



## Cellular Responses to Mechanical Stress

### Invited Review: Pulmonary capillary stress failure

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**West, John B.** Invited Review: Pulmonary capillary stress failure. *J Appl Physiol* 89: 2483–2489, 2000.—The pulmonary blood-gas barrier is an extraordinary bioengineering structure because of its vast area but extreme thinness. Despite this, almost no attention has been given to its mechanical properties. The remarkable area and thinness come about because gas exchange occurs by passive diffusion. However, the barrier also needs to be immensely strong to withstand the very high stresses in the capillary wall when capillary pressure rises during exercise. The strength of the thin region of the barrier comes from type IV collagen in the basement membranes. When the stresses in the capillary walls rise to high levels, ultrastructural changes occur in the barrier, a condition known as stress failure. Physiological conditions that alter the properties of the barrier include severe exercise in elite human athletes. Animals that have been selectively bred for high aerobic activity, such as Thoroughbred racehorses, consistently break their pulmonary capillaries during galloping. Pathophysiological conditions causing stress failure include high-altitude pulmonary edema and overinflation of the lung, which frequently occurs with mechanical ventilation. Remodeling of the capillary wall occurs in response to increased wall stress in diseases such as mitral stenosis. The barrier is able to maintain its extreme thickness with sufficient strength as a result of continual regulation of its wall structure. How it does this is a central problem in lung biology.

blood-gas barrier; basement membrane; extracellular matrix; type IV collagen; pulmonary edema; pulmonary hemorrhage; endothelial cells; epithelial cells

THE STUDY OF PULMONARY MECHANICS has a long, colorful history. For example, Galen (131–201 CE) understood how the expansion of the lungs follows that of the thorax, and he recognized that the diaphragm is innervated by nerves that originate high in the neck (16). It is extraordinary that, despite this long history, what is arguably the most remarkable mechanical structure in the mammalian lung, that is, the blood-gas barrier (BGB), has received almost no attention from bioengineers or physiologists interested in pulmonary mechanics.

Consider the following: the total area of the BGB in the human lung is 50–100 m<sup>2</sup> (17). In more than half of this enormous area, the thickness of the BGB is only

0.2–0.3 μm. That such an **incredibly thin** membrane can extend over such a **vast area without breaking** always amazes my **structural engineering** friends, and attempts to reproduce a similar gas-exchanging surface in **artificial** lungs **fall ludicrously short**. The vulnerability of such a vast but extremely thin membrane is obvious. Moreover, the sequelae of failure are potentially disastrous, since plasma or blood will enter the alveolar spaces, putting an end to gas exchange in that region. It is truly remarkable that so little attention has been devoted to the mechanics of this extraordinary structure.

Our interest in possible failure of the BGB was initially aroused by the puzzle of the pathogenesis of high-altitude pulmonary edema (**HAPE**). We knew that a high pulmonary artery pressure was a critical factor in the development of HAPE (25). This clearly suggested a pressure-related basis. It was then found

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that the alveolar fluid in HAPE was of the high-permeability type, with high concentrations of large molecular weight proteins and many cells (19, 43). This was strong evidence of damage to the BGB. We therefore wondered what capillary pressures would be required to cause ultrastructural changes in their walls (57), and our laboratory carried out a systematic study of the electron microscope appearances of the BGB as capillary transmural pressure was raised (49).

Surprisingly, some disruptions of the capillary endothelium and alveolar epithelium were found to occur at capillary transmural pressures as low as 24 mmHg, although consistent failure required pressures that approached 40 mmHg. These findings initially received a frosty reception from some people who argued that the pressures were far too high to be of any physiological significance. However, important misconceptions exist about how high the capillary pressure can rise under physiological conditions (53). For example, the chapter on pulmonary circulation in the American Physiological Society's *Handbook of Physiology* includes the statement "The pulmonary wedge pressure is unaffected by mild exercise but may increase slightly as the intensity of the exercise increases" (14). In addition, a popular current textbook of physiology states "Because the left atrial pressure in a healthy person almost never rises above +6 mmHg even during the most strenuous exercise, the changes in left atrial pressure have virtually no effect on pulmonary circulatory function except when the left side of the heart fails" (18). However, these are misconceptions. Direct measurements of mean pulmonary arterial wedge pressure in normal subjects during severe exercise indicate that this rises to over 20 mmHg (51) with the result that the capillary transmural pressure at the base of the lung must exceed 25 mmHg (57). Such pressures must cause very high hoop stresses in the thin-walled capillaries according to the Laplace relationship (Fig. 1).

