*Am J Physiol Lung Cell Mol Physiol* 285: L501–L513, 2003;
10.1152/ajplung.00117.2003.

# Thoughts on the pulmonary blood-gas barrier

**John B. West**
*Department of Medicine, University of California San Diego, La Jolla, California 92093-0623*

**West, John B.** Thoughts on the pulmonary blood-gas barrier. *Am J Physiol Lung Cell Mol Physiol* 285: L501–L513, 2003; 10.1152/ajplung.00117.2003.—The pulmonary blood-gas barrier is an extraordinary structure because of its extreme thinness, immense strength, and enormous area. The essential components of the barrier were determined early in evolution and have been highly conserved. For example, the barriers of the African, Australian, and South American lungfish that date from as much as 400 million years ago have essentially the same structure as in the modern mammal or bird. In the evolution of vertebrates from bony fishes through amphibia, reptiles, and ultimately mammals and birds, changes in the pulmonary circulation occurred to limit the stresses in the blood-gas barrier. Only in mammals and birds is there a complete separation of the pulmonary and systemic circulations, which is essential to protect the extremely thin barrier from the necessary high-vascular pressures. To provide the blood-gas barrier with its required strength, evolution has exploited the high ultimate tensile strength of type IV collagen in basement membrane. Nevertheless, stress failure of the barrier occurs under physiological conditions in galloping Thoroughbred racehorses and also apparently in elite human athletes at maximal exercise. The human blood-gas barrier maintains its integrity during all but the most extreme physiological conditions. However, many pathological conditions cause stress failure. The structure of the blood-gas barrier is apparently continually regulated in response to wall stress, and this regulation is essential to maintain the extreme thinness but adequate strength. The mechanisms of this regulation remain to be elucidated and constitute one of the fundamental problems in lung biology.

vertebrate evolution; stress failure; type IV collagen; exercise-induced pulmonary hemorrhage; lung overinflation; pulmonary circulation

IT IS CERTAINLY AN HONOR to give this lecture in memory of Dr. Julius Comroe. I was fortunate enough to know him fairly well. I visited my many friends at the Cardiovascular Research Unit at University of California San Francisco on several occasions while he was there and gave a couple of lectures. But Dr. Comroe's influence on my career started long before this. Back in 1955, when I was newly graduated in medicine and working in London, Charles Fletcher recommended that I spend a year at the Medical Research Council Pneumoconiosis Research Unit (PRU) in South Wales to learn respiratory physiology so that I could join a new program that was planned for the Postgraduate Medical School, Hammersmith Hospital, in London. While I was at the PRU, the first edition of Dr. Comroe's *The Lung* (9) came out, and I still have my copy dated August 1955. That book made an enormous impression on me and was one of the main reasons why I chose a career in respiratory physiology. When I joined the Postgraduate Medical School a year later, we worked on alveolar gas analysis using the newly invented respiratory mass spectrometer (55). This introduced me to pulmonary gas exchange and, particularly, the fascinating world of ventilation-perfusion inequality. The second most-influential book of that time for me was *A Graphical Analysis of the Respiratory Gas Exchange* by Rahn and Fenn (41). I still have my copy, dated May 1956. Parenthetically, I wish the name of Hermann Rahn was better known to younger respiratory physiologists because he was also a giant in the field, with seminal contributions to both pulmonary gas exchange and mechanics.

## DISCOVERY OF THE BLOOD-GAS BARRIER

The first intimation of the blood-gas barrier (BGB) was by Marcello Malpighi (1628–1694) in 1661 when he wrote two letters to his friend Giovanni Borelli (1608–1679) about his first microscopic observations of frog lung. He wrote ". . . by careful investigation I have discovered that the whole mass of the lung . . . is an aggregate of very fine thin membranes . . ." (*levissimis et tenuissimis membranis*) (37). However, further progress on the structure of the BGB was stymied by the fact that it is so thin that it is beyond the resolution of the light microscope. In fact, the French physician Albert Policard (40) wrote that "The respiratory surface is like the flesh of an open wound" (*La surface respiratoire est assimable à une plaie à vif*), by which he meant that the pulmonary capillaries with their endothelium were directly exposed to the alveolar gas. It was not until Frank Low (32) prepared the first electron micrographs of the BGB that it became clear that on the thin side, the barrier consisted only of a single layer of alveolar epithelium, the capillary endothelium, and the intervening extracellular matrix (ECM), which contained the basement membranes (BM) of the two cell layers. Modern electron micrographs show the ultrastructure of the BGB with dramatic clarity (19, 53).

## DESIGN CHALLENGE OF THE BGB

The BGB has a basic dilemma. On one hand, it must be extremely thin for adequate gas exchange because

Address for reprint requests and other correspondence: J. B. West, UCSD Dept. of Medicine 0623A, 9500 Gilman Dr., La Jolla, CA 92093-0623 (E-mail: jwest@ucsd.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

 1040-0605/03 $5.00 Copyright © 2003 the American Physiological Society 

Downloaded from ajplung.physiology.org on July 28, 2010

this occurs through passive diffusion, and the diffusion resistance is directly proportional to the thickness of the barrier. Moreover, the BGB needs to have a very large area because the diffusion conductance of the barrier is directly proportional to its area. Evolution has been enormously successful in providing for the human lung. As an example, the BGB is of the order of 0.2 μm in thickness over much of its area of 50–100 $m^2$ (19).

But this is only part of the challenge. In spite of its extreme thinness, the barrier also needs to be immensely strong because, as discussed later, the tensile stresses in the barrier become extremely high under certain conditions. These include intense exercise when the hydrostatic pressure in the pulmonary capillaries rises and also high states of lung inflation when the capillary wall is under tension because of the longitudinal stresses in the alveolar walls. Thus the dilemma of the BGB is to combine extreme thinness with immense strength, and this has to be done over a very large area.

It is interesting to consider the necessary components of the BGB. First, since developmentally all blood vessels are basically endothelial derivatives, the blood capillaries themselves are lined by an endothelium. Next, because the respiratory surface develops by invagination from the epithelial lining of the primitive pharynx, an epithelial covering exists. However, these two cellular layers by themselves cannot provide the necessary tensile strength. Fortunately, the type IV collagen of the BM of the cellular layers is extremely strong, so this is exploited to confer the required tensile strength for the BGB. As electron micrographs show (Fig. 1), the two BM of the endothelial and epithelial cell layers fuse in the thinnest parts of the BGB, forming a central band of type IV collagen, perhaps only 50 nm thick. The net result of this ingenious design is that the BGB may be only 0.2 μm thick in the human lung but it is also able to withstand high tensile stresses.

## STRENGTH OF THE BGB

What is the evidence that the strength of the BGB comes from its BM and, in particular, the type IV collagen that is an important component of these? The evidence can be summarized as follows. *1*) In animal preparations with high capillary transmural pressures, disruptions often occur in the epithelial and endothelial layers, but the BM remains intact (59). *2*) Experiments on isolated rabbit renal tubules show that the elastic properties of the tubules are determined by the BM (54). *3*) The distensibility of frog mesentery capillaries is attributable to the mechanical properties of the BM (45). *4*) Renal glomerular capillaries routinely withstand a high transmural pressure and have a correspondingly thick BM. *5*) Measurements show that the thickness of the BM of systemic capillaries increases down the body as their transmural pressure rises, notably in the giraffe (62).

Type IV collagen itself has a triple helix structure like that of other matrix collagens, but it is distinctive in that the COOH-terminal end has an NC1 globular domain that allows two of the ~400-nm-long molecules to join to form a doublet (Fig. 2). The other $NH_2$ terminus contains the 7S domain, which allows four doublet molecules to form a matrix configuration similar to chicken wire. This configuration apparently combines great strength with porosity. The 7S domain allows the collagen to link with integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ (30).

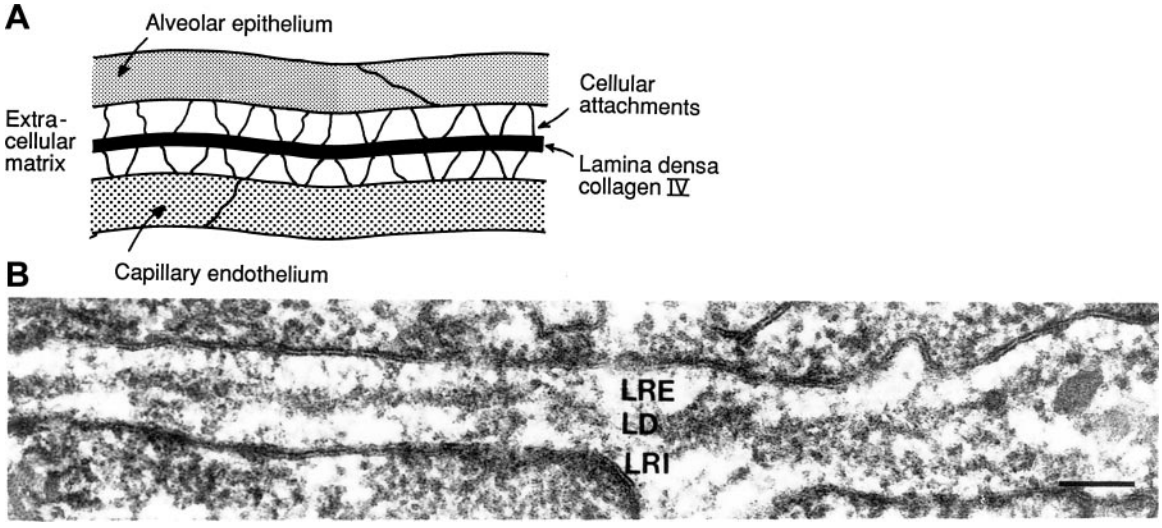


Fig. 1. *A*: diagram of the thin part of the blood-gas barrier (BGB). Most of the type IV collagen responsible for the strength of the BGB is located in the lamina densa (LD). This is only ~50 nm thick and is sandwiched in the middle of the extracellular matrix (ECM). [From West and Mathieu-Costello (56).] *B*: ultrastructure of the thin part of the BGB in rat with portions of alveolar epithelial cell and capillary endothelial cell. Note that the ECM has a central LD flanked by a lamina rara externa (LRE) and lamina rara interna (LRI). The LD is formed by fusion of the basement membranes of the 2 cellular layers. Bar, 0.1 μm. [Modified from Vaccaro and Brody (51).]



