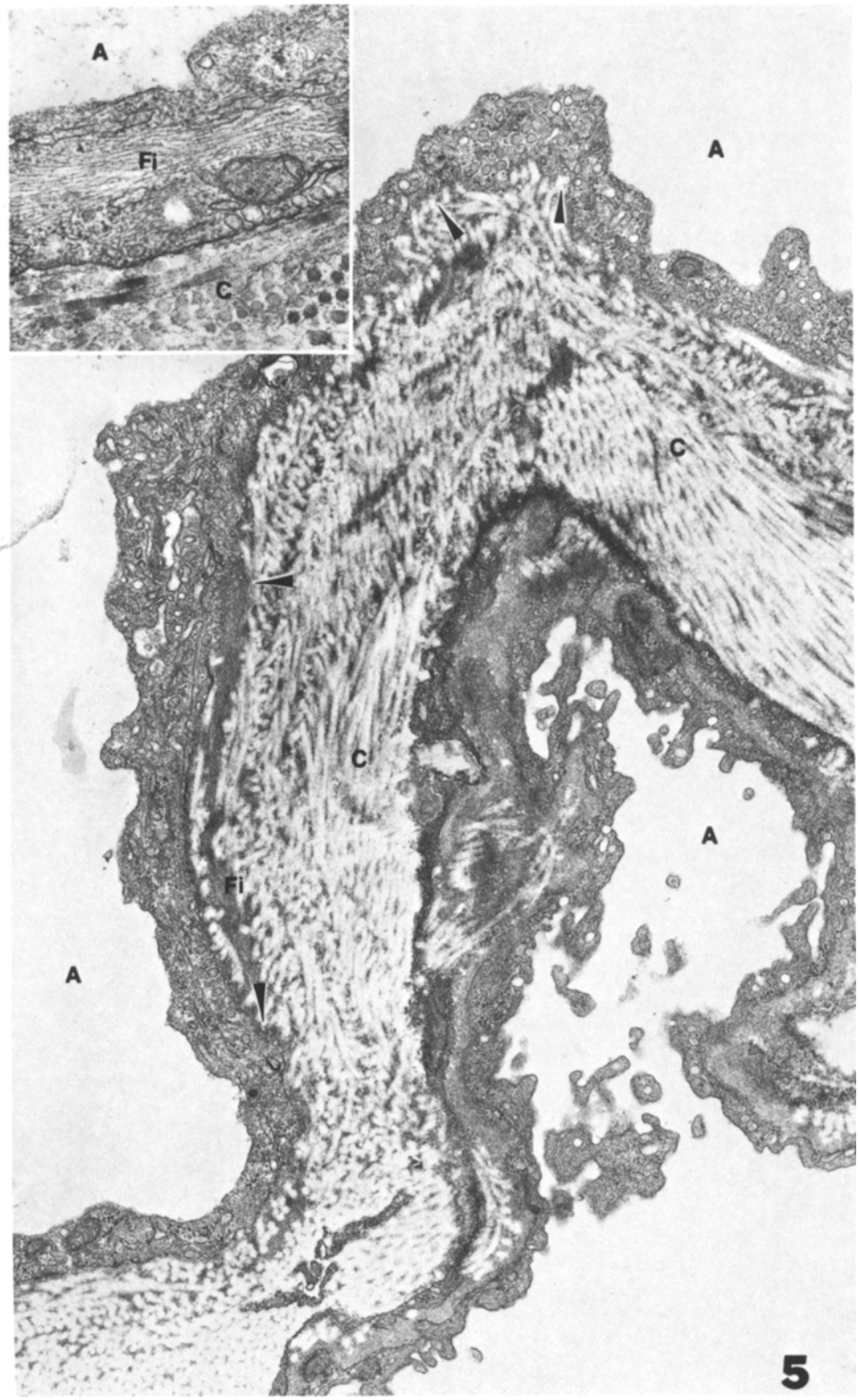
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resembled fibroblasts since they contained numerous organelles and fibrils and exhibited typical cytoplasmic processes (Figs. 2, 3, and 4). The walls of numerous air spaces focally lacked basement membranes demonstrable by electron microscopy. Consequently, some attenuated lining cells appeared to rest directly on connective tissue (Fig. 5). Fibrillar extensions from these cells were intimately associated with underlying collagenous tissue (Fig. 5).

