"CONTRACTILE INTERSTITIAL CELLS" IN PULMONARY ALVEOLAR SEPTA: A POSSIBLE REGULATOR OF VENTILATION/PERFUSION RATIO?

Ultrastructural, Immunofluorescence, and In Vitro Studies

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ABSTRACT

In the lungs of healthy rats, humans, lambs, and monkeys, about 50% of the alveolar interstitial cells—resembling fibroblasts—contain bundles of fibrils measuring 30–80 Å in diameter. Immunofluorescence studies on frozen sections of rat lung demonstrate that many interstitial cells bind sera containing antiactin antibodies. On account of these two sets of findings and our additional in vitro studies suggesting alveolar tissue contraction due to hypoxia or epinephrine, we postulate that the alveolar septa contain contractile cells different from that of smooth muscle. For these cells we propose the name of "contractile interstitial cells." Such cells lie within the thick portion of the air-blood barrier and around the pre- or postcapillary vessels. Hence it is possible that they play a role in the autoregulation of ventilation/perfusion ($\dot{V}/\dot{Q}$) ratio, particularly in hypoxic pulmonary hypertension. These findings, demonstrating a contractile system other than bronchial and arterial smooth muscle, suggest that the alveolus should no more be conceived as a passive "organ."

INTRODUCTION

The mechanism of pulmonary ventilation/perfusion ($\dot{V}/\dot{Q}$) autoregulation is poorly understood. The prevailing hypothesis maintains that arteries and bronchi regulate the blood flow and air supply to the alveoli, their reactions being determined by the partial pressure of alveolar gases, and particularly of oxygen. The alveolus is considered as a passive organ, involved in $\dot{V}/\dot{Q}$ regulation only by passive deformation consequent to changes in pleural and intra-alveolar pressures (1–7). Indeed the lack of substantial musculature in the normal alveolar septa (8, 9) has been used as an argument to rule out any possible active participation of the sacculi in this phenomenon (2).

The present study demonstrates that in alveolar septa of normal rat lungs there are many interstitial cells containing contractile structures. In addition, it shows that, in vitro, strips of lung parenchyma contract in hypoxic aqueous media or in the presence of epinephrine. The possible

role of what seems to be alveolar tissue contraction in $\dot{V}/\dot{Q}$ regulation and in hypoxic pulmonary hypertension is discussed.

MATERIALS AND METHODS

Electron Microscopy

The lungs of 20 Wistar rats (150 g) deeply anesthetized by intraperitoneal (i.p.) Nembutal (17 mg/100 g) were fixed by perfusion (10–12); 6 other rat lungs were fixed by conventional intratracheal instillation. We used 2% glutaraldehyde adjusted with phosphate buffer and saccharose to pH 7.4 and 350 mosmol. The perfusion was carried out under 39 cm H_2O pressure; airway pressure was maintained at 15 cm H_2O; instillation was performed under 20 cm H_2O pressure. Tissues were postfixed in cacodylate-buffered 1% OsO_4 and embedded in Epon. Sections were cut on an LKB Ultrotome, and stained with uranyl acetate and lead citrate (13). Preparations from human, lamb, and monkey lungs were also examined; they were picked at random from the material of previous studies (14, 15). These studies were performed on a Philips 300 electron microscope.

Immunofluorescence

The trachea of 10 deeply anesthetized (Nembutal, i.p.; 17 mg/100 g) Wistar rats (150–200 g) was opened and cannulated. After bilateral pneumothorax 3.5–4 cc of 10% gelatin solution in 0.9% NaCl at 38°C were injected into the lungs through the intratracheal cannula. The lungs were then removed and dipped into liquid nitrogen; after 10 min they were brought to a temperature of −20°C, adequate tissue blocks were prepared, and 5-μm serial sections were cut on a cryostat. Thanks to the injection of gelatin, which distended the alveoli, tissue topography was perfectly preserved.

Two consecutive sections were mounted on each slide and fixed in acetone for 5 min. The first section was treated for 30 min with human serum containing antiactin antibodies (AAA) obtained from patients with chronic aggressive hepatitis (16). This serum was diluted $\frac{1}{20}$ with phosphate-buffered saline (PBS). The second section mounted on the same slide was treated with normal human serum in PBS. Both sections were then washed in PBS and stained for 30 min with fluorescein-conjugated IgG fraction of goat antiserum to human IgG. After rewashing in PBS they were mounted in 90% glycerol (17). A third and fourth sections were mounted on another slide. One was treated with AAA serum, the other with the same serum previously absorbed by thrombosthenin-A, i.e., the actin moiety of thrombosthenin (16). The fluorescein staining was performed as described above. The intensity of fluorescence was determined by comparing sections treated with AAA serum and those treated with normal human, or with actin-absorbed AAA sera. Photographs were taken on Zeiss-UV photomicroscope with UG_1 or UG_2 excitor filter and Zeiss $\frac{5}{65}$ barrier filter, using Anscochrome color slide film 500 daylight (GAF Corp., New York). The sections were then fixed in formaldehyde and stained with HE or toluidine blue.

In Vitro Studies

These experiments were carried out with the lungs of 47 Wistar rats (150–250 g) killed by decapitation, 2 human lungs (surgical), and 15 fresh bovine lungs. A total of 96 parenchymal, 23 arterial, and 20 bronchial strips were prepared. Parenchymal strips (rat, human, bovine) were obtained from transversal subpleural sections in order to avoid large arteries and bronchi. The pleura was cut off with a razor blade and a strip of parenchyma devoid of grossly visible bronchi or arteries was isolated. Arterial (human and bovine) and bronchial (bovine) strips were prepared through dissection of a sixth generation artery (1–2 mm ϕ) or bronchus (2–3 mm ϕ) in order to obtain parenchyma-free tubes. They were cut transversally into rings and then opened, thus forming 3–6-mm long strips. No rat arteries and bronchi were tested, since they were too small to be used in our system.

All samples were quickly immersed into previously oxygenated Tyrode solution (18) adjusted to pH 7.4. In a Petrie dish, filled with this medium the strips were attached to a glass support and then were placed into the contraction observation microbath (Fig. 1). This is a tall and narrow chamber with flat transparent sides placed vertically in front of a horizontal microscope. It contains 10 ml Tyrode solution saturated by 95% O_2 + 5% CO_2. The top end of the strip was attached by a silk thread (SSC Nr 6/0 United States Patent) to a horizontal bar fixed on to the glass support, while the other end was tied to the free end of a 3 cm long calibrated glass filament also anchored into the support. This filament was adjusted to exert a traction of 10 mg on parenchymal strips and 50 mg on arterial and bronchial strips. The microscope was focused onto its free tip, the movements of which were measured by means of a graduated eyepiece (Olympus P7X micro). When the microbath was placed in front of the microscope the position of the tip of the filament was recorded (time zero of recordings). Then 95% O_2 + CO_2 was bubbled through the Tyrode solution for 10 or 20 min. In some experiments the strips were left for 10 min in Tyrode solution with only passive room air diffusion before O_2 diffusion (room-air stabilization). Thereafter hyperoxic (95% O_2 + 5% CO_2), anoxic or hypoxic gas mixtures (0%, 5%, 10% O_2 ± 5% CO_2

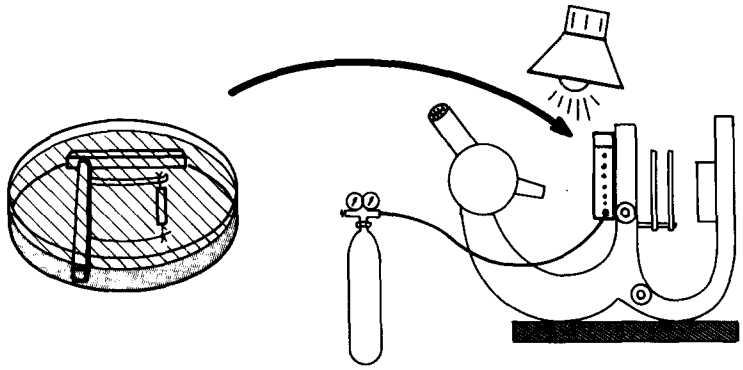

FIGURE 1 Schematic diagram of the setup used for the in vitro studies. Left: a strip mounted on the support in a Petrie dish. Right: horizontal microscope on which a microbath containing the support has been placed for the observation of contraction and relaxation.

in N_2) were bubbled through the medium for periods varying between 40 min and 3 h. The effects of hyper- and hypoxia (or anoxia) were studied on 64 parenchymal, 7 arterial, and 13 bronchial strips. A further 32 parenchymal, 16 arterial, and 7 bronchial strips were tested as to their reaction to epinephrine (10^{-6} g/ml) and/or serotonin (10^{-5} g/ml); these substances were injected into the microbath during the hypoxic or hyperoxic phases of the experiments. All experiments ended 10–20 min after addition of 10^{-4} g/ml of Papaverine HCl 2% (Vifor Co., Geneva, Switzerland).