Downloaded from ajplung.physiology.org on July 28, 2010

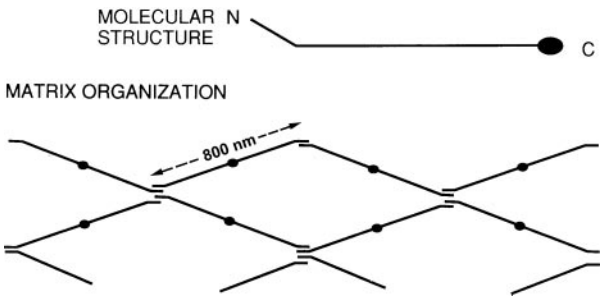


Fig. 2. Some features of type IV collagen. Each molecule is ~400 nm long. Two join at the COOH-terminal region (C) and 4 at the $NH_2$-terminal region (N) to give a matrix that has a very high ultimate tensile strength. [Modified from Timpl et al. (48).]

It is well known that collagens are some of the strongest soft tissues in the body, although there have been few studies of the ultimate tensile strength of BM. However, studies of BM from cat lens capsule (15) and measurements of mechanical properties of BM from isolated rabbit renal tubules (54) suggest that the ultimate tensile strength of type IV collagen is $\sim 1 \times 10^6$ $N \cdot m^{-2}$, a very high value. As discussed below, calculated stresses in the type IV collagen layer of the BGB can approach these values under some extreme physiological conditions.

## PHYLOGENESIS OF THE BGB

A fascinating aspect of the BGB is how evolution fixed on the basic tripartite structure shown in Fig. 1 very early after vertebrate transition to land and the adoption of air breathing, and how this has apparently altered very little over the ensuing 400 million years or so. Figure 3 shows the BGB of the lungs of the lungfishes (Dipnoi), one of the earliest air-breathing taxa. Figure 3*A* is from the South American lungfish *Lepidosiren paradoxa*, and it can be seen that the structure is similar to that shown in Fig. 1 and that the capillary endothelial cell, prominent ECM, and epithelial cell are very evident. There is also an obvious surface lining layer in this perfusion-fixed preparation. Figure 3*B* shows the BGB of the Australian lungfish *Neoceratodus forsteri*, and again the epithelial, endothelial, and ECM layers are clearly seen. Interestingly, the epithelial cell body has prominent microvilli and also osmophilic bodies, features that show uncompleted differentiation of pulmonary pneumocytes in primitive lungs, and resemble the type II alveolar epithelial cell of mammals. Figure 3*C* shows the BGB of the African lungfish *Protopterus aethiopicus*, and again the three layers are obvious. Thus all three extant genera of lungfishes, which are some of the earliest air-breathing vertebrates and are taxa that have been separated for about 300 million years, show the same basic structure of the BGB as in Fig. 1.

The same BGB structure is also seen in amphibia, and Fig. 4 shows the BGB of the tree frog *Chiromantis petersi* where the electron-dense band in the center of the ECM is prominent and very much like the appearance shown in Fig. 1. A similar structure occurs in reptiles, and Fig. 5 shows the BGB of the black mamba snake *Dendroaspis polylepis*. Here the endothelial layer is unusually thick, partly because of the overlapping junction of two endothelial cells. However, the electron-dense band in the center of the ECM is remarkably prominent.

A particularly informative structure is seen in the avian lung (for example, that of the domestic chicken *Gallus gallus* variant *domesticus*) (Fig. 6). Note that the stress-bearing ECM forms an unbroken ring around the blood capillary to counter the stresses caused by the capillary transmural pressure (Fig. 7). However, where two air capillaries adjoin, the ECM with its type IV collagen component is conspicuously lacking because the avian lung is virtually rigid and has a negligibly small change in volume during ventilation (27). This is additional evidence confirming the critical importance of type IV collagen in the ECM in maintaining the integrity of the BGB.

It is remarkable that evolution has conserved the basic structure of the BGB over such a long period of time in spite of the fact that the gross anatomy of the lung has changed out of all recognition (35). As an example, the lung of the South American lungfish is long and spindly and completely different from a morphological point of view compared with the chicken parabronchial lung (Fig. 6), which is itself very different from the mammalian bronchoalveolar lung. Nevertheless, the basic structure of the BGB is almost unchanged.

## STRESSES IN THE BGB

Figure 7 shows the two mechanisms by which stresses can be generated in the BGB. The first is the hoop or circumferential stress that develops as a result of the transmural pressure across the curved capillary wall according to the Laplace relationship. If we regard the capillary as part of a thin-walled cylindrical tube, the stress S is given by Pr/t where P is the transmural pressure, r is the radius of the capillary, and t is the thickness of the load-bearing structure.

The transmural pressure of the capillaries of the human lung during exercise is not known with any accuracy, but pulmonary arterial wedge pressures as high as 21.1 mmHg have been measured (52). Consistent with this, mean pulmonary artery pressures have been shown to increase from 13.2 mmHg at rest to 37.2 mmHg during exercise (12, 20, 52). Micropuncture studies of the pressures in small pulmonary blood vessels in anesthetized cats have shown that capillary pressure is about halfway between arterial and venous pressure, and much of the pressure drop in the pulmonary circulation occurs in the capillary bed (3). This means that at midlung during heavy exercise, the capillary pressure is about halfway between 37 and 21 mmHg, that is 29 mmHg. Because the capillaries at the bottom of the upright lung are ~10 cm lower, adding the hydrostatic gradient gives a capillary pressure there of ~36 mmHg (59).