Associations between certain alveolar lining cells and interstitial elements were found without difficulty in each of the 25 biopsies from patients with pulmonary fibrosis. Detailed studies of tissue from the ten seemingly normal "control" lungs by both the senior author and the consultant electron microscopist consistently failed to reveal these changes. Thus, they appeared to be present only in the diseased pulmonary parenchyma. To our knowledge, the alterations described herein have not been referred to in publications concerned with the fine structural anatomy of human lung (Bensch, 1968; Ryan, 1969; Weibel, 1971; Okada, 1972; Nagaishi, 1972).

Discussion

Fibrosis of the lung parenchyma represents a stage in the reparative process that follows a wide variety of pulmonary insults. The lesion is consequent to damaging influences affecting the interstitium or the alveolar lining cells, but also results from the organization of intra-alveolar exudates. In this context it might be expected that mesenchymal elements in the alveolar and bronchiolar walls of the fibrotic lung develop a relationship with the cells that line the surfaces of air spaces. This report documents some of these heretofore unrecorded features.

Although a diversity of associations between mesenchymal and epithelial components were present, they appeared to represent patterns of two distinct types. In the first, cuboidal and attenuated epithelial cells made contact with interstitial cells through gaps in an otherwise well-defined basement membrane (Figs. 2, 3, and 4). In the second, attenuated cells that lined the walls of some air spaces appeared to lie directly upon connective tissue (Fig. 5). Although a number of these latter cells had cytoplasmic organelles commonly observed in fibroblasts (fibrils and rough endoplasmic reticulum), their identity remains to be established.

The significance of the associations between epithelial cells and interstitial elements is unclear. They were found commonly in lesions where there was extensive accumulation of interstitial connective tissue (Figs. 1, 2, 3, and 4). Often, alveolar walls seemed normal by light microscopy, but they were comprised largely of dense collagenous tissue (Fig. 5). In this situation, attenuated

Fig. 5. The wall of an air space in the lung of a 57-year-old man with diffuse idiopathic pulmonary fibrosis. The interstitium is comprised of collagen (*C*) and fine fibrils (*Fi*), and the attenuated cells lining the air space (*A*) are intimately associated (arrowheads) with this connective tissue. ×21,000. The inset demonstrates the fibrillar (*Fi*) nature of an attenuated lining cell and that this cell lies directly upon collagenous tissue (*C*) with no intervening basement membrane. ×38,750