In all experiments the reactions of the strips were followed by continuous observations of the movements of the glass filament, the position of which was recorded each 5 min. The pH of the solution was maintained at 7.4 and its pO_2 during hypoxic or anoxic gas mixture bubbling was measured and recorded. At the end of each experiment the strips were measured and weighted, then fixed in 8% neutral formaldehyde, embedded in paraffin, and examined histologically. Thus the muscular nature of the arterial strips was checked and in the parenchymal strips the number of vessels and bronchi measuring 100 μm or more in diameter was counted.

RESULTS

Electron Microscope Studies

GENERAL CONSIDERATIONS: The ultrastructural features of the respiratory tissue of normal mammalian lung have been described by previous authors (11, 14, 19–21). The alveoli are lined by a continuous layer of epithelial cells comprising membranous (type I cells) and granular (type II cells) pneumocytes, as well as a few alveolar brush cells (22). In cross section, capillaries appear as tubules lined by one or two contiguous endothelial cells, seldom by more. Recently, the presence of pericytes in the wall of alveolar capillaries has been reported (23). At the thick portion of the air-blood barrier, interstitial cells—fibroblasts, and some macrophages—as well as connective tissue fibers are situated between the capillary and the alveolar basement membranes (11, 14, 19, 20). These cells are most abundant at the junction of three or more alveoli, where pre- or postcapillary vessels can be seen.

Lungs fixed by perfusion, as in this study, have a peculiar appearance which might mislead an unwary observer, because many alveoli are collapsed and the large empty spaces seen on the sections correspond to capillaries (Fig. 2). A good description of lung tissue fixed by perfusion is given in recent publications (11, 12).

ALVEOLAR INTERSTITIAL CELLS: An interstitial cell is shown in Fig. 3. It is located within the thick portion of the air-blood barrier and is not surrounded by a basement membrane but by few collagen fibers. Such cells have abundant rough endoplasmic reticulum, many free ribosomes, a prominent Golgi apparatus, and resemble conventional fibroblasts (24). Indeed, in the lungs they have been identified up until now, on light and electron microscope criteria, as fibroblasts (11, 14, 19, 20). However, they also contain compact bundles of parallel fibrils comparable to those of smooth muscle cells (Figs. 2, 4–8). These bundles were usually seen beneath the plasma membrane and obliquely "inserted" onto it. The individual fibrils measured 30–80 Å in diameter, the bundles 0.25–0.50 μm (Fig. 4). The section of a single cell could contain as many as 5–10 bundles (Fig. 3).

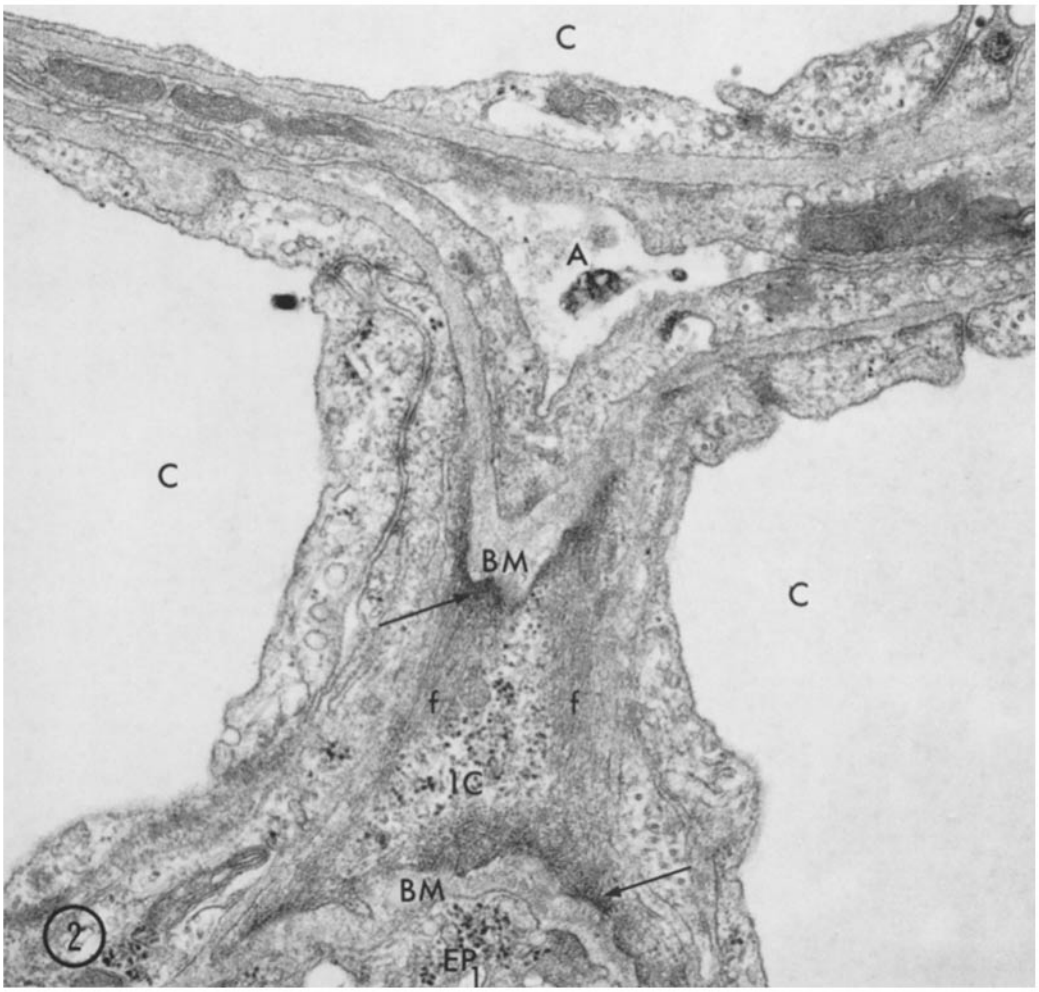

FIGURE 2 A collapsed alveolus (*A*) surrounded by three capillaries (*C*) is visible. At the bottom of the picture the epithelium (EP_1) of another alveolus is present. Between the two alveolar basement membranes (*BM*) there is a portion of an interstitial cell (*IC*) containing fibrillar bundles (*f*) with dense bodies (arrows). (Rat lung fixed by perfusion.) × 38,000.