Downloaded from ajplung.physiology.org on July 28, 2010

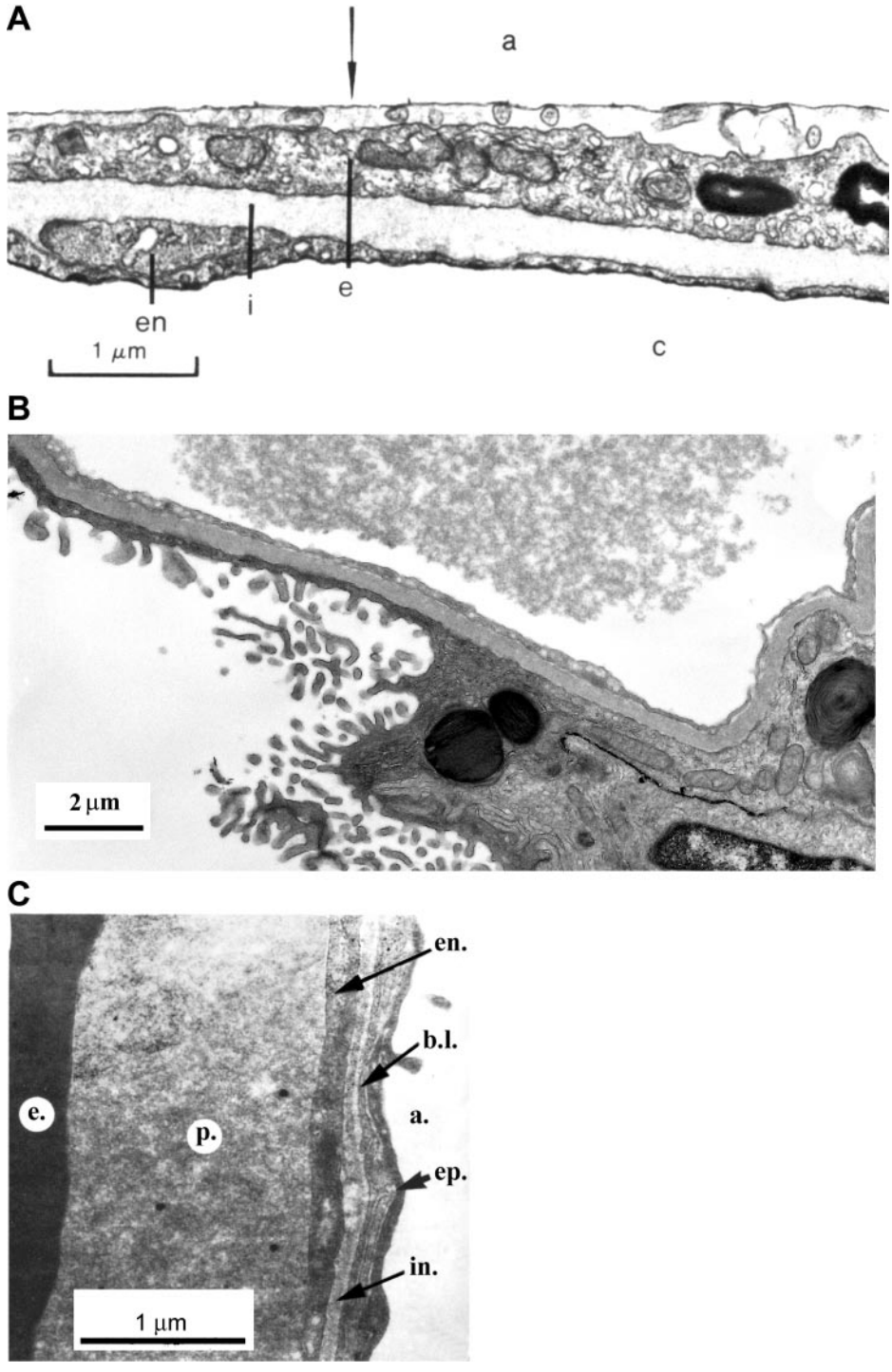


Fig. 3. *A*: BGB from the lung of the South American lungfish *Lepidosiren paradoxa*. Note that the BGB of this animal that appeared ~400 million years ago shows the 3 layers consisting of air space epithelium, capillary endothelium (en), and ECM. A surface lining layer is also seen. a, air space; en, endothelium; i, interstitium; e, epithelium; c, capillary. [Modified from Hughes (25).] *B*: BGB of the Australian lungfish *Neoceratodus forsteri* showing the same 3 layers. Note that the epithelial cell body has microvilli and osmophilic bodies as in the mammalian type II alveolar epithelial cell. Courtesy of John H. T. Power. *C*: BGB of the African lungfish *Protopterus aethiopicus* showing the same basic structure. e, erythrocyte; p, plasma; en, endothelium; b.l., basal lamina; a, air space; ep, epithelium; in, interstitium. [Modified from Maina and Maloiy (36).]

We do not have good data on the radius of human pulmonary capillaries at high capillary pressures, but using the average for rabbits and dogs gives a value of 3.5 μm (4). As indicated earlier, there is evidence that the stress-bearing structure is the thin band of type IV collagen in the electron-dense region of the ECM as shown in Fig. 1. The thickness of this is of the order of 50 nm. With the use of these numbers, which are admittedly approximate, the tensile stress in the type IV collagen layer is calculated to be $\sim 3 \times 10^5$ N·m$^{-2}$. This is approaching the ultimate tensile strength of type IV collagen of $\sim 1 \times 10^6$ N·m$^{-2}$ as discussed above. Therefore, it appears that the normal lung does not have a great deal of reserve in terms of the strength of the BGB, and this fits with the apparent changes in the integrity of the BGB that are seen in elite athletes at very high levels of exercise, as discussed later.

The second mechanism responsible for increasing stress in the BGB is increased tension in the alveolar wall as occurs at high states of lung inflation. In this



Downloaded from ajplung.physiology.org on July 28, 2010

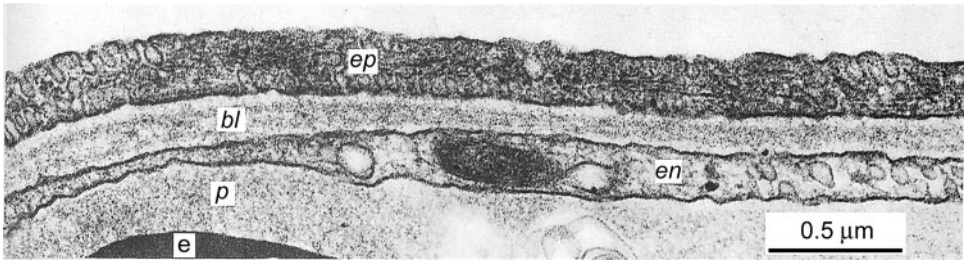


Fig. 4. BGB of the tree frog *Chiromantis petersi*. This amphibian shows the 3 layers of the BGB very clearly with a central LD in the ECM. ep, epithelium; bl, basal lamina; en, endothelium; p, plasma; e, erythrocyte. [Modified from Maina (33).]

context, we can think of the alveolar wall as a string of capillaries with at least part of the longitudinal tension of the wall being transmitted to the capillary wall. As discussed later, there is evidence that when the lung is inflated to very high volumes, for example, as a result of high levels of positive end-expiratory pressure (PEEP) in the intensive care unit, the integrity of the BGB is impaired.

### STRESS FAILURE OF THE BGB

To determine the ultrastructural changes that occur in the walls of pulmonary capillaries when the stresses are greatly increased, measurements were made in anesthetized rabbits where the pulmonary artery and left atrium were cannulated so that the capillary transmural pressure could be accurately measured. The lungs were then fixed for electron microscopy using buffered glutaraldehyde. An example of the ultrastructural changes is shown in Fig. 8, *top*. Note that there is disruption of the capillary endothelial cell, although its intact BM can be clearly seen. Close inspection of the two ends of the endothelial layer shows that the structures are smoothly rounded, suggesting that the plasmalemmal layer is intact. The alveolar epithelial layer is also intact as is its BM. Another example of stress failure is shown in Fig. 8, *bottom*. In this case there is disruption of the alveolar epithelial layer (Fig. 8, *top*). In addition, close inspection reveals disruption of the capillary endothelial layer (Fig. 8, *bottom*), and a blood platelet appears to be adhering to the exposed BM. This is not surprising because the BM is electrically charged and highly reactive.

An interesting question is whether the disruptions occur at intercellular junctions. We do not have good information on this point for the capillary endothelium, but scanning electron micrographs show that for the alveolar epithelium, almost no breaks occurred at intercellular junctions, but within the cells themselves breaks did occur (10). This suggests that the junctions themselves have considerable mechanical strength

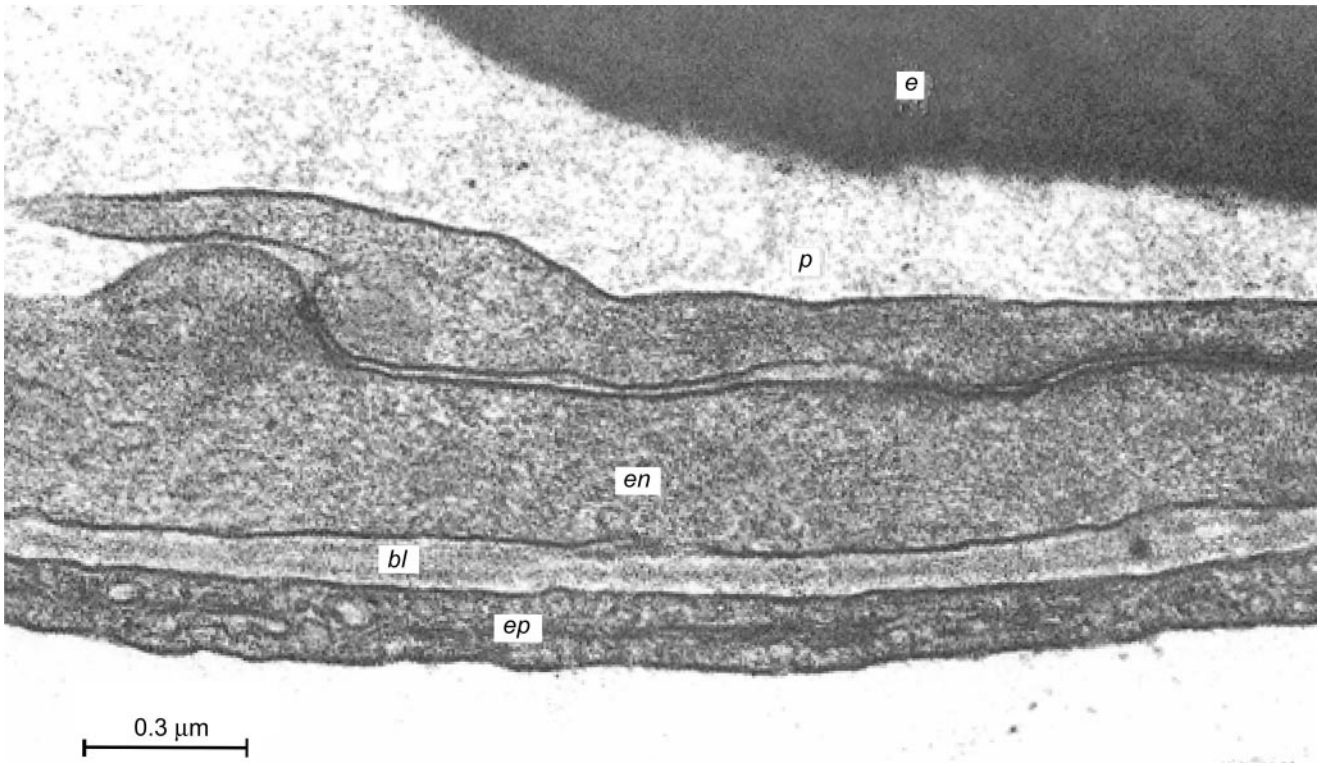


Fig. 5. BGB of the black mamba snake *Dendroaspis polylepis*. This reptile shows the tripartite structure very clearly, and the electron-dense layer in the center of the ECM is prominent. e, erythrocyte; p, plasma; en, endothelium; bl, basal lamina; ep, epithelium. [Modified from Maina (35).]