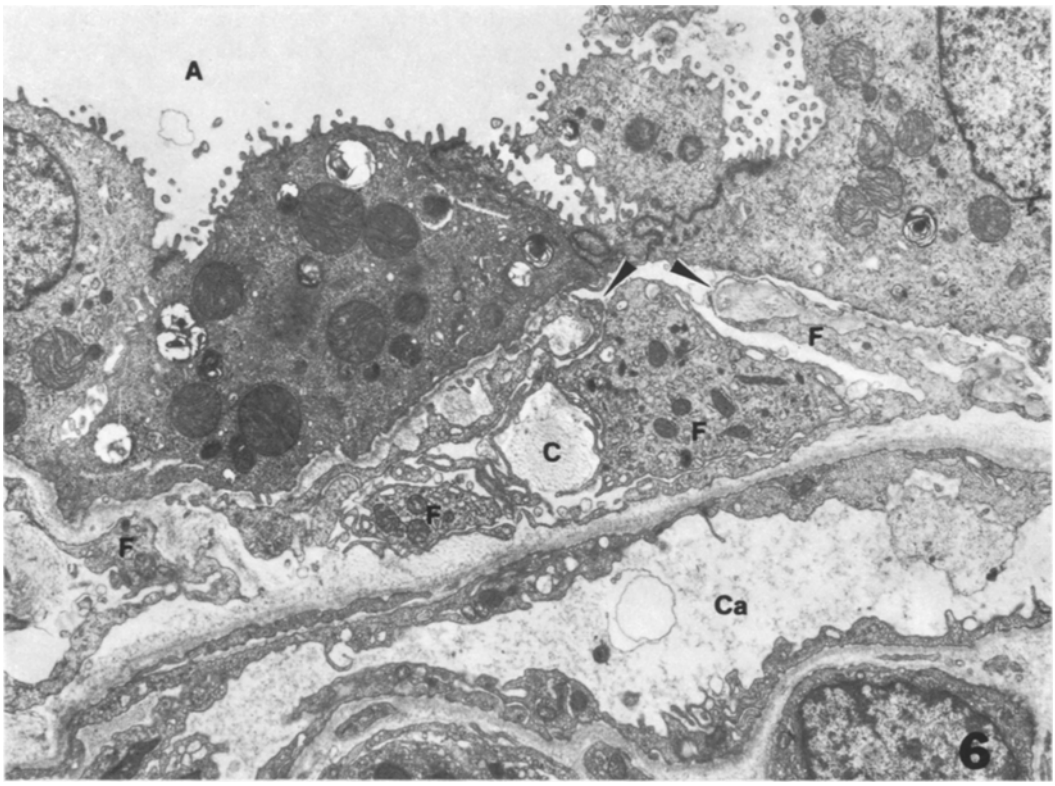
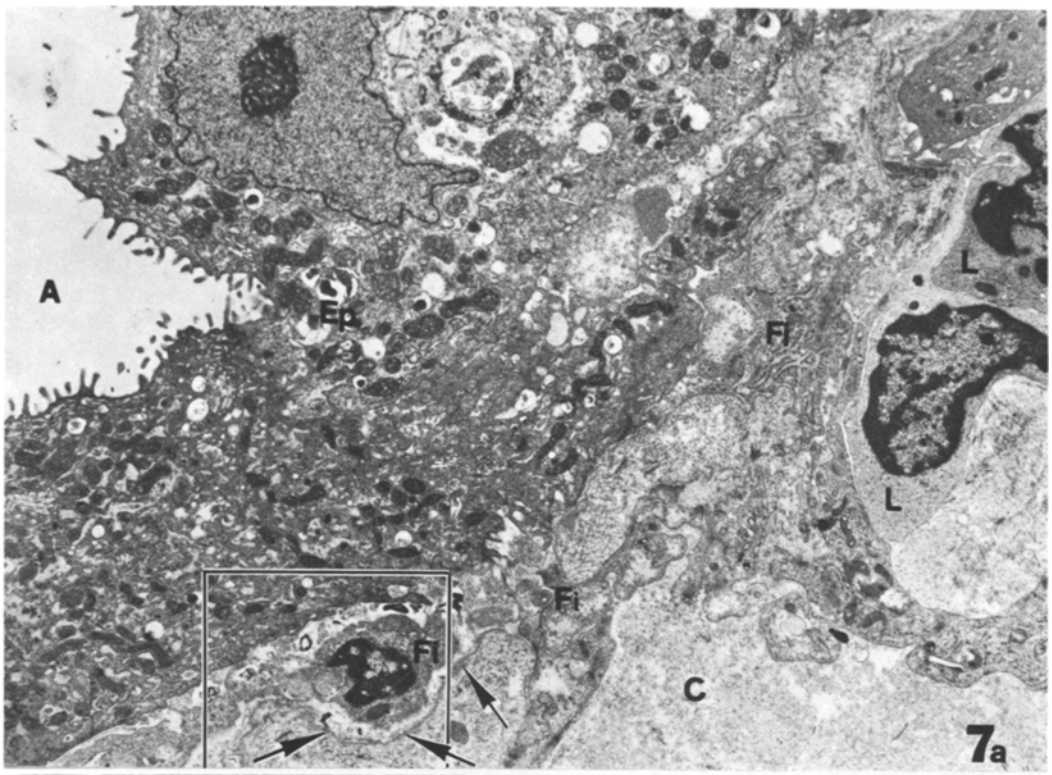


Fig. 6. Lung from a 19-year-old girl with lymphoid interstitial pneumonitis. Several of the cells which line the air space (*A*) are not separated by a basement membrane from fibroblast-like interstitial cells (*F*). The latter cells have attenuated processes (arrowheads) that extend from the interstitium to a space beneath the lining cells. A capillary (*Ca*) and collagenous tissue (*C*) are observed within the interstitium. ×6300

cells that resembled fibroblasts lined portions of the air spaces (Figs. 1b and 5). There is no direct evidence that these cells are fibroblasts. Obviously, we are limited by morphologic techniques. On the other hand, recent studies carried out with collaborating laboratories have shown that fibroblast-like cells can be cultured from fluids recovered by bronchofiberscopic lavage from patients with diffuse interstitial fibrosis, but not from normal individuals (Davis et al., 1976). It was concluded that these cells were dislodged from the walls of airspaces during the lavage procedure.