In view of these high capillary pressures during normal exercise, is there any evidence that the integrity of the BGB is altered? Yes, indeed. Hopkins et al. (23) studied six elite competition cyclists who sprinted uphill over 4 km at maximal effort, giving them a mean heart rate of 177 beats/min. Bronchoalveolar lavage (BAL) performed within 1 h of completion of the exercise showed higher concentrations of red blood cells, total protein, and leukotriene  $B_4$  than that found in normal, sedentary subjects who did not exercise before BAL. In a companion study, a similar group of six elite cyclists exercised at 77% of their maximal oxygen consumption for 1 h and showed no changes in their BAL fluid (22). Thus it appears that maximal exercise in elite athletes causes alterations in the BGB.

It is remarkable that Thoroughbred racehorses that have been selectively bred for very high levels of exercise consistently develop alveolar bleeding on exercise. For example, it has been shown that all Thoroughbreds in training contain hemosiderin-laden macrophages in their tracheal washings (58). Selective breeding has endowed these animals with extraordinarily high car-

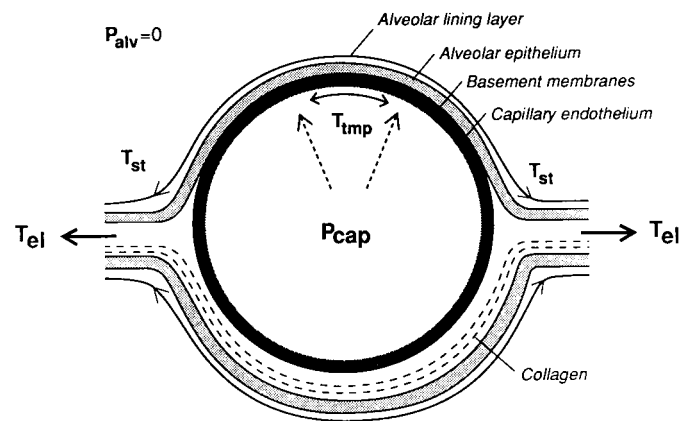


Fig. 1. Three forces act on the blood-gas barrier (BGB).  $T_{tmp}$  is the circumferential or hoop tension caused by the capillary transmural pressure.  $T_{el}$  is the longitudinal tension in the alveolar wall elements associated with lung inflation; part of this is transmitted to the BGB.  $T_{st}$  is the surface tension of the alveolar lining layer; this apparently exerts an inward-acting force to support the capillary when the latter bulges into the alveolar space at high capillary transmural pressures.  $P_{alv}$ , alveolar pressure;  $P_{cap}$ , capillary pressure. [From West et al. (57).]

diac outputs with the result that the filling pressures of the left ventricle are enormous. In animals galloping on a treadmill, left atrial pressure measured directly with an indwelling catheter can be as high as 70 mmHg and mean pulmonary artery pressure can be 120 mmHg (12, 26, 31). This means that the pulmonary capillary pressures in these animals approach 100 mmHg!

Why has the human lung evolved to produce a BGB that is so vulnerable that maximal exercise in elite athletes apparently alters its integrity? The answer is that the BGB has to satisfy two conflicting requirements. On the one hand, it has to be extremely thin for adequate gas exchange to occur by passive diffusion. Here, extreme thinness and large area are essential. However, even so, there is evidence that the diffusion rate through the BGB is not sufficiently fast for the blood to be fully oxygenated within the lung in some elite human athletes because it is known that the arterial  $P_{O_2}$  falls during high levels of exercise as a result of diffusion limitation (8, 51). Diffusion limitation is even more dramatic in the Thoroughbred racehorse, which develops severe arterial hypoxemia during galloping, partly because of limited pulmonary diffusion (52). Thus there would be an advantage in having a thinner BGB in these situations, and presumably there is continuous evolutionary pressure to keep the barrier as thin as possible.

However, at the same time, the BGB must be strong enough to prevent mechanical failure. Increasing strength can only be accomplished at the penalty of increasing thickness. Note that the Thoroughbred lung fails on both counts. The BGB is not thin enough for adequate diffusion but also not strong enough to prevent it breaking during exercise. Thus the human BGB faces a dilemma in that it needs to be extremely thin for gas exchange but just strong enough to maintain its

integrity when the capillary pressure rises maximally during exercise.