Often interstitial cells were in contact with the alveolar or capillary basement membranes. In such cases, where the bundles of microfilaments joined the cell membrane, there was frequently a dense zone within the microfilaments immediately beneath the plasmalemma, thus resulting in a hemidesmosome-like complex (Figs. 2, 4–7). Such structures were similar to those described between endothelial cells, pericytes, smooth muscle cells, or myofibroblasts and their basal lamina (25–27). They were seen also when interstitial cells were in contact with collagen or elastic fibers (Figs. 4, 5). Although we have not formally excluded the possibility that a grazing section near the cell surface is responsible for this "anchoring" appearance, it seems likely that these hemidesmosome-like complexes—in analogy with endothelial cells, pericytes, and smooth muscle (25, 26)—participate in the transmission of cellular pull to other tissue components. In fact, the alveolar profiles were at times distorted, suggesting that the fibrils of an interstitial cell were pulling onto the alveolar basement membrane (Figs. 2 and 5). In lungs fixed by perfusion, and to a lesser degree in those fixed by instillation, some alveoli had angular contours (Figs. 2, 5, and 7), wherever

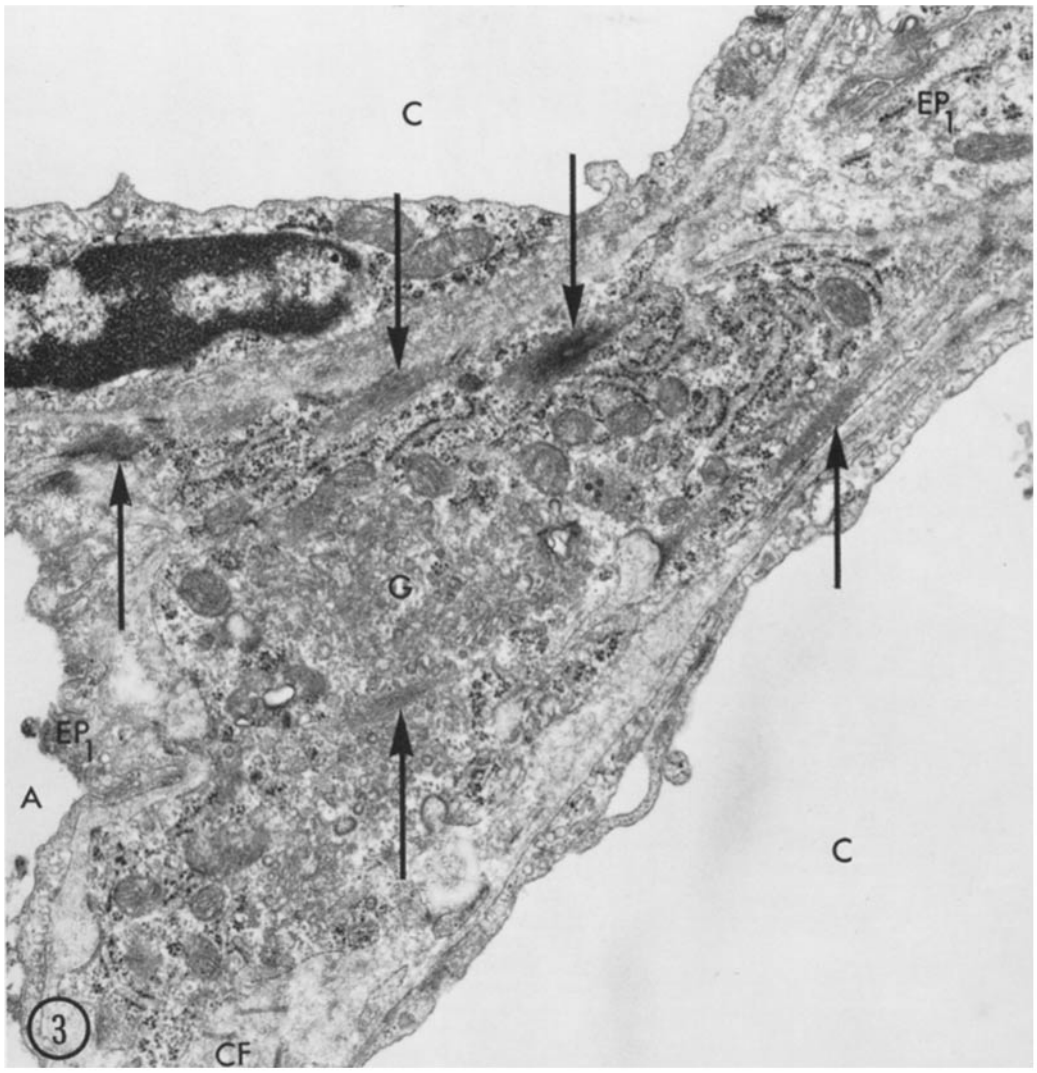

FIGURE 3 Interstitial cell containing fibrillar bundles (arrows). This cell lies within the thick portion of the air-blood barrier. It contains fibrils which are arranged in small bundles beneath the plasma membrane. This feature plus the abundance of the rough endoplasmic reticulum and the prominent Golgi apparatus (*G*) distinguishes this cell from a "usual smooth muscle cell." *A*, alveolus; EP_1, alveolar epithelium; *C*, capillaries; *CF*, collagen fibers. (Rat lung fixed by perfusion.) × 24,400.

an interstitial cell containing fibrillar bundles was located. Long pseudopodal projections of such cells extended between the alveolar and the capillary basement membranes (Fig. 7). The intracellular situation of the fibrillar bundles was not always easy to recognize, and they could be mistaken for extracellular connective tissue microfibrils (Fig. 6).

It should be noted that bundles of fibrils with hemidesmosome-like structures were not found in cells other than those interpreted as of "fibroblast type." In our sections we did not encounter fully differentiated smooth muscle cells. Very occasionally between an interstitial cell and the capillary endothelium a small portion of a cell containing many fibrils with dense bodies was seen (Fig. 7). Such a structure could be interpreted as being a portion of a pericyte since it appeared to be surrounded by a basement membrane. However, from the study of many consecutive