Downloaded from ajplung.physiology.org on July 28, 2010

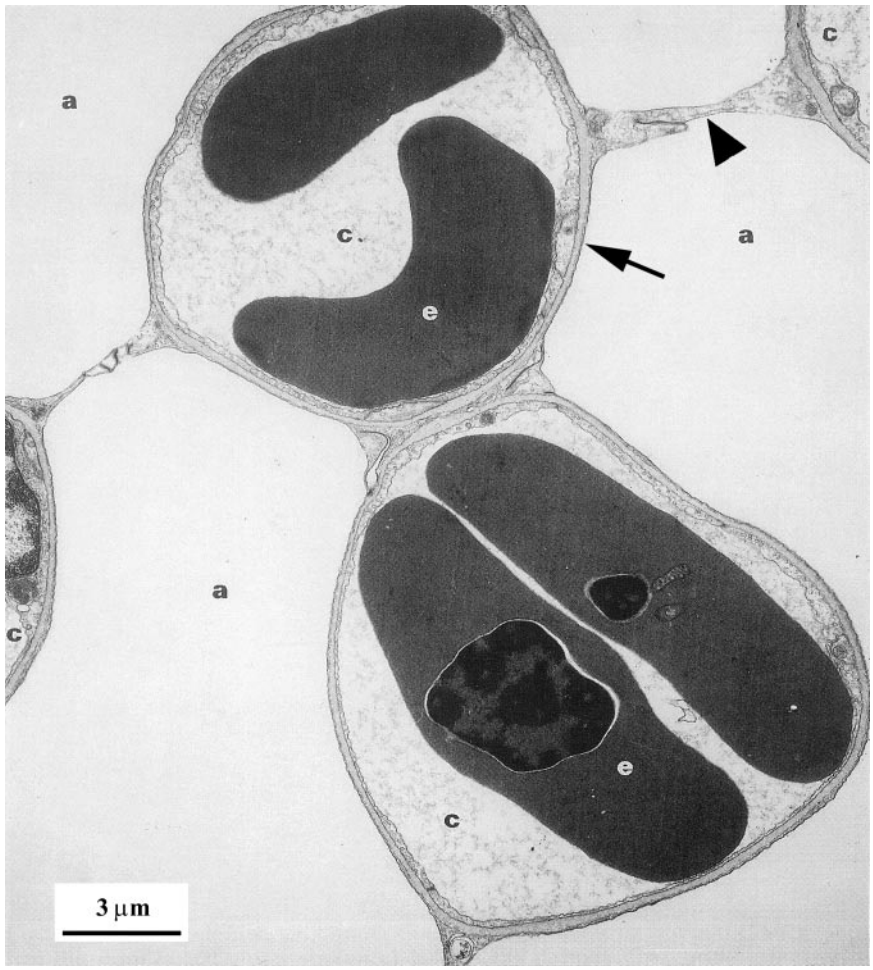


Fig. 6. Lung of the domestic chicken *Gallus gallus* variant *domesticus* showing the 3 layers of the BGB and, in particular, the ECM, which forms a "cable" around the blood capillaries (arrow). However, this band of type IV collagen is not seen in the tissue separating adjacent air capillaries (arrowhead). a, air capillary; c, blood capillary; e, erythrocyte. [Modified from Maina (34).]

consistent with the highly organized intercellular junctions of type I alveolar epithelial cells. This finding that the disruptions are within instead of between cells is consistent with reports that when the capillary transmural pressure of frog mesentery is raised, >80% of the breaks in the endothelium are transcellular rather than intercellular (38).

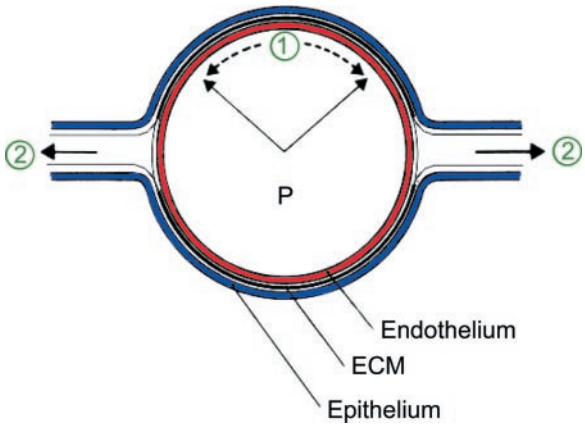


Fig. 7. Diagram showing 2 mechanisms that can cause an increased stress in the BGB. Circled 1, the hoop or circumferential stress caused by the capillary transmural pressure; circled 2, results from linear tension in the alveolar wall, which increases as the lung is inflated. P, capillary hydrostatic pressure. [From West et al. (59).]

In our rabbit preparation, the first indications of stress failure were seen at a capillary transmural pressure of 24 mmHg, but the number of breaks was much increased at a pressure of 39 mmHg, and the frequency was even greater at 53 mmHg (50). The fact that some disruptions were seen at a pressure as low as 24 mmHg is remarkable in view of the evidence presented above that some of the capillaries of the human lung during severe exercise have a transmural pressure as high as 36 mmHg. However, of course, we cannot assume that stress failure occurs at the same pressures in rabbit and human lung, and indeed we have shown that different pressures are required in rabbit, dog, and horse lung (4).

A remarkable feature of these disruptions is that many are rapidly reversible when the capillary transmural pressure is reduced. This was shown in our rabbit preparation by first raising the pressure to a high level and then reducing it to a low level, followed by intravascular fixation at the low pressure. The results showed that ~70% of both the endothelial and epithelial breaks closed within a few minutes (13). The breaks that closed were predomi-