Where does the strength of the BGB come from? The thin region of the BGB with a thickness of only 0.2–0.3  $\mu\text{m}$  has only three layers: the capillary endothelium, the alveolar epithelium, and the extracellular matrix (ECM) formed by the fusion of the two basement membranes. Several pieces of evidence indicate that the strength comes from the ECM. For example, we frequently see ultrastructural evidence of disruptions of the capillary endothelium and alveolar epithelium while the ECM remains intact (49). The ECM contains four principal molecules, including type IV collagen, laminin, entactin/nidogen, and heparan sulfate proteoglycans (7). Type IV collagen has a triple helix structure like that of other matrix collagens but is unusual in that the COOH-terminal end has an NC1 globular domain that allows two of the  $\sim 400\text{-nm}$ -long molecules to join, forming a doublet. The  $\text{NH}_2$  terminus contains the 7S domain, which enables four doublet molecules to form a matrix configuration similar to chicken wire (47, 59). It is likely that the strength of the BGB comes principally from the type IV collagen. Measurements of the ultimate tensile strength of basement membrane are sparse but suggest a value of around  $2 \times 10^6 \text{ N/m}$ , which is not very different from that of the enormously strong type I collagen (13, 45).

There is evidence that the type IV collagen in the thin region of the BGB is not uniformly distributed throughout the ECM. Crouch and colleagues (7), using anti-human type IV collagen antibodies, showed that the distribution of type IV collagen is closely associated with the lamina densa in the center of the ECM (Fig. 2A). Thus it appears that the enormous strength of the thin part of the BGB is attributable to an extremely thin layer of type IV collagen, perhaps only 50 nm thick, which is sandwiched in the middle of the ECM (Fig. 2B). Note that, because the individual molecules are  $\sim 400 \text{ nm}$  in length, this implies that they have the configuration of layers of chicken wire that are placed

on a flat surface, thus providing the great tensile strength in the plane of the surface. This arrangement is well suited to withstanding the large hoop stresses that develop when the capillary transmural pressure is raised (Fig. 1).

We do not fully understand the micromechanics of failure of the BGB. Often, we see disruption of capillary endothelial and/or alveolar epithelial cells while the ECM remains intact. Scanning electron micrographs of the alveolar epithelium show that the disruptions occur within the cells, not at the intercellular junctions (6). Similar intracellular disruptions have been described in endothelial cells of microvessels of frog mesentery when the pressure is raised (37). An important observation is that most of the disruptions are rapidly reversible when the capillary transmural pressure is reduced. In fact, Elliott et al. (11) showed that  $\sim 70\%$  of both the endothelial and epithelial disruptions closed within a few minutes of reducing the capillary transmural pressure. Incidentally, this finding is consistent with the clinical observation that patients with HAPE typically recover very rapidly when brought to a lower altitude and the pulmonary vascular pressures are reduced.

Possibly distortion of the type IV collagen matrix allows some lengthening to occur under stress, and this is the basis for the cellular disruptions and their recovery when the stress is reduced (Fig. 3). It is known that type IV collagen molecules have sites that allow bending to occur. In human  $\alpha 1(\text{IV})$  and  $\alpha 2(\text{IV})$  polypeptide chains, about 25 irregularly spaced sites have been described that impart flexibility to the whole molecule (46). A 90-nm-long segment of high flexibility near the 7S domain has also been described (21). Thus it is possible, as Fig. 3 shows, that the chicken wire-like matrix is distorted when stressed, allowing intracellular disruptions of the capillary endothelial and alveolar epithelial cells to occur, and that these disruptions reunite when the stress is relieved and the matrix resumes its normal configuration.