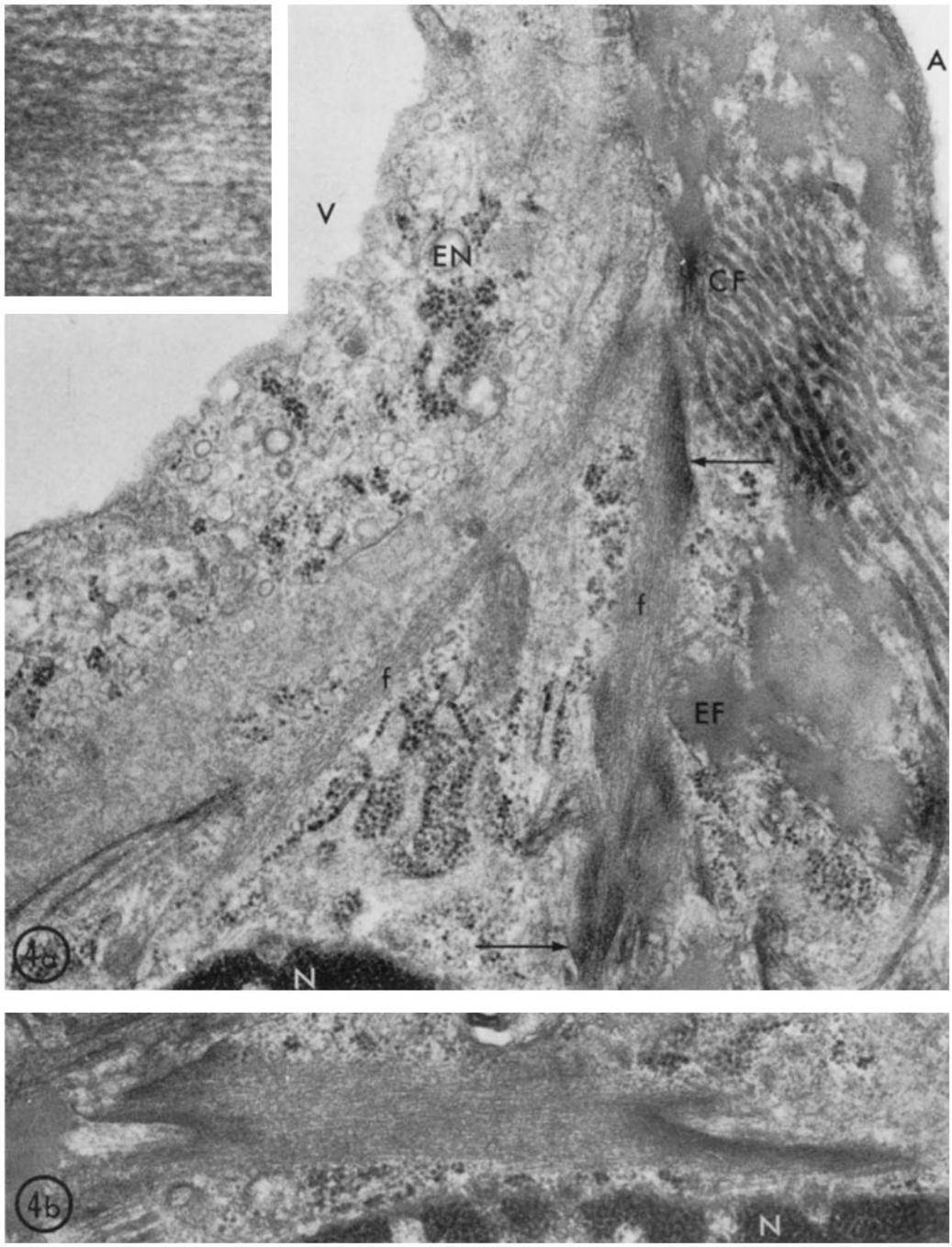

FIGURE 4 (*a*) Prominent fibrillar bundles (*f*) in an interstitial cell adjacent to a pre- or postcapillary vessel (*V*). Note the dense bodies (arrows) appearing as if they were anchoring the cell to collagen (*CF*) and elastic (*EF*) fibers. High magnification of the intracellular fibrils shows that they measure 30–80 Å in diameter (*inset*). (*b*) An individual fibrillar bundle with dense bodies at each extremity. *N*, nuclei; *EN*, endothelial cell; *A*, alveolus. (Rat lung fixed by perfusion.) *a*, × 41,500; *b*, × 43,200; *inset*, × 179,220.

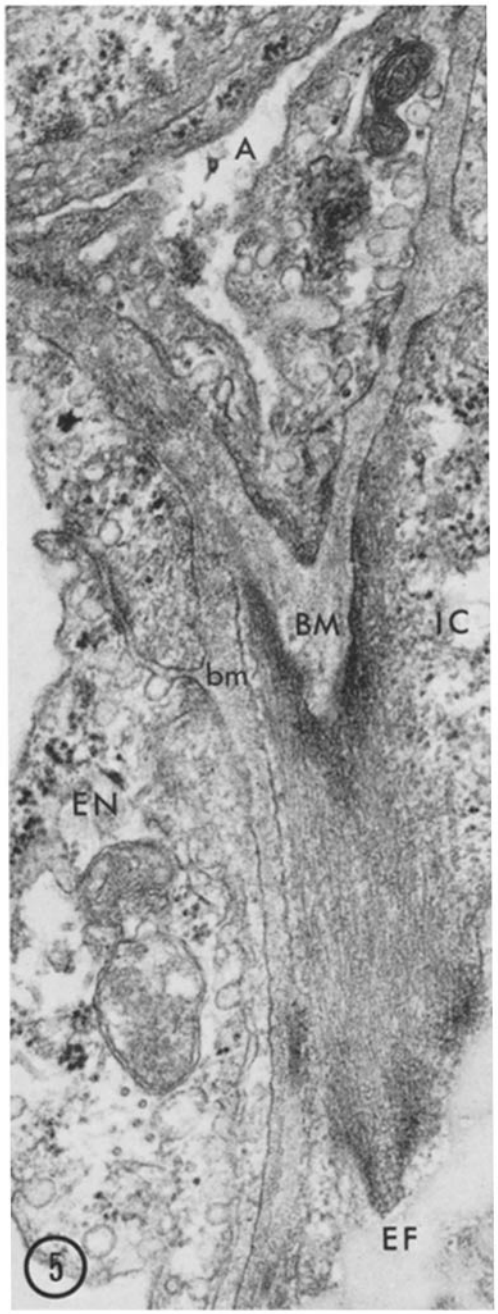

FIGURE 5 A fibrillar bundle "anchoring" the cell onto an alveolar basement membrane (*BM*) and onto an elastic fiber (*EF*). The alveolus (*A*) is collapsed; its basement membrane (*BM*) appears as if it were pulled by the intracellular fibrils, *bm*, capillary basement membrane; *EN*, endothelium; *IC*, interstitial cell. (Rat lung fixed by perfusion.) × 51,800.

sections it seems probable that such cell portions correspond to a transversal or oblique cut of an interstitial-cell pseudopod engulfed by the capillary basement membrane.

Intracellular fibrils and hemidesmosome-like structures could be detected in about 50% of the interstitial cells. The two main regions where such cells could be seen were: first, the connective tissue surrounding the pre- or postcapillary vessels (Fig. 4); second, the thick portion of the air-blood barrier at the alveolar septa (Figs. 2, 3, 5–8). Human, monkey, and lamb lungs showed similar structures in interstitial cells of lung alveoli; they were more abundant and more dense in alveolar interstitial cells of man (Fig. 8).

Immunofluorescence Studies

Cryostat sections treated with AAA serum showed obvious specific fluorescence within cells in the alveolar walls (Figs. 9–11); fluorescence of bronchial and arterial smooth muscles served as a specific control in each preparation. Fluorescent cells were scattered in the alveolar septa and around the pre- or postcapillary vessels (Figs. 9–10). In the peribronchial and perivascular connective tissue on fluorescent cells were visible. The fluorescein-labeled cells were oval or fusiform. A few linear structures also appeared; they were interpreted as pseudopodal projections of the fluorescent cells (Fig. 10). When preparations were treated with AAA serum absorbed by purified thrombosthenin-A, the staining of interstitial cells as well as that of smooth muscle cells were abolished (Fig. 11), demonstrating that the material stained by the fluorescent antibody was really actin (16). As an incidental finding it was observed that ciliated cells in bronchial epithelium were stained with AAA serum (Fig. 11 *a*). This was thought to indicate the presence of contractile proteins, related to ciliar function of these cells. A general discussion about actin-containing epithelial cells is reported elsewhere (16).

In Vitro Studies

PARENCHYMAL STRIPS (81 rat, 3 human, 12 bovine): Rat, human, and bovine lungs reacted similarly. When the medium was saturated with 95% O_2 + 5% CO_2 a relaxation resulting in elongation of the strips was observed. This relaxation was rapid during the first 5 min and reached a

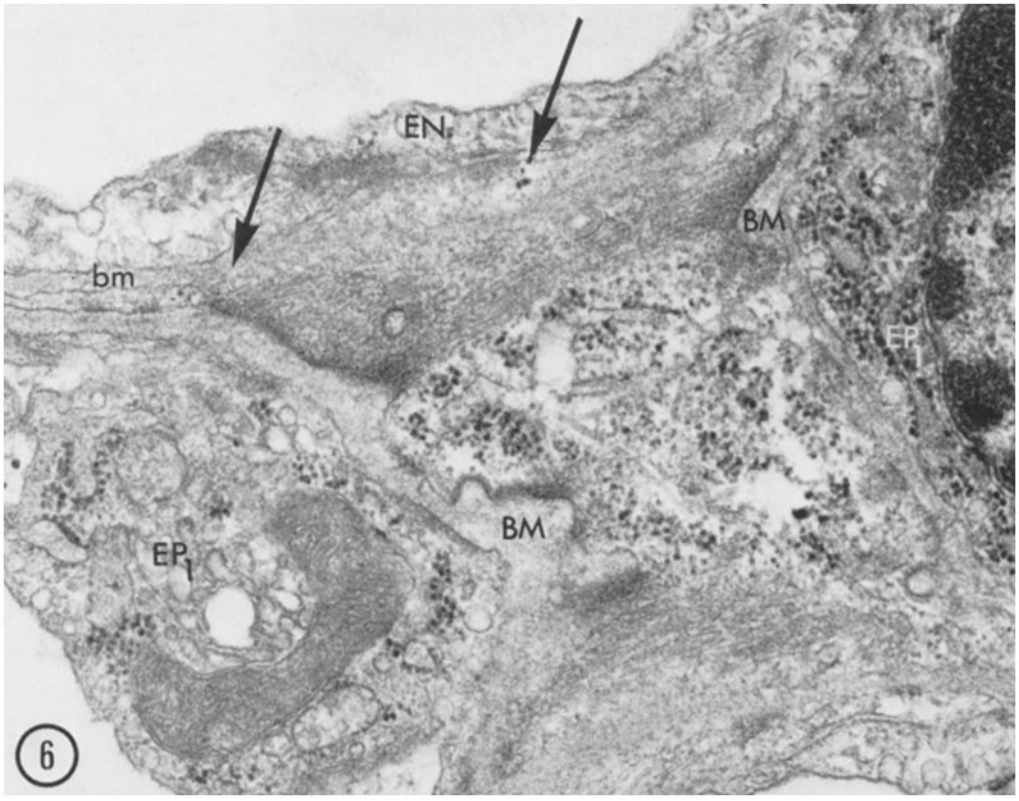

FIGURE 6 Intracellular fibrillar bundles located between two alveolar basement membranes (*BM*). Since the cell boundaries (arrows) are indistinct, these fibrils may be mistaken for interstitial microfibrils. *bm*, capillary basement membrane; *EN*, endothelium; EP_1, alveolar epithelium. (Rat lung fixed by perfusion.) × 48,500.

base line after about 20 min (Fig. 12 *a*). Since some elongation occurred also during passive room-air diffusion (room-air stabilization), this initial relaxation phase was attributed to a combined effect of stretch and hyperoxia; indeed, after room-air stabilization, if O_2 was bubbled through the medium, further elongation of the strips occurred (Fig. 12 *b*).