Downloaded from ajplung.physiology.org on July 28, 2010

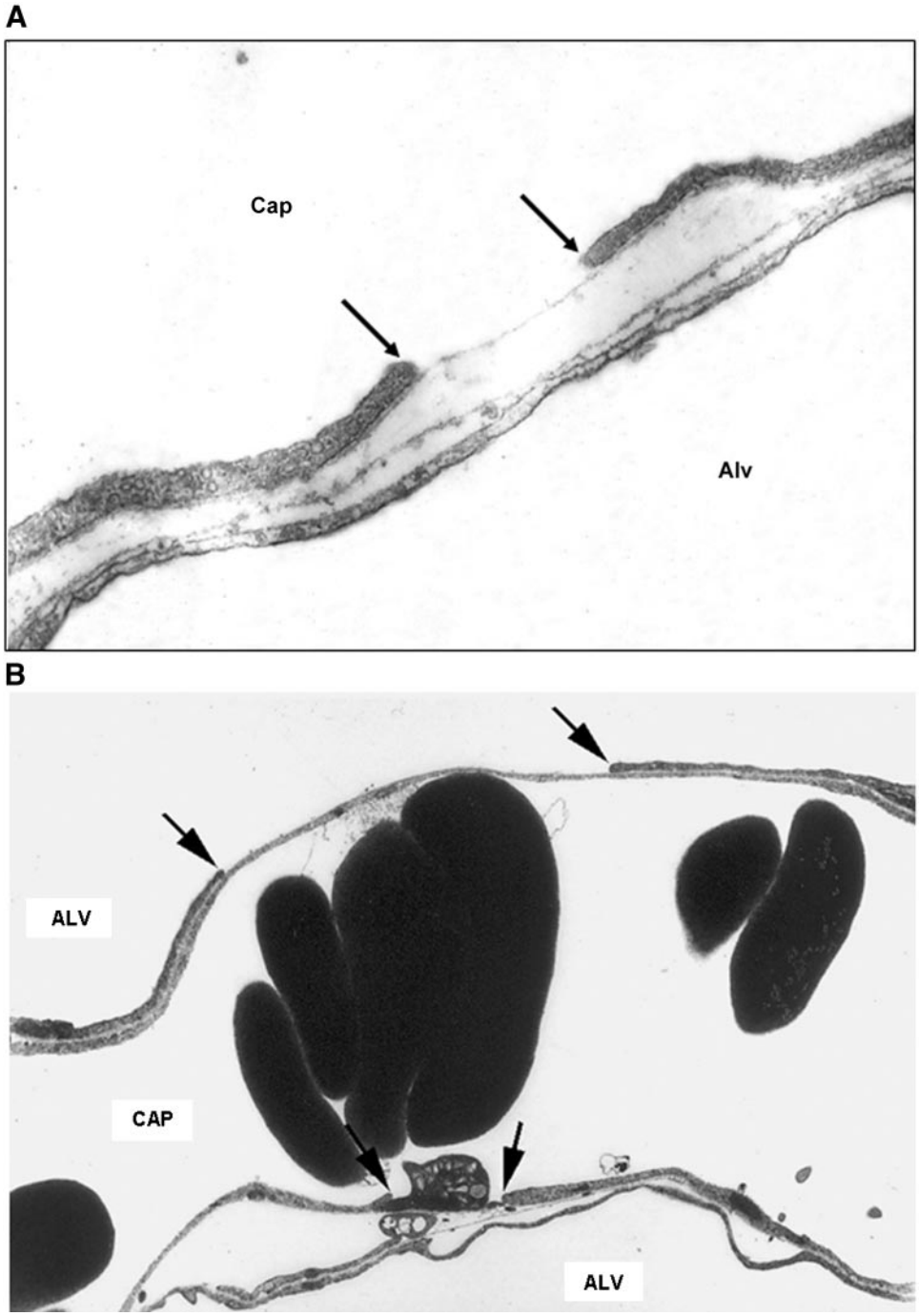


Fig. 8. *A*: the effects of raising the capillary transmural pressure in the rabbit. Note that there is a disruption in the capillary endothelial layer (Cap), but the alveolar epithelium (Alv) and 2 basement membranes are intact. *B*: disruptions of both the alveolar epithelial layer (*top*) and capillary endothelial layer (*bottom*). A blood platelet is adhering to the exposed basement membrane below. [From West et al. (59).]

nantly those that were initially small and also associated with an intact BM.

The micromechanics underlying the mechanism of stress failure are poorly understood, but one possibility is that the high hoop stress in the type IV collagen layer causes this to stretch, thus distorting the matrix arrangement shown in Fig. 2. It is known that type IV collagen molecules have sites that allow bending to occur. For example, in human $\alpha_1$(IV) and $\alpha_2$(IV) polypeptide chains, ~25 irregularly spaced sites have been described that impart flexibility to the whole molecule (47), and a 90-nm-long segment of high flexibility near the 7S domain also exists (22). Thus it may be that the BM elongates with the result that the overlying cell disrupts. If the BM regains its original configuration when the capillary transmural pressure is reduced, this could explain the rapid reversibility of many of the breaks.

## PHYLOGENETIC STRATEGIES TO AVOID STRESS FAILURE

It is instructive to look at the evolution of vertebrates to understand how they avoid stress failure of lung or



Downloaded from ajplung.physiology.org on July 28, 2010

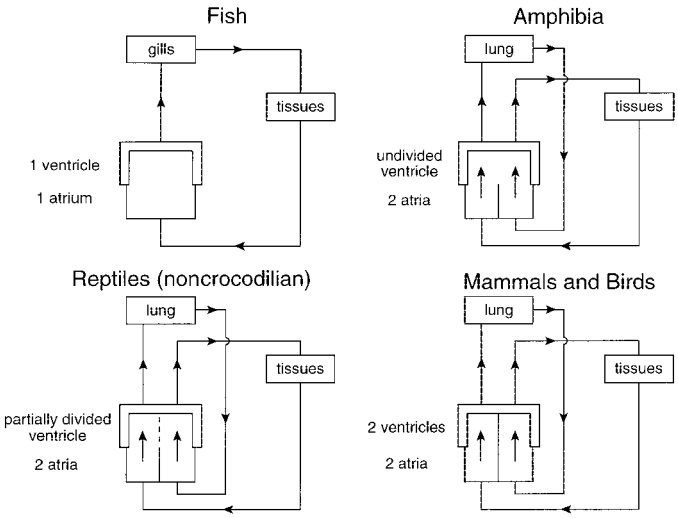


Fig. 9. Stages in the evolution of the pulmonary circulation. In fishes, the gill capillaries are exposed to the full dorsal aorta pressure. In amphibia and noncrocodilian reptiles, the pulmonary circulation is functionally partly separated by streaming of blood within the heart for more efficient gas exchange. However, only the fully endothermic mammals and birds have achieved total separation, thus protecting the vulnerable pulmonary capillaries from the high vascular pressure. [From West and Mathieu-Costello (57).]

gill capillaries. Figure 9 shows that in fishes, the heart consists of only a single atrium and single ventricle in series, and blood is pumped to the gills and then distributed to the tissues via the dorsal aorta. This arrangement means that the hydrostatic pressure in the gill capillaries must exceed that in the dorsal aorta. It is known that in some athletic fish, such as the albacore tuna *Thunnus alalunga*, the mean dorsal aortic pressure is as high as 79 mmHg (6). Therefore, the capillary transmural pressure in the gill of this animal greatly exceeds that which is necessary to cause stress failure in some mammals. However, the wall of the gill capillary can afford to be much thicker and, therefore, stronger than in the mammal because the maximal oxygen consumption of the fish is considerably less than in a mammal of the same size. In other words, the diffusion requirements of the barrier are less, and, therefore, it can afford to be thicker.

With gradual evolution through the amphibia and reptiles to the highly aerobic mammals and birds, the pulmonary circulation is gradually separated from the systemic circulation so that the pulmonary capillaries are eventually exposed to much lower pressures. In the modern amphibian, such as the frog, the beginning of a separation of the two circulations is seen because the atria are completely separate, although there is only one undivided ventricle. It is known that streaming of blood within the heart results in much of the oxygenated blood from the lungs finding its way into the aorta, but since the ventricle is undivided, the lung capillaries presumably see a high pressure. Again, however, the low maximal oxygen consumption of these exothermic animals and the fact that some respiration occurs through the skin means that the BGB can afford to be thicker.

In the noncrocodilian reptile, the ventricle is now partially divided, and streaming of oxygenated and poorly oxygenated blood results in a functionally well-developed double circulation. However, the fact that the ventricles do communicate means that the pulmonary capillaries are potentially exposed to a high pressure. A remarkable exception is the monitor lizard *Varanus exanthematicus*, which has an unusually high aerobic scope and in which there is a ridge in the ventricle that separates the two outflow tracts during systole. The result is that the pressure in the pulmonary artery is much lower than in the aorta (8).

Only in the fully endothermic vertebrates, such as mammals and birds, is it essential that the pressures in the pulmonary circulation be much lower than in the systemic circulation. This is because the high levels of maximal oxygen consumption are only possible when the pulmonary capillary walls are extremely thin, to allow rapid diffusion of oxygen. Thus full endothermy requires complete separation of the two ventricles, and this explains why this is mandatory in mammals and birds.

## ONTOGENY RECAPITULATES PHYLOGENY

It is interesting that the changes that occur in the pulmonary and systemic circulations of the mammal at birth reflect those seen in vertebrate evolution. In the fetus, the outflow tracts of the left and right ventricles are connected by the large patent ductus arteriosus. As in amphibians and reptiles, streaming of blood occurs in the heart, with the result that the best oxygenated blood from the placenta via the inferior vena cava tends to be directed to the brain. However, the pressure in the pulmonary artery is high, being the same as that in the aorta. At first sight, this suggests that the pulmonary capillaries would be prone to stress failure. However, the blood flow through the lung is only ~15% of the cardiac output, and this restricted flow is accomplished by having considerable tone in the highly muscularized pulmonary arteries. The result is that the pulmonary capillaries are protected from the high pressure.

At birth, the blood flow through the lung has to increase to 100% of the cardiac output. This is brought about by a striking fall in pulmonary vascular resistance, partly caused by release of hypoxic pulmonary vasoconstriction, and also by expansion of the lung. The pulmonary capillaries would, therefore, be at risk if it was not for the fact that the pulmonary artery pressure simultaneously falls as a result of constriction of the ductus arteriosus. It is extraordinary that these two events, opening of the pulmonary arterial throttle and closure of the ductus arteriosus, are so well synchronized in the majority of births. It is also interesting that recent work in our laboratory has shown that the capillaries in the newborn rabbit lung are much more



Downloaded from ajplung.physiology.org on July 28, 2010

fragile and vulnerable to stress failure than in the adult animal (17, 18).

## PHYSIOLOGICAL CONDITIONS CAUSING STRESS FAILURE

The most remarkable physiological example of stress failure of the BGB is seen in galloping Thoroughbred racehorses. It is now known that all Thoroughbreds in training bleed into their alveolar spaces based on the finding of hemosiderin-laden macrophages in their tracheal washings (60). This so-called exercise-induced pulmonary hemorrhage has been known for hundreds of years but the mechanism has been obscure until recently. It is now known that galloping Thoroughbreds have extremely high pulmonary vascular pressures. For example, measurements with animals galloping on a treadmill show that the left atrial pressure measured directly with a catheter can be as high as 70 mmHg, and this is associated with a mean pulmonary artery pressure as high as 120 mmHg (14, 28). Other vascular pressures are equally astonishing, with a mean systemic arterial pressure as high as 240 mmHg and a mean right atrial pressure of 40 mmHg.