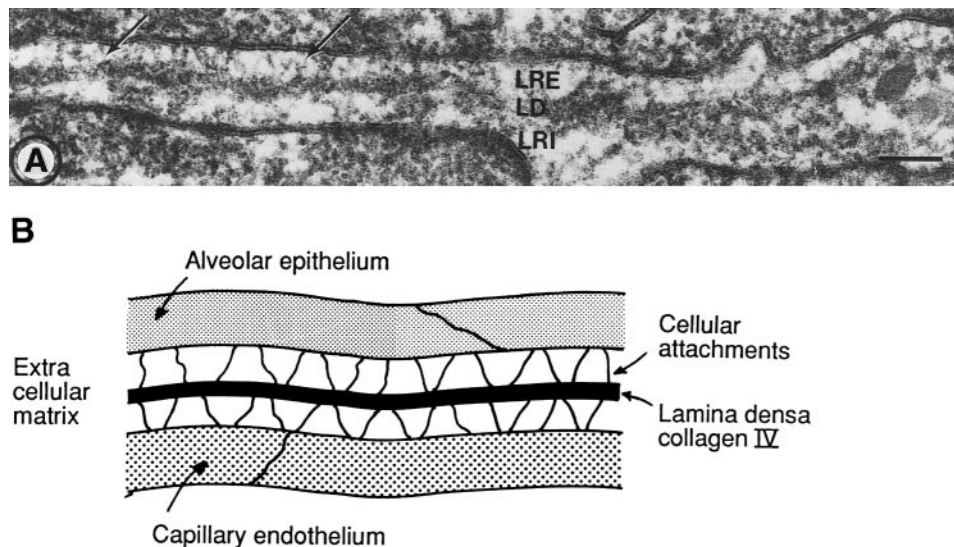
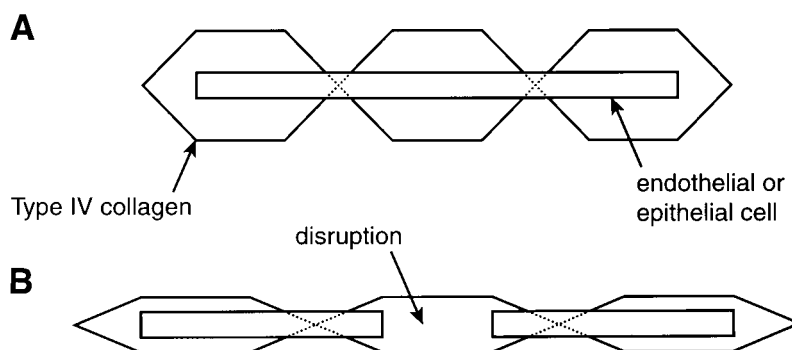


Fig. 2. A: ultrastructure of the thin part of the BGB in rat, with portions of the alveolar epithelial cell (top) and capillary endothelial cell (bottom). Note that the extracellular matrix (ECM) has a central lamina densa (LD) flanked by a lamina rara externa (LRE) and lamina rara interna (LRI). The arrows point to filaments in the lamina rara externa that connect the lamina densa to the basal epithelial cell surface. Bar = 0.1  $\mu\text{m}$ . [From Vaccaro and Brody (50).] B: diagram of the thin part of the BGB. Most of the type IV collagen, which is believed to be responsible for the strength of the BGB, is located in the lamina densa. This is only  $\sim 50 \text{ nm}$  thick and is sandwiched in the middle of the ECM (54).



Fig. 3. Diagram to indicate how distortion of the type IV collagen matrix could cause disruptions in the capillary endothelial and alveolar epithelial layers. *A*: situation at a normal capillary transmural pressure. *B*: situation at high pressure when the matrix is stretched. [Modified from West and Mathieu-Costello (55).]



Under what conditions does stress failure of pulmonary capillaries occur? We can identify both physiological and pathophysiological causes. The physiological conditions were referred to earlier when it was pointed out that elite human athletes at very high exercise levels apparently develop some changes in the BGB that allow red blood cells, protein, and leukotriene B<sub>4</sub> to enter the alveolar spaces (23). The failure is much more obvious in Thoroughbred racehorses because these animals routinely break their capillaries and bleed into their alveolar spaces (58). We have been able to demonstrate ruptured pulmonary capillaries in these animals after they have galloped at top speed on a treadmill (56).

Pathological conditions associated with stress failure of pulmonary capillaries include diseases in which the capillary pressure is increased to unphysiologically high levels. Reference has already been made to HAPE, in which the increase in pressure in some capillaries is apparently the result of uneven hypoxic pulmonary venous constriction, as originally suggested by Hultgren (24). Another pathological condition associated with stress failure of pulmonary capillaries is neurogenic pulmonary edema. This condition is known to be associated with very high pulmonary vascular pressures (41, 42) and the alveolar edema fluid is of the high-permeability type, with high concentrations of large-molecular-weight proteins and red blood cells (4). In addition, Minnear and colleagues (35, 36) have described disruptions of both capillary endothelial and alveolar epithelial cell layers in experimental neurogenic pulmonary edema. Other pathological conditions associated with unphysiologically high capillary pressures accompanied by pulmonary capillary stress failure include severe left ventricular failure and mitral stenosis.