Saturation of the medium with hypoxic (5% and 10% O_2) or anoxic gas mixture was followed by contraction of parenchymal strips (Fig. 13). The 5–6% shortening recorded represented "work" since the tissue samples were mounted in the chamber under 10 mg of traction. Contraction of parenchymal strips in hypoxic media occurred regardless whether the gas mixtures contained 5% CO_2 (12 experiments) or no CO_2 (52 experiments). The strips started to contract when pO_2 of the medium was 80 mm Hg or below; the slope and the amplitude of the contraction curve seemed to depend on the degree of O_2 depletion (Fig. 13). Readmission of 95% O_2 + 5% CO_2 or addition of papaverine into the medium was followed by relaxation (Fig. 13). Alternate bubbling of hyperoxic and hypoxic gas mixtures (six experiments) was followed by alternate relaxation and contraction, respectively (Fig. 14).

Pharmacological experiments were performed with 27 rat and 5 bovine parenchymal preparations. In some cases epinephrine (10^{-6} g/liter) and serotonin (10^{-5} g/liter) were tested alternately on the same sample. Addition of *epinephrine* to the medium saturated with 95% O_2 + 5% CO_2 was followed by an immediate and sustained contraction of the parenchymal strips (Fig. 15). In hypoxic media, as the strips were already contracted, no further reaction could be observed. Occasionally epinephrine seemed to delay the

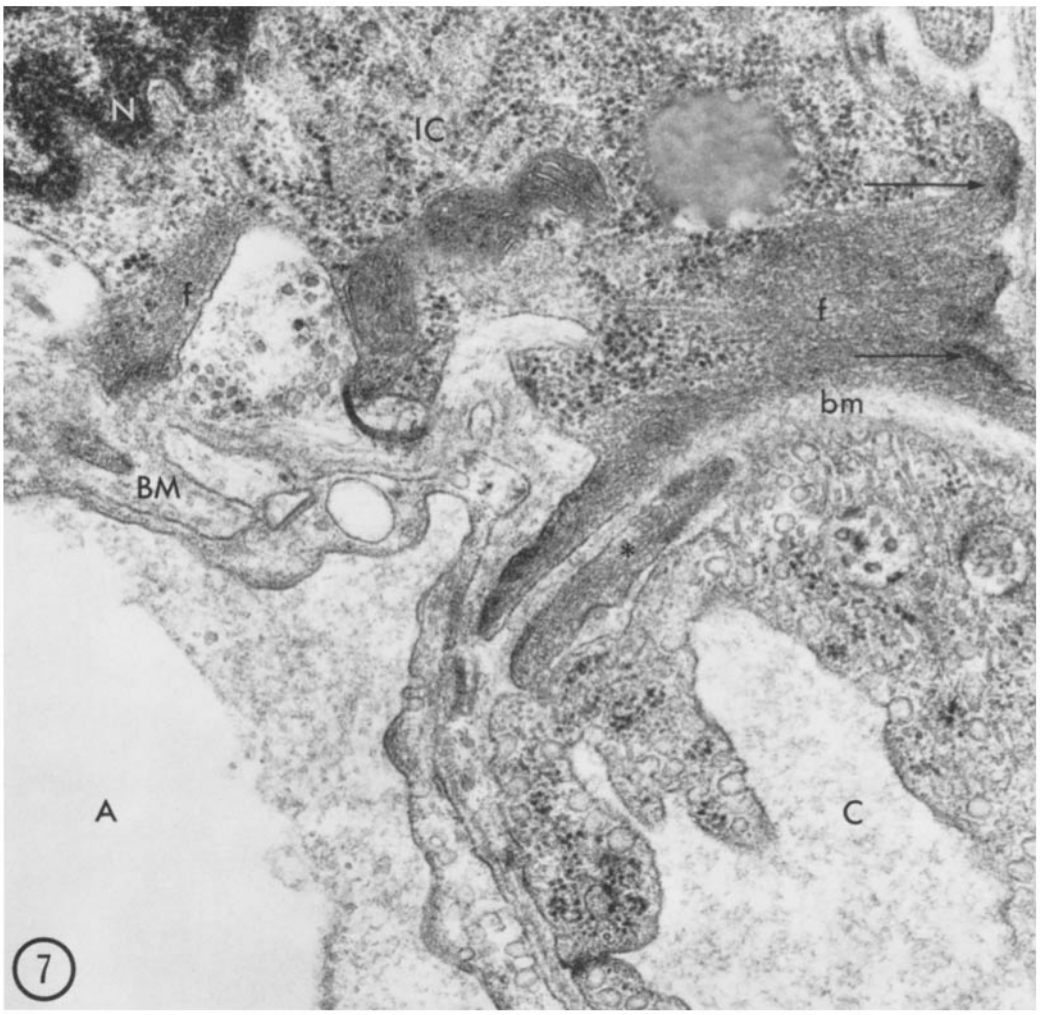

FIGURE 7 An interstitial cell (*IC*) with fibrillar bundles (*f*). Some fibrils are seen next to the alveolar basement membrane (*BM*) and a larger bundle next to the capillary basement membrane (*bm*). At the right edge of the figure another basement membrane adjacent to dense bodies (arrows) can be distinguished. The nuclear membrane (*N*) is slightly folded. Between the interstitial cell and the capillary (*C*) endothelium, a portion of a cell (*) is visible (portion of the interstitial cell ? Pericyte ? See text). The alveolus (*A*) is lined by the base layer of surfactant. (Rat lung fixed by instillation.) × 40,000.

relaxation of these strips upon readmission of 95% O_2 + 5% CO_2 into the chamber. Addition of *serotonin* produced no significant effect on the parenchyma neither in hyperoxic nor in hypoxic media. However, in a few instances a small and transient peak of contraction was observed (Fig. 16 *a*). This contraction occurred also in strips previously contracted by epinephrine or hypoxia; its magnitude was comparable whether the parenchymal sample was previously stimulated or not with epinephrine (Fig. 16 *a* and *b*). Such observations suggested that serotonin acted on structures other than those stimulated by epinephrine. In fact, histological examination of the strips which reacted to serotonin showed that they contained some muscular arteries and bronchi over 100 μm in diameter with substantial musculature (particularly bovine lungs). It should be noted that although some individual amplitude variations occurred in the response of the strips to pharma-