The basic reason for these extraordinarily high pressures is that the animals have been selectively bred for hundreds of years for extremely high aerobic performances. For example, they have maximal oxygen consumptions of up to 180 $ml \cdot min^{-1} \cdot kg^{-1}$ and cardiac outputs as high as 750 $ml \cdot min^{-1} \cdot kg^{-1}$. These enormous cardiac outputs demand very high filling pressures in the left ventricle and, therefore, very high left atrial pressures, and the pulmonary artery pressure is passively raised in response. There is no evidence of increased pulmonary vascular resistance. However, it is clear that the transmural pressure of the pulmonary capillaries must be of the order of 100 mmHg. Under these conditions, it is not at all surprising that stress failure of the BGB occurs; indeed, it would be astonishing if it did not. Of course, with these enormous maximal oxygen consumptions, the BGB has to be very thin to allow rapid diffusion. Direct observations of the ultrastructure of the lung in Thoroughbreds after galloping on the treadmill show unequivocal evidence of breaks in the BGB (58).

These remarkable findings in Thoroughbred racehorses prompt the question of whether elite human athletes during maximal exercise ever have ultrastructural changes in their BGB. There is now strong evidence for this. We studied six elite cyclists who sprinted uphill at maximal effort sufficient to give a mean heart rate of 177 $beats \cdot min^{-1}$. Within an hour of finishing the exercise, the volunteers underwent bronchoalveolar lavage (BAL), and the results were compared with normal sedentary subjects who did not exercise before BAL. It was found that the athletes had significantly higher concentrations of red blood cells, total protein, albumin, and leukotriene $B_4$ ($LTB_4$) in their BAL fluid than control subjects (24). In other words, brief but very intense exercise in elite human athletes causes changes in the integrity of the BGB.

Do the same changes occur if similar athletes exercise for a longer period of time at submaximal exercise levels? We tested this by carrying out a further study on a similar group of six elite cyclists who exercised at 77% of their maximal oxygen consumption for 1 h and then underwent BAL. The controls were eight normal nonathletes who did not exercise before BAL. In contrast to the results in the previous study, the concentrations of red blood cells, total protein, and $LTB_4$ in the BAL fluid of the exercising athletes were not different from those of the control subjects (23). There were higher concentrations of surfactant apoprotein A in the athletes, but this is known to occur with exercise. The overall conclusion of these two studies in elite human athletes is that the integrity of the BGB is altered only at extreme levels of exercise, and indeed, this is what might be expected on general evolutionary lines. It is reasonable that the structure of the organism evolves to cope with all but the most extreme stresses to which it is subjected.

## PATHOLOGICAL CONDITIONS CAUSING STRESS FAILURE

Stress failure of the BGB occurs under many pathological conditions, and these are summarized in Table 1. As we saw in the previous section, the normal human BGB retains its integrity up to virtually the highest stresses that occur under physiological conditions. However, if the capillary transmural pressure rises to unphysiologically high levels, stress failure is inevitable. This occurs in high-altitude pulmonary edema (HAPE), which is discussed in more detail later, and also heart diseases, such as mitral stenosis, and left ventricular failure that raise pulmonary capillary pressure.

Another cause of stress failure of the BGB is abnormally high states of lung inflation, as predicted in Fig. 7. This is frequently seen in the intensive care unit if high levels of PEEP are used (11). 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 (16). Consistent with this, a controlled trial of low vs. traditional high tidal volumes during mechanical ventilation in intensive care units showed reduced mortalities with the low tidal volumes (7).

Table 1. *Pathological conditions causing stress failure of the blood-gas barrier*

| |
|---|
| 1) High capillary pressure resulting in high-permeability edema |
| e.g., high-altitude pulmonary edema, neurogenic pulmonary edema |
| 2) High capillary pressure causing edema and hemorrhage |
| e.g., mitral stenosis, left ventricular failure |
| 3) High state of lung inflation |
| e.g., positive end-expiratory pressure in the intensive care unit |
| 4) Abnormal extracellular matrix |
| e.g., Goodpasture's syndrome |



Downloaded from ajplung.physiology.org on July 28, 2010

Stress failure also occurs if the type IV collagen of the ECM, which is responsible for the strength of the BGB (Fig. 1), is weakened by disease. This is the case with Goodpasture's syndrome (61) in which bleeding occurs into the alveolar spaces.

The mechanism of HAPE is interesting because it was disputed for many years. In fact, it was this condition that initially sparked our interest in the possibility of stress failure of the BGB. It is well known that HAPE is strongly associated with a high pulmonary artery pressure, but the pulmonary venous pressure is normal. A crucial finding was that the alveolar edema in HAPE is of the high-permeability type, with a large concentration of high-molecular-weight proteins and red blood cells (43). Therefore, it seemed likely that in some way, the high pulmonary artery pressure was damaging the walls of the capillaries. One perplexing feature was how an increase in pulmonary artery pressure could be transmitted to some of the capillaries since the site of hypoxic vasoconstriction is believed to be upstream of the capillaries. However, a reasonable explanation was given by Hultgren (26) more than 30 years ago when he suggested that if the hypoxic pulmonary vasoconstriction is uneven, some of the capillaries would be exposed to a high pressure. Consistent with this, it is known that there is a meager amount of vascular smooth muscle in small pulmonary arteries in the adult lung after the involution that occurs after birth, and the muscle that remains is unevenly distributed (42). Convincing evidence that HAPE is caused by stress failure of pulmonary capillaries was obtained by Swenson et al. (46) when they showed that in very early HAPE, BAL fluid showed increased red blood cell and protein concentrations with no evidence of inflammatory markers.

## REGULATION OF THE BGB

As pointed out earlier, the pulmonary BGB needs to satisfy two conflicting requirements. It must be extremely thin for efficient gas exchange but also immensely strong to withstand the high stresses in the capillary wall when capillary pressure rises during exercise. The human BGB maintains its integrity except under conditions of extreme exercise in elite athletes. However, pathological conditions associated with an abnormally high capillary pressure cause stress failure.

How is the structure of the BGB optimized so that it is just strong enough to withstand almost all the normal mechanical stresses but at the same time remain extremely thin? Our hypothesis is that the capillary wall senses the wall stress in some way and then regulates its structure, especially the ECM, which appears to be primarily responsible for its strength.

Pulmonary vascular remodeling is well known to occur in larger pulmonary blood vessels and has been extensively reviewed, for example, by Stenmark and Mecham (44). A particularly interesting study was carried out by Tozzi et al. (49), who applied mechanical tension to explants of rings of rat main pulmonary artery and showed increases in collagen synthesis (incorporation of [$^{14}$C]proline), elastin synthesis (incorporation of [$^{14}$C]valine), mRNA for pro-$\alpha_1$(I) collagen, and mRNA for protooncogene v-sis within 4 h. These changes were endothelium dependent because they did not occur when the endothelium was removed from the arterial rings.

However, in contrast to the extensive literature on vascular remodeling in pulmonary arteries and veins, remodeling of pulmonary capillaries has been almost completely ignored. We know that it occurs because, as

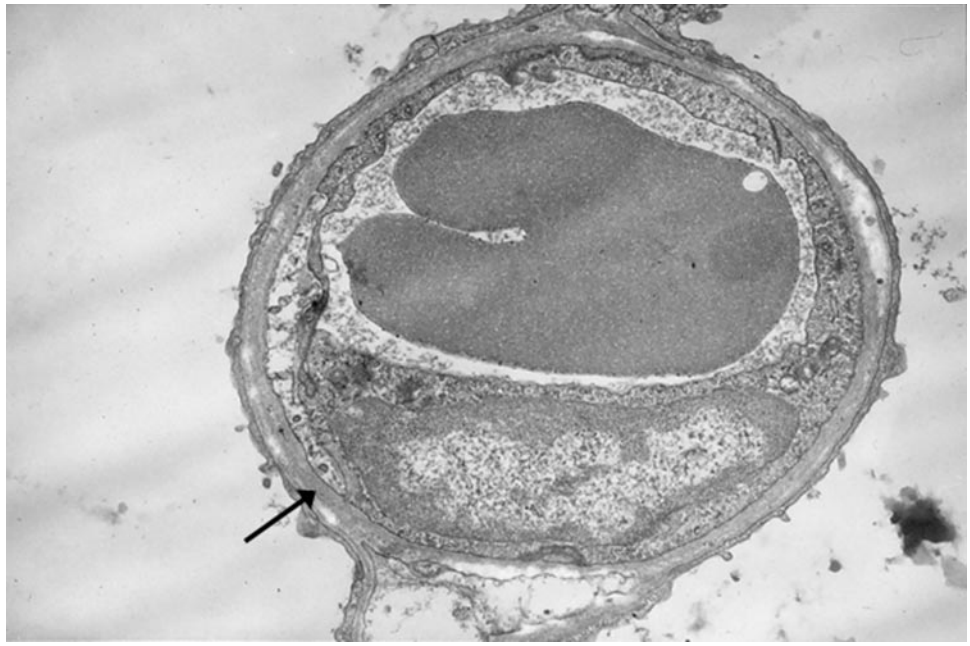

Fig. 10. Electron micrograph of a pulmonary capillary from a young patient with mitral stenosis. Note thickening of the basement membranes of the capillary endothelial and alveolar epithelial cells, particularly the former (arrow). Courtesy of S. G. Haworth.