A different pathological condition is Goodpasture's syndrome, in which the type IV collagen is abnormal because autoantibodies are produced that attack the NC1 globular domain. It is interesting that bleeding occurs both into the alveolar spaces and into the glomerular spaces. This serves to remind us that the glomerular capillaries are similar to the alveolar capillaries in that both can be exposed to high transmural pressures and, in both cases, the strength is attributable to the basement membrane.

A particularly important pathological condition involving stress failure of pulmonary capillaries is the

damage to the lung caused by overinflation. It has been known for many years that inflation of the lung to high volumes increases the permeability of pulmonary capillaries (5, 9, 10, 29, 39). This is a well-known serious problem in the intensive care unit, where high levels of positive end-expiratory pressure (PEEP) are required to maintain adequate levels of arterial PO<sub>2</sub>.

Figure 1 shows that the wall stress of pulmonary capillaries can be increased both by raising the transmural pressure of the capillaries and by increasing the longitudinal tension in the alveolar wall. In this context, the alveolar wall can be regarded as a string of pulmonary capillaries with the result that some of the increased tension in the alveolar wall caused by high states of lung inflation is transmitted to the capillary wall. We have shown in animal preparations that increasing lung volume from normal to high levels, while keeping the capillary transmural pressure constant, results in a great increase in the number of disruptions in both the capillary endothelial and alveolar epithelial layers (15). Consistent with this, a recent controlled trial of low and traditional tidal volumes during mechanical ventilation in intensive care units showed reduced mortalities with the low tidal volumes (3).

How is it that the BGB has evolved to be extremely thin, as required for adequate gas exchange by passive diffusion, but with just sufficient strength to maintain its integrity under all (or nearly all) physiological conditions? We believe the answer is that the structure of the BGB is continually regulated in response to capillary wall stress in some way. This regulation is known as remodeling.

There is a large literature on pulmonary vascular remodeling that deals with the changes in both the pulmonary arteries and veins (for example, see Refs. 32, 33, 40, 44, 48). Typical are the studies by Meyrick and Reid (33, 34), in which they showed that rats exposed to hypoxic gas developed new smooth muscle in the pulmonary arteries after 2 days. In a particularly interesting study, Tozzi et al. (48) used explants of rat pulmonary artery rings and applied mechanical tension equivalent to a transmural pressure of 50 mmHg for 4 h. They reported increases in collagen synthesis, elastin synthesis, mRNA for pro- $\alpha$ 1(I) collagen, and mRNA for protooncogene V-sis. They also showed that the changes were endothelium dependent because they did not occur when the endothelium was removed from the arterial rings.

It is extraordinary that, despite the extensive literature on vascular remodeling in pulmonary arteries and veins, the possible remodeling of pulmonary capillaries has been almost completely ignored. We know that this occurs because, as Fig. 4 shows, striking thickening of the basement membranes of the capillary endothelial and alveolar epithelial cells is seen in the pulmonary capillaries of patients with mitral stenosis (20, 28, 30) and pulmonary venoocclusive disease (27). Careful inspection of Fig. 4 suggests that most of the thickening of the basement membranes is associated with the capillary endothelial cell rather than the alveolar epithelial cell. This may be relevant to the observation of Tozzi et al. (48) referred to above in that, in the pulmonary arterial rings, the remodeling is endothelium dependent. The mechanisms by which physical forces are converted to biological signals (mechanotransduction) are poorly understood and are considered in other reviews in this series. Putative mechanisms include distortion of the cell membrane with consequent stimulation of ion channels and distortion of the cytoskeleton affecting the nucleus and thus alterations in transcription.

During the past few years, my colleagues and I have been studying the molecular consequences of increasing stress in the walls of pulmonary capillaries. As shown in Fig. 1, there are two obvious ways of increasing the wall stress of pulmonary capillaries. The first is to increase capillary transmural pressure and the second is to use high levels of lung inflation. As indicated earlier, it is known that the latter raises capillary wall stress because it greatly increases the frequency of endothelial and epithelial cell disruptions.

In one set of experiments, the volume of one lung of anesthetized open-chest rabbits was greatly increased, whereas the other lung was ventilated at a normal volume (2). Additional control animals had both lungs ventilated at normal, low levels. It was found that high states of lung inflation over 4 h resulted in increased gene expression for  $\alpha 1(\text{III})$  and  $\alpha 2(\text{IV})$  procollagens,

fibronectin, basic fibroblast growth factor, and transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ). In contrast, mRNA levels for  $\alpha 1(\text{I})$  procollagen and vascular endothelial growth factor (VEGF) were unchanged. An unexpected finding was that these changes in mRNA were identical in both the overinflated lung (9 cmH<sub>2</sub>O PEEP) and the normally inflated lung (1 cmH<sub>2</sub>O PEEP) for the preparation in which one lung was overinflated and the other was normally inflated. This suggests a generalized organ-specific response after the localized (unilateral) application of mechanical force, but the mechanism for this was not identified.