Fig. 7a and b. Lung tissue from a 54-year-old man with diffuse fibrosis. **a** Hyperplastic epithelial cells (*Ep*) line an air space (*A*). Immediately beneath these cells are several fibroblasts (*Fi*) which have cytoplasmic extensions into the underlying connective tissue (*C*). An attenuated fibroblast is lying between an epithelial cell and basement membrane (arrows). Interstitial lymphocytes (*L*) are seen. The area enclosed in the rectangle is reproduced at higher magnification in Figure 7b. **b** A fibroblast (*Fi*) is insinuated between an epithelial cell (*Ep*) and the underlying basement membrane (arrows). Pre-collagen fibrils are seen within the fibroblast (arrowheads), and mature collagen (*Co*) is distributed throughout the interstitium. (7a) ×6000; (7b) ×35,000



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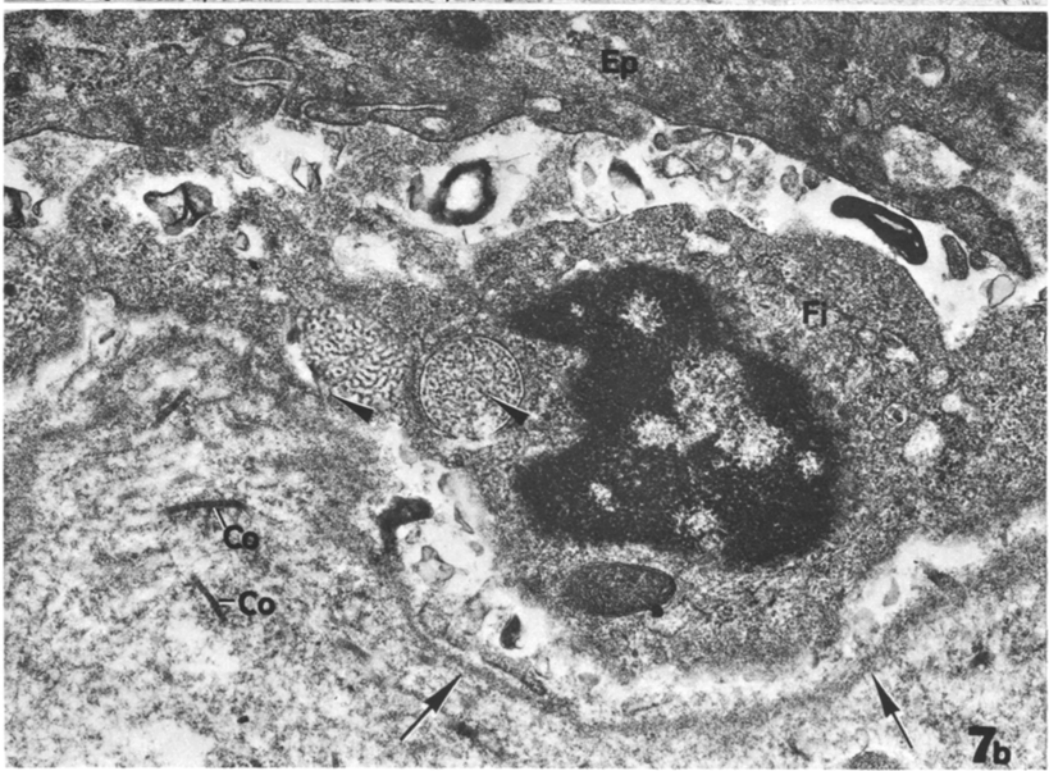

7b

Inasmuch as mesenchymal elements participate in the organization of intra-alveolar exudates, fibroblasts would be expected in the resulting scar. In an experimental mode of bleomycin toxicity, Adamson and Bowdon (1974) demonstrated fibroblasts surrounding organizing exudates. Thus, these mesenchymal cells lined small air spaces. Freeman and co-workers (1974) found fibroblasts lining the alveolar ducts and respiratory bronchioles of rats after pulmonary injury by nitrogen dioxide and ozone. In addition, observations on two of our cases demonstrate that cells resembling fibroblasts are found in air spaces, insinuated between epithelial lining cells and the basement membranes (Figs. 6 and 7).

Previous investigators have directed attention to the cell populations that line the walls of air spaces in interstitial fibrosis. Using light microscopy, Fraire et al. (1973) described the hyperplasia and metaplasia of alveolar epithelial cells in this condition. A number of workers (Kapanci et al., 1969; Evans et al., 1973) have shown that the hyperplastic type II pneumocyte is the primary source of repair in the injured or diseased respiratory epithelium. Our observations in no way dismiss these results, but suggest that mesenchymal cells also may contribute to the lining of some air spaces. A similar view has been expressed by Geever et al. (1943) and by Freeman et al. (1974) as discussed above. An analogy can be drawn from the work of Spaet et al. (1975). These investigators provided evidence that new cells lining the vascular spaces in healing rabbit aortas are derived from smooth muscle cells of the media.

It remains to be determined if the changes described here are associated with specific types of insults to the lung. Their common presence in our material suggests that this is unlikely.

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