Downloaded from ajplung.physiology.org on July 28, 2010

Fig. 10 shows, marked thickening of the BM of the capillary endothelial cells and alveolar epithelial cells is seen in the pulmonary capillaries of patients with mitral stenosis (21) and pulmonary veno-occlusive disease (29). In both conditions, pulmonary capillary pressure is raised, and it is reasonable to infer that the thickening occurs in response to the increased capillary wall stress. Careful inspection of Fig. 10 suggests that most of the thickening of the BM is associated with the capillary endothelial cell instead of the alveolar epithelial cell, implicating the endothelium as the main source. As noted earlier, Tozzi et al. (49) showed that some aspects of vascular remodeling in pulmonary artery were endothelium dependent. Of course, the fact that the increase in BM thickness apparently comes from the endothelial cell does not necessarily mean that this cell is the primary sensor of the increased wall stress. It is possible that some other cell in the parenchyma, such as an epithelial cell, fibroblast, or some other pericyte responds to the increased tension and sends a signal to the endothelial cell. It is known that cultures of type II alveolar epithelial cells are responsive to stretch (63), and cultures of pulmonary fibroblasts respond to stretch with an increase in proliferation through an autocrine growth factor mechanism (5) that involves platelet-derived growth factor-B (PDGF-B) (31).

We have designed experiments in which the pulmonary capillary wall stress was raised, and gene expression for ECM proteins and the concentrations of the proteins themselves were measured in peripheral lung tissue, where most of the blood vessels are pulmonary capillaries. Table 2 summarizes some of the results from three separate experiments. In the first experiment, the volume of one lung of an anesthetized open-chest rabbit was raised while the other lung was ventilated at a normal volume (2). Additional control animals had both lungs ventilated at normal states of lung inflation. It was found that high states of lung inflation over 4 h resulted in increased gene expression for $\alpha_1$(III) and $\alpha_2$(IV) procollagens, fibronectin, basic fibroblast growth factor, and transforming growth factor-β1 (TGF-β1). By contrast, mRNA levels for $\alpha_1$(I) procollagen and vascular endothelial growth factor were unchanged. An unexpected finding was that the changes in mRNA listed above were identical in both the overinflated lung (9 $cmH_2O$ PEEP) and normally inflated lung (1 $cmH_2O$ PEEP) in the rabbit preparation in which one lung was overinflated and the other lung was normally inflated. This observation suggests that a generalized organ-specific response occurred after the localized, unilateral application of mechanical force. The mechanism for this was not identified, but one possibility was that information was transferred via the circulation from the overinflated lung to the normally inflated lung.

In a second experiment carried out by Parker and colleagues (39), capillary transmural pressure was increased by raising the venous pressure in isolated perfused rat lungs. To avoid producing pulmonary edema, the venous pressure was increased cyclically to 28 $cmH_2O$ for 15 s every minute for 4 h. Controls were similar lungs perfused at low pressure and also unperfused lungs. As Table 2 summarizes, this study showed significant increases in gene expression for $\alpha_1$(I) and $\alpha_1$(III) procollagens, fibronectin, laminin, and TGF-β1.

In a third experiment, alveolar hypoxia was used to raise the pressure in some of the capillaries (1). The rationale for this was described in the earlier section on high-altitude pulmonary edema, and an assumption was that the hypoxic pulmonary vasoconstriction was uneven so that some of the capillaries were exposed to an increased hydrostatic pressure. Rats were exposed to 10% oxygen for 6 h or 3 days (short-term group) and 3 or 10 days (long-term group). The results showed that in peripheral lung tissue, levels of mRNA for $\alpha_2$(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 PDGF-B doubled after 6 h of hypoxia but returned to control values after 3 days. mRNA levels for $\alpha_1$(I) and $\alpha_1$(III) procollagens and fibronectin were increased after 3 days of hypoxia and then decreased toward control values after 3 days. The results are consistent with capillary remodeling in response to increased wall stress.

As shown in Table 2, the results of these three different experiments were somewhat variable, and a clear picture has not yet emerged. One of the practicable problems is the difficulty of raising the wall stress in the capillaries without increasing the pressures and, therefore, the wall stresses in larger pulmonary blood vessels. An attempt to obviate this problem was made by only collecting tissue from the outer few millimeters of lung. However, more studies are clearly needed.

In conclusion, the structure of the BGB presents a fascinating challenge because of the basic dilemma of combining extreme thinness with immense strength. How the barrier is regulated to satisfy these conflicting demands so that, for example, stress failure only occurs under the most extreme conditions in the human lung remains a central issue for lung biology research.

Table 2. *Increased gene expression after a rise in capillary wall stress*

| | Procollagens | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\alpha_1$(I) | $\alpha_1$(II) | $\alpha_2$(IV) | fibronectin | laminin | bFGF | TGF-β1 | PDGF-B |
| High lung inflation (Ref. 2) | | + | + | + | | + | + | |
| Increased capillary pressure (Ref. 39) | + | + | | + | + | | | |
| Increased capillary pressure, hypoxia (Ref. 1) | | | + | + | | | | + |

bFGF, basic fibroblast growth factor; TGF-β1, transforming growth factor β1; PDGF-B, platelet-derived growth factor B.



Downloaded from ajplung.physiology.org on July 28, 2010

I am indebted to numerous colleagues, especially Zhenxing Fu, Odile Mathieu-Costello, and John Maina.

## DISCLOSURES

This work was supported by National Heart, Lung, and Blood Institute Grant RO1-HL-60698.