In another set of experiments, capillary transmural pressure was increased by raising the venous pressure in isolated perfused rat lungs (38). To limit the production of pulmonary edema, the venous pressure was increased cyclically to 28 cmH<sub>2</sub>O every 15 s of every minute for 4 h. This allowed fluid to leave the pulmonary capillaries when the venous pressure was raised and return to the capillary lumen when the pressure was reduced. Controls were similar lungs perfused at low pressure and also unperfused lungs. This study showed significant increases in gene expression for  $\alpha 1(\text{I})$  and  $\alpha 3(\text{III})$  procollagens, fibronectin, laminin, and TGF- $\beta_1$ .

A third method of increasing capillary wall stress, that is, alveolar hypoxia, was also investigated (1). This method increases pulmonary artery pressure in rats within minutes as a result of pulmonary vasoconstriction, and, if the vasoconstriction is uneven, some capillaries will be exposed to increased transmural pressures. Rats were exposed to 10% oxygen for 6 h or 3 days (short-term group) and 3 or 10 days (long-term group). Peripheral lung tissue was then collected, and mRNA levels for ECM proteins and growth factors were measured, as well as collagen content by hydroxyproline. mRNA levels for  $\alpha 1(\text{IV})$  procollagen increased sixfold after 6 h of hypoxia and sevenfold after 3 days. The levels then decreased after 10 days of exposure. mRNA levels for platelet-derived growth fac-

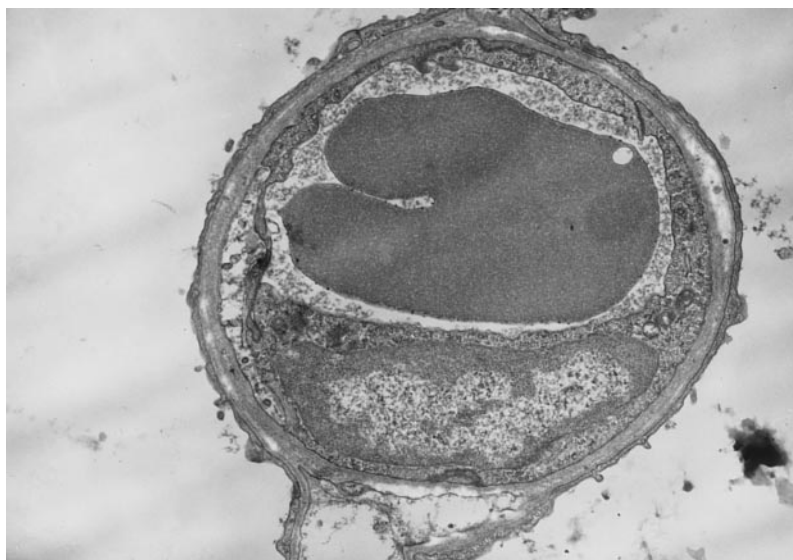


Fig. 4. Electron micrograph of a pulmonary capillary from a young patient with mitral stenosis. Note thickening of the basement membranes of the capillary endothelial and alveolar epithelial cells, particularly the former. [Courtesy of S. G. Haworth.]

tor B doubled after 6 h of hypoxia but returned to control values after 3 days. In addition, mRNA levels for  $\alpha 1(I)$  and  $\alpha 1(III)$  procollagens and fibronectin were increased after 3 days of hypoxia but then decreased toward control values after 10 days. In contrast, levels of VEGF mRNA and collagen content did not change.

Interpretation of the above experiments is complicated by the fact that we have not yet been able to devise a method of increasing capillary wall stress without increasing wall stress in other structures such as larger blood vessels or airways. To some extent, this objection was mitigated by sampling only peripheral lung parenchyma, which is mainly made up of alveolar tissue. At any event, these are initial steps in attempting to understand a central issue in lung biology, that is, how does the BGB maintain its extreme thinness with just enough strength to withstand the maximal stresses to which it is exposed under physiological conditions.

Odile Mathieu-Costello, PhD, had a major role in these studies.

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