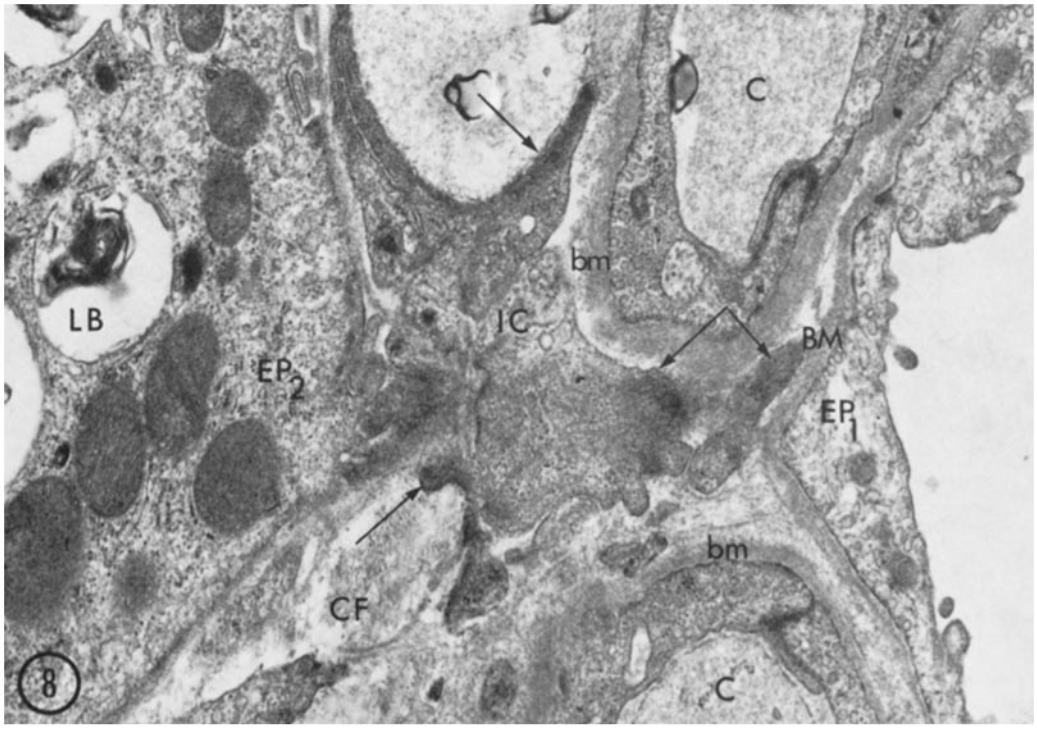

FIGURE 8 Alveolar septum in a normal human lung. An interstitial cell (*IC*) containing fibrils is situated at the thick portion of the air-blood barrier. Dense bodies are indicated by arrows. *CF*, collagen fibers; *C*, capillaries; *BM*, basement membrane; *bm*, capillary basement membrane; EP_1, membranous pneumocytes; EP_2, granular pneumocytes; *LB*, lamellated body. (Lung of a 49 year old man fixed by instillation 30 min after death.) × 29,000.

cological stimuli, a given substance always produced the same type of response.

ARTERIAL STRIPS (19 bovine, 4 human): Arteries relaxed in hyperoxic and hypoxic (or anoxic) media (Fig. 17 *a*). This relaxation did not seem to be related to the oxygen tension in the medium but more probably to the stretch applied (50 mg traction). Stimulation of such relaxed arteries with serotonin resulted in an immediate and violent contraction (about 40% shortening) while epinephrine caused only a slight and transient shortening of the strips (Fig. 18).

BRONCHIAL STRIPS (20 bovine): Bronchi contracted (30–40% shortening) in hyperoxic media and relaxed as soon as hypoxic (5% or 10% O_2) or anoxic gas mixtures were bubbled through the bath. Alternate hyperoxia and anoxia was followed by alternate contraction and relaxation, respectively (Fig. 17 *b*). This finding was exactly contrary to that observed when parenchymal samples were treated in the same manner (Fig. 14). Two bronchial preparations were left in Tyrode solution for 10 min at passive room-air diffusion (room-air stabilization) and then the solution was saturated with 95% O_2 + 5% CO_2: an immediate contraction occurred; admission of 5% O_2 was followed by relaxation. Epinephrine caused relaxation of bronchial strips and, as expected, serotonin produced a violent contraction of these samples (about 40% shortening). Both arterial and bronchial strips relaxed after the addition of papaverine HCl 2% into the medium (Figs. 17 and 18).

DISCUSSION

The human antiactin antibody (AAA) serum (16) used in this study was the smooth muscle autoantibody serum (SMA) obtained from patients with chronic aggressive hepatitis (16, 17). It reacted with different smooth muscle samples obtained from rats (17, 28) and stained also granu-

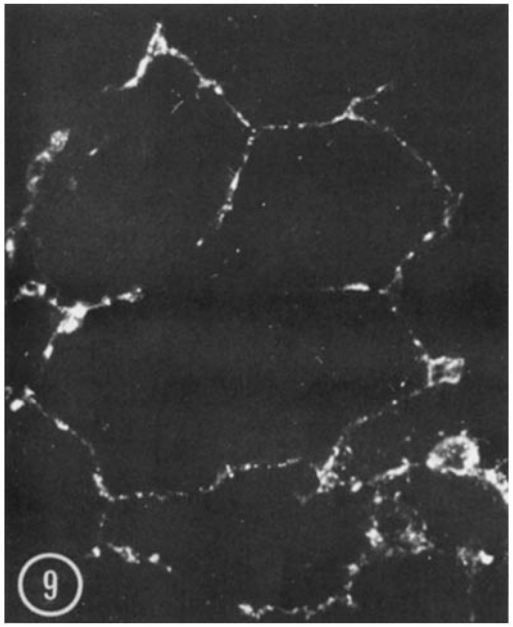

FIGURE 9 Frozen section of rat lung treated with human AAA serum followed by fluorescein-conjugated goat antihuman IgG serum. Numerous fluorescent cells in the alveolar septa and at the junction between three alveoli are visible × 160.

lation tissue fibroblasts, which have been shown to possess contractile properties (28). More recently it was demonstrated that the antibodies present in SMA sera are antiactin antibodies (16). In fact, incubation of SMA sera with purified thrombosthenin-A suppresses the labeling of actin-containing structures such as smooth muscle cells or granulation tissue fibroblasts (16). Therefore it seems plausible that the fluorescent cells seen in the walls of the alveoli (Figs. 9–11) are endowed with contractile proteins. Of course, on the basis of light microscope immunofluorescence studies alone, it would not be possible to determine whether we are dealing with smooth muscle or with other cell types. However, our electron microscope studies demonstrate that the alveolar septa contain cells identified until now as fibroblasts (11, 14, 19, 20), while also possessing fibrillar bundles similar to those of smooth muscle fibers. These cells differ from "usual smooth muscle" mainly because of their abundant ribosomes and rough endoplasmic reticulum, and also because the fibrils do not fill the cytoplasm, but appear as bundles scattered beneath the cell membrane (Fig. 3). Moreover, such cells are surrounded by interstitial fibers and are devoid of a basal lamina. They are possibly attached to the capillary and to

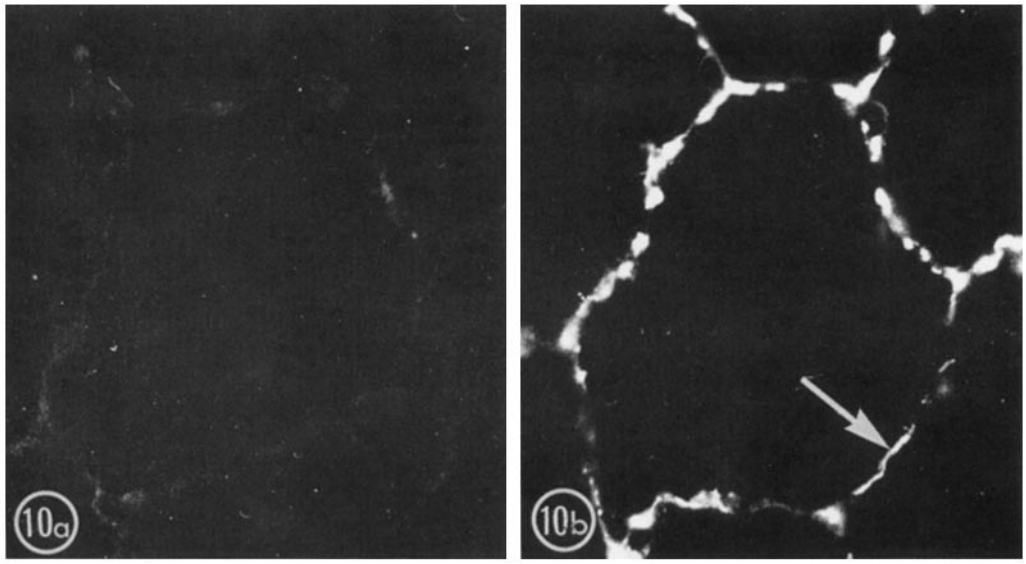

FIGURE 10 Frozen sections of rat lung. (*a*) Treated with normal human serum (control); (*b*) adjacent section treated with AAA serum. The arrow points to a linear fluorescent structure interpreted as a pseudopodal projection of an interstitial cell. × 500.