## REFERENCES


1. **Berg JT, Breen EC, Fu Z, Mathieu-Costello O, and West JB.** Alveolar hypoxia causes increased gene expression of extracellular matrix proteins and PDGF-B in lung parenchyma. *Am J Respir Crit Care Med* 158: 1920–1928, 1998.
2. **Berg JT, Fu Z, Breen EC, Tran HC, Mathieu-Costello O, and West JB.** High lung inflation increases mRNA levels of ECM components and growth factors in lung parenchyma. *J Appl Physiol* 83: 120–128, 1997.
3. **Bhattacharya J and Staub NC.** Direct measurement of microvascular pressures in the isolated perfused dog lung. *Science* 210: 327–328, 1980.
4. **Birks EK, Mathieu-Costello O, Fu Z, Tyler WS, and West JB.** Comparative aspects of the strength of pulmonary capillaries in rabbit, dog and horse. *Respir Physiol* 97: 235–246, 1994.
5. **Bishop JE, Mitchell JJ, Absher PM, Baldor L, Geller HA, Woodcock-Mitchell J, Hamblin MJ, Vacek P, and Low RB.** Cyclic mechanical deformation stimulates human lung fibroblast proliferation and autocrine growth factor activity. *Am J Respir Cell Mol Biol* 9: 126–133, 1993.
6. **Breisch EA, White F, Jones HM, and Laurs RM.** Ultrastructural morphometry of the myocardium of *Thunnus alalunga*. *Cell Tissue Res* 233: 427–438, 1983.
7. **Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, and Wheeler A.** Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 342: 1301–1308, 2000.
8. **Burggren W and Johansen K.** Ventricular haemodynamics in the monitor lizard *Varanus exanthematicus*: pulmonary and systemic pressure separation. *J Exp Biol* 96: 343–354, 1982.
9. **Comroe JH.** *The Lung: Clinical Physiology and Pulmonary Function Tests*. Chicago, IL: Year Book Publishers, 1955.
10. **Costello ML, Mathieu-Costello O, and West JB.** Stress failure of alveolar epithelial cells studied by scanning electron microscopy. *Am Rev Respir Dis* 145: 1446–1455, 1992.
11. **Dreyfuss D, Basset G, Soler P, and Saumon G.** Intermittent positive-pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. *Am Rev Respir Dis* 132: 880–884, 1985.
12. **Ekelund LG and Holmgren A.** Central hemodynamics during exercise. *Circ Res* 30: I33–I43, 1967.
13. **Elliott AR, Fu Z, Tsukimoto K, Prediletto R, Mathieu-Costello O, and West JB.** Short-term reversibility of ultrastructural changes in pulmonary capillaries caused by stress failure. *J Appl Physiol* 73: 1150–1158, 1992.
14. **Erickson BK, Erickson HH, and Coffman JR.** Pulmonary artery and aortic pressure changes during high intensity treadmill exercise in the horse: effect of frusemide and phentolamine. *Equine Vet J* 24: 215–219, 1992.
15. **Fisher RF and Wakely J.** The elastic constants and ultrastructural organization of a basement membrane (lens capsule). *Proc R Soc Lond B Biol Sci* 193: 335–358, 1976.
16. **Fu Z, Costello ML, Tsukimoto K, Prediletto R, Elliott AR, Mathieu-Costello O, and West JB.** High lung volume increases stress failure in pulmonary capillaries. *J Appl Physiol* 73: 123–133, 1992.
17. **Fu Z, Heldt G, and West JB.** Increased fragility of pulmonary capillaries in newborn rabbit. *Am J Physiol Lung Cell Mol Physiol* 284: L703–L709, 2003.
18. **Fu Z, Heldt GP, and West JB.** Thickness of the blood-gas barrier in premature and 1-day-old newborn rabbit lungs. *Am J Physiol Lung Cell Mol Physiol* 285: L130–L136, 2003.
19. **Gehr P, Bachofen M, and Weibel ER.** The normal human lung: ultrastructure and morphometric estimation of diffusion capacity. *Respir Physiol* 32: 121–140, 1978.
20. **Groves BM, Reeves JT, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM, and Houston CS.** Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. *J Appl Physiol* 63: 521–530, 1987.
21. **Haworth SG, Hall SM, and Patel M.** Peripheral pulmonary vascular and airway abnormalities in adolescents with rheumatic mitral stenosis. *Int J Cardiol* 18: 405–416, 1988.
22. **Hofmann H, Voss T, Kuhn K, and Engel J.** Localization of flexible sites in thread-like molecules from electron micrographs. Comparison of interstitial, basement membrane and intima collagens. *J Mol Biol* 172: 325–343, 1984.
23. **Hopkins SR, Schoene RB, Henderson WR, Spragg RG, and West JB.** Sustained submaximal exercise does not alter the integrity of the lung blood-gas barrier in elite athletes. *J Appl Physiol* 84: 1185–1189, 1998.
24. **Hopkins SR, Schoene RB, Martin TR, Henderson WR, Spragg RG, and West JB.** Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090–1094, 1997.
25. **Hughes G.** *The Vertebrate Lung*. Burlington, NC: Oxford/Carolina Biological Supply Company, 1979.
26. **Hultgren HN.** High altitude pulmonary edema. In: *Biomedicine of High Terrestrial Elevations*, edited by Hegnauer AH. New York: Springer-Verlag, 1969, p. 131–141.
27. **Jones JH, Effmann EL, and Schmidt-Nielsen K.** Lung volume changes during respiration in ducks. *Respir Physiol* 59: 15–25, 1985.
28. **Jones JH, Smith BL, Birks EK, Pascoe JR, and Hughes TR.** Left atrial and pulmonary arterial pressures in exercising horses. *FASEB J* 6: A20201992. (Abstract).
29. **Kay JM, De Sa DJ, and Mancer JF.** Ultrastructure of lung in pulmonary veno-occlusive disease. *Hum Pathol* 14: 451–456, 1983.
30. **Kern A, Eble J, Golbik R, and Kühn K.** Interaction of type IV collagen with the isolated integrins a1b1 and a2b1. *Eur J Biochem* 215: 151–159, 1993.
31. **Liu M, Liu J, Buch S, Tanswell AK, and Post M.** Antisense oligonucleotides for PDGF-B and its receptor inhibit mechanical strain-induced fetal lung cell growth. *Am J Physiol Lung Cell Mol Physiol* 269: L178–L184, 1995.
32. **Low FN.** Electron microscopy of the rat lung. *Anat Rec* 113: 437–443, 1952.
33. **Maina JN.** The morphology of the lung of the black mamba *Dendroaspis polylepis* (Reptilia: Ophidia: Elapidae). A scanning and transmission electron microscopic study. *J Anat* 167: 31–46, 1989.
34. **Maina JN.** *Functional Morphology of the Vertebrate Respiratory Systems*. Enfield, UK: Science Publishers, 2002.
35. **Maina JN.** Fundamental structural aspects and features in the bioengineering of the gas exchangers: comparative perspectives. *Adv Anat Embryol Cell Biol* 163: 1–112, 2002.
36. **Maina JN and Maloiy GMO.** The morphometry of the lung of the African lungfish *Protopterus-Aethiopicus* and its structural-functional correlations. *Proc R Soc Lond B Biol Sci* 224: 399–420, 1985.
37. **Malpighi M.** *Duae epistolae de pulmonibus*. Florence, Italy: 1661.
38. **Neal CR and Michel CC.** Openings in frog microvascular endothelium induced by high intravascular pressures. *J Physiol* 492: 39–52, 1996.
39. **Parker JC, Breen EC, and West JB.** High vascular and airway pressures increase interstitial protein mRNA expression in isolated rat lungs. *J Appl Physiol* 83: 1697–1705, 1997.
40. **Policard A.** Les nouvelles idées sur la disposition de la surface respiratoire pulmonaire. *Presse Med* 37: 1293–1295, 1929.
41. **Rahn H and Fenn WO.** *A Graphical Analysis of the Respiratory Gas Exchange*. Washington, DC: American Physiological Society, 1955.
42. **Reid LM.** The pulmonary circulation: remodeling in growth and disease. *Am Rev Respir Dis* 119: 531–546, 1979.
43. **Schoene RB, Swenson ER, Pizzo CJ, Hackett PH, Roach RC, Millis WJ Jr, Henderson WR Jr, and Martin TR.** The lung at high altitude: bronchoalveolar lavage in acute mountain




Downloaded from ajplung.physiology.org on July 28, 2010

sickness and pulmonary edema. *J Appl Physiol* 64: 2605–2613, 1988.
44. **Stenmark KR and Mecham RP.** Cellular and molecular mechanisms of pulmonary vascular remodeling. *Annu Rev Physiol* 59: 89–144, 1997.
45. **Swayne GT, Smaje LH, and Bergel DH.** Distensibility of single capillaries and venules in the rat and frog mesentery. *Int J Microcirc Clin Exp* 8: 25–42, 1989.
46. **Swenson ER, Maggiorini M, Mongovin S, Gibbs JS, Greve I, Mairbaurl H, and Bartsch P.** Pathogenesis of high-altitude pulmonary edema: inflammation is not an etiologic factor. *JAMA* 287: 2228–2235, 2002.
47. **Takami H, Burbelo PD, Fukuda K, Chang HS, Phillips SL, and Yamada Y.** Molecular organization and gene regulation of type IV collagen. *Contrib Nephrol* 107: 36–46, 1994.
48. **Timpl R, Wiedemann H, van Delden V, Furthmayr H, and Kühn K.** A network model for the organization of type IV collagen molecules in basement membranes. *Eur J Biochem* 120: 203–211, 1981.
49. **Tozzi CA, Poiani GJ, Harangozo AM, Boyd CD, and Riley DJ.** Pressure-induced connective tissue synthesis in pulmonary artery segments is dependent on intact endothelium. *J Clin Invest* 84: 1005–1012, 1989.
50. **Tsukimoto K, Mathieu-Costello O, Prediletto R, Elliott AR, and West JB.** Ultrastructural appearances of pulmonary capillaries at high transmural pressures. *J Appl Physiol* 71: 573–582, 1991.
51. **Vaccaro CA and Brody JS.** Structural features of alveolar wall basement membrane in the adult rat lung. *J Cell Biol* 91: 427–437, 1981.
52. **Wagner PD, Gale GE, Moon RE, Torre-Bueno JR, Stolp BW, and Saltzman HA.** Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260–270, 1986.
53. **Weibel ER.** Morphological basis of alveolar-capillary gas exchange. *Physiol Rev* 53: 419–495, 1973.
54. **Welling LW and Grantham JJ.** Physical properties of isolated perfused renal tubules and tubular basement membranes. *J Clin Invest* 51: 1063–1075, 1972.
55. **West JB, Fowler KT, Hugh-Jones P, and O'Donnell TV.** Measurement of the ventilation-perfusion ratio inequality in the lung by the analysis of a single expirate. *Clin Sci* 16: 529–547, 1957.
56. **West JB and Mathieu-Costello O.** Strength of the pulmonary blood-gas barrier. *Respir Physiol* 88: 141–148, 1992.
57. **West JB and Mathieu-Costello O.** Pulmonary circulation. In: *Exercise and Circulation in Health and Disease*, edited by Saltin B, Boushel R, Secher NH, and Mitchell J. Champaign, IL: Human Kinetics, 2000, p. 79–91.
58. **West JB, Mathieu-Costello O, Jones JH, Birks EK, Logemann RB, Pascoe JR, and Tyler WS.** Stress failure of pulmonary capillaries in racehorses with exercise-induced pulmonary hemorrhage. *J Appl Physiol* 75: 1097–1109, 1993.
59. **West JB, Tsukimoto K, Mathieu-Costello O, and Prediletto R.** Stress failure in pulmonary capillaries. *J Appl Physiol* 70: 1731–1742, 1991.
60. **Whitwell KE and Greet TR.** Collection and evaluation of tracheobronchial washes in the horse. *Equine Vet J* 16: 499–508, 1984.
61. **Wieslander J and Heinegard D.** The involvement of type IV collagen in Goodpasture's Syndrome. *Ann NY Acad Sci* 460: 363–374, 1985.
62. **Williamson JR, Vogler NJ, and Kilo C.** Regional variations in the width of the basement membrane of muscle capillaries in man and giraffe. *Am J Pathol* 63: 359–370, 1971.
63. **Wirtz HR and Dobbs LG.** Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells. *Science* 250: 1266–1269, 1990.



Downloaded from ajplung.physiology.org on July 28, 2010