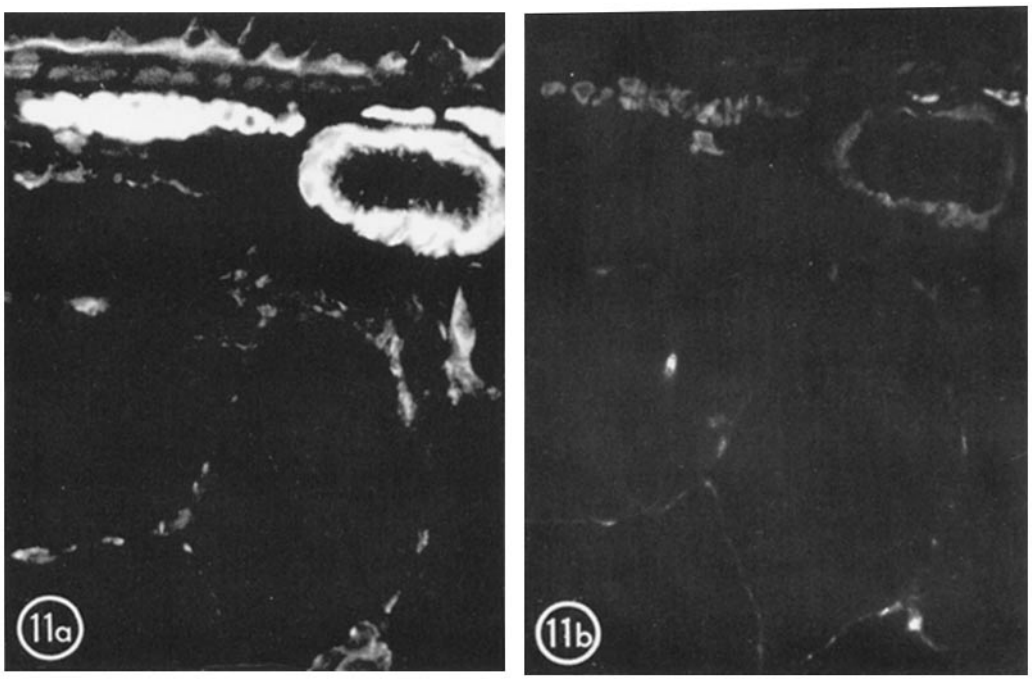

FIGURE 11 Frozen section of rat lung. (*a*) AAA serum stains arterial and bronchial smooth muscle as well as many alveolar interstitial cells. As an accidental finding, note the weak but definite fluorescence of bronchial epithelium. (*b*) Adjacent section stained with AAA serum previously absorbed with thrombosthenin-A; no fluorescence is visible. × 500.

the alveolar basement membranes through a hemidesmosome-like structure. (Figs. 2, 4, and 7). Thus, by combining the immunofluorescence and electron microscope data, it is concluded that the alveoli possess cells provided with potentially contractile structures; these cells are neither smooth muscle cells nor pericytes as commonly described.

Cells other than "usual muscle fibers" with contractile properties have been known for about a hundred years (29). Fibrillar structures resembling those of smooth muscle cells were first identified in the endothelium and pericytes (30–33) and later in some connective tissue cells (27, 34–36). Parallely actomyosin was demonstrated in cells other than muscle cells (28, 37, 38). The fibroblasts of granulation tissue contain prominent fibrillar bundles (27); immunologic and pharmacologic investigations suggested that these cells were contractile (28, 36). On account of their morphological features, intermediate between those of a muscle cell and those of a fibroblast, they were called myofibroblasts (27, 34). Other investigators have described similar fibrils (39, 40) and actomyosin (41) in normal glomerular mesangial cells; on the other hand, it was demonstrated that in some circumstances the mesangial cells might lay down collagen, behaving thus as fibroblasts (42).

Our in vitro studies show that parenchymal strips contract in hypoxic media as well as with epinephrine stimulation. A similar in vitro response of parenchyma to decreased O_2 tension was reported by previous authors (43); the contraction of the strips was attributed to the response of arterial or less probably to that of extra bronchovascular smooth muscle fibers (43). Our findings indicate that small arteries and bronchi contained in parenchymal preparations are not responsible for the contraction of these strips (44). This is supported by the three following observations: (*a*) Isolated arterial strips are not affected by changes in O_2 tension in the medium, whereas during hypoxia bronchial strips relax (Fig. 17) and parenchymal strips contract (Fig. 13). Epinephrine, which triggers a contraction of parenchymal strips (Fig. 15), has a lesser effect

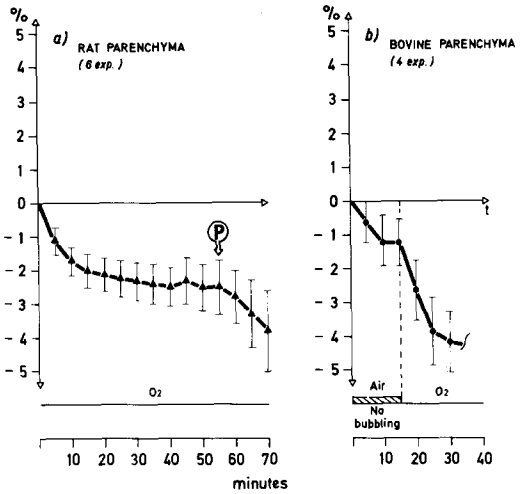

FIGURE 12 Relaxation of parenchymal strips in hypoxic media. (*a*) Relaxation is produced by bubbling 95% O_2 + 5% CO_2 and a further relaxation by injection of papaverine (*P*) into the bath (means and standard errors of six experiments). (*b*) A relaxation, in two steps, is produced by room-air stabilization and subsequent bubbling of 95% O_2 + 5% CO_2 (means and standard errors of four experiments). Negative values indicate the percentage of elongation with respect to the strip length, the level zero representing the initial recording.

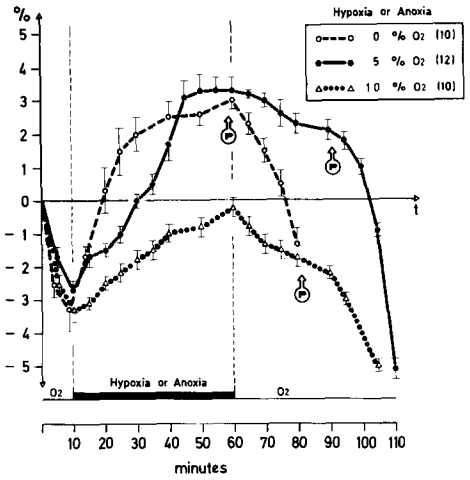

FIGURE 13 Contraction of rat lung parenchymal strips in hypoxic or anoxic media. Concentrations of O_2 in N_2 and the number of experiments (in brackets) are indicated in the *inset*. On the graph, means with standard errors are reported. Hypoxia (or anoxia) produces contraction of the strips, hyperoxia, and/or papaverine (*P*) produces relaxation. The positive values indicate the percentage of shortening and the negative values the percentage of elongation with respect to the strip length, the level zero representing the initial recording.

on arteries (Fig. 18) and relaxes the bronchi. If the contraction of parenchymal strips depended on the reaction of small arteries and bronchi within the strips, one should expect no parenchymal response to hypoxia and only a minimal response to epinephrine; as stated above, this is not what was recorded. Of course this first argument, based on the assumption that small arteries and bronchi within the parenchymal strips react similarly to the larger ones (1–2 mm Φ) tested, might be objected. However, (*b*) serotonin triggers a violent contraction of arterial (Fig. 18) and bronchial strips; it has practically no effect on parenchymal samples. Serotonin is a known stimulator of smooth muscle fibers. If the contractibility of parenchymal strips depended on the arterial and/or bronchial musculature, one should observe a more appreciable contraction of serotonin-stimulated parenchymal samples. (*c*) In hypoxic media, the strips already being in a state of contraction, stimulation with epinephrine does not produce further shortening. In hyperoxic media, when contraction of the parenchymal strips has been induced by epinephrine, stimulation by serotonin still produces a small peak of contraction (Fig. 16), particularly if tested samples contained substantial arterial and/or bronchial musculature. The amplitude of this peak is of the same order of magnitude whether serotonin is given before or after epinephrine stimulation (Fig. 16). These findings indicate that serotonin stimulates other structures than the targets of hypoxia and epinephrine action. Since serotonin triggers violent contraction of arterial and/or bronchial musculature, we conclude that in epinephrine-induced as well as hypoxia-induced contractions, tissue other than arterial and bronchial musculature are implicated. Can this be so-called "extra bronchovascular lung musculature" (45–47)? The answer is negative since such muscle would have reacted to serotonin and furthermore the quantity of typical smooth muscle fibers in pulmonary tissue is too small to be functionally significant (2). Indeed, although such fibers exist in the walls of alveolar ducts, their supposed existence in alveolar septa (45, 47) is denied (8, 9). In our own experience, with lungs of man and monkeys, completely differentiated smooth muscle cells are very seldom observed in normal alveolar septa (14, 15); they do not exist in the rat lung. We

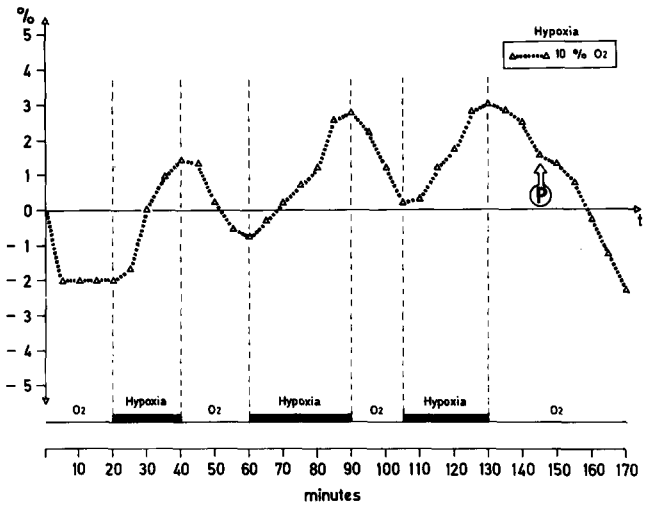

FIGURE 14 A human lung parenchymal strip tested alternately with hyperoxic (95% O_2 + 5% CO_2) and hypoxic (10% O_2 + 90% N_2) gas mixtures. Alternate relaxation and contraction phases are observed, papaverine (*P*) causing relaxation.

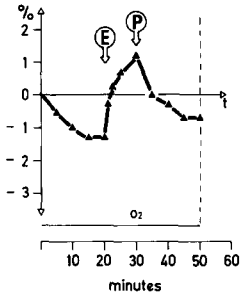

FIGURE 15 Contraction of rat lung parenchymal strip by epinephrine (*E*) and relaxation by papaverine (*P*).

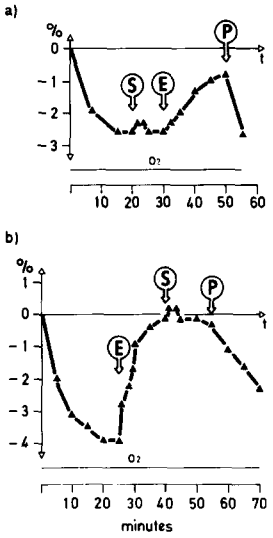

FIGURE 16 Effects of serotonin (*S*), epinephrine (*E*), and papaverine (*P*) on two adjacent rat lung parenchymal strips. (*a*) Serotonin injected into the bath before epinephrine induces a slight contraction. (*b*) Serotonin injected into the bath after epinephrine induces a similar contraction.

therefore conclude that the contraction of parenchymal strips induced by hypoxia or epinephrine is due essentially to the contraction of alveolar tissue itself (18, 44), and more precisely to the cells which contain fibrillar bundles as well as actin, as described herein. We propose to call such cells "contractile interstitial cells."

Our studies suggesting that the sacculi are actively contractile open a new topic of investigation in pulmonary physiology. If one admits that the contraction of parenchymal strips in hypoxic media is at least partly achieved by the play of interstitial cells, one may conclude that the alveoli participate actively in the autoregulation of ventilation/perfusion ratio. In fact, there are physiological observations suggesting that the degree of alveolar opening regulates the perfusion in the lungs; when the alveoli close, as in collapsed lung, the resistance to flow increases (3–6, 48–52). Hence the closing of the alveoli, by contraction of interstitial cells, might contribute to ventilation/perfusion autoregulation. The mechanism of this regulation is now being studied in our laboratories.

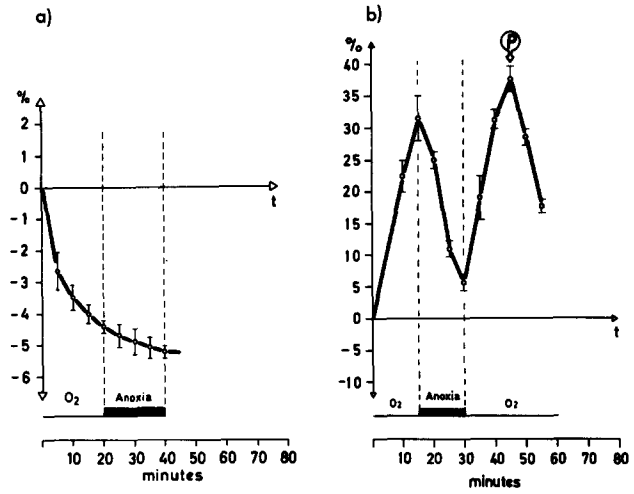

FIGURE 17 (*a*) Relaxation of the strips of bovine pulmonary artery after the bubbling of 95% O_2 + 5% CO_2 or 100% N_2 (anoxia) through the bath. Means and standard errors of five experiments. (*b*) Contraction of bovine bronchial strips after the bubbling of 95% O_2 + 5% CO_2. When 100% N_2 (anoxia) is introduced, the same strips relax. Readmission of O_2 induces a new contraction which is followed by relaxation after injection of papaverine (*P*) into the bath.

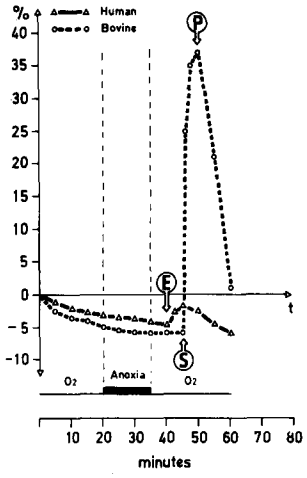

FIGURE 18 Contraction of two strips of pulmonary artery (one human, one bovine) after epinephrine (*E*) and serotonin (*S*) stimulation and relaxation by papaverine (*P*). Reaction to serotonin is much more violent than to epinephrine.

It has been demonstrated that hypoxic pulmonary hypertension is more severe during alveolar hypoxia than during arterial pO_2 decrease (53–58), suggesting a "vasopressor response" at the very distal portion of the pulmonary vascular tree (53, 54, 58). Our study demonstrates that the alveolar contractile cells are abundant in the septa and around the pre- and postcapillary vessels. On account of their anatomic distribution, these cells are perfect candidates for reacting instantly to changes in the composition of alveolar gas. By virtue of their presumed attachment to the basement membranes of alveoli, capillaries, and pre- or postcapillary vessels, these cells may modify the vascular conductance.

In conclusion, it seems probable that the contraction of interstitial cells may at least partly explain the acute pulmonary hypertension due to hypoxia. At present, the reasons why the pulmonary vascular bed and the systemic vessels react in opposite manner to decreased oxygen tension are not established (2, 59–61). Our findings suggest that the "paradoxical response of lung vessels to hypoxia" (2, 60) may be a matter of different reacting structures, i.e., vascular musculature vs. contractile interstitial cells of the pulmonary alveolar septa.

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