Very high pressures are required to cause stress failure of pulmonary capillaries in Thoroughbred racehorses

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Birks, Eric K., Odile Mathieu-Costello, Zhenxing Fu, Walter S. Tyler, and John B. West. Very high pressures are required to cause stress failure of pulmonary capillaries in Thoroughbred racehorses. J. Appl. Physiol. 82(5):1584–1592, 1997.—Thoroughbred horses develop extremely high pulmonary vascular pressures during galloping, all horses in training develop exercise-induced pulmonary hemorrhage, and we have shown that this is caused by stress failure of pulmonary capillaries. It is known that the capillary transmural pressure (Ptm) necessary for stress failure is higher in dogs than in rabbits. The present study was designed to determine this value in horses. The lungs from 15 Thoroughbred horses were perfused with autologous blood at Ptm values (midlung) of 25, 50, 75, 100, and 150 mmHg, and then perfusion fixed, and samples (dorsal and ventral, from caudal region) were examined by electron microscopy. Few disruptions of capillary endothelium were observed at Ptm = 75 mmHg, and 5.3 ± 2.2 and 4.3 ± 0.7 breaks/mm endothelium were found at 100 and 150 mm Hg Ptm, respectively. Blood-gas barrier thickness did not change with Ptm. At low Ptm, interstitial thickness was greater than previously found in rabbits but not in dogs. We conclude that the Ptm required to cause stress failure of pulmonary capillaries is between 75 and 100 mmHg and is greater in Thoroughbred horses than in both rabbits and dogs.

 METHODS

Animals

Fifteen Thoroughbred horses (445 ± 59 (SD) kg; 7.0 ± 2.7 yr; 9 mares and 6 geldings) were used for this project. They had been donated to the University of California, Davis, for reasons unrelated to the respiratory system (lameness, reproductive or neurological difficulties), and the protocols for these experiments were approved by the Animal Subjects Committees of the University of California, San Diego, and the University of California, Davis.

Lung Perfusion

The horses were heparinized (50 IU/kg), anesthetized with pentobarbital sodium (60 mg/kg), and then killed by exsanguination via a cannulated carotid arterial catheter. The lung and heart were removed en bloc; cannulas were placed in the trachea, pulmonary artery, and left atrium; and the entire preparation was floated in a tank filled with water, in a position as close as possible to that in the standing animal. The lungs were inflated three times to a pressure of 25 mmHg with compressed air and then maintained at 11 mmHg positive pressure for the duration of the procedure. The pulmonary vasculature was perfused via the pulmonary artery with the blood previously collected from the animal. After ~3 min (total volume 10–20 liters), the blood perfusion was replaced by a saline mixture (11.06 g NaCl/l, 350 mosM; 10 IU/ml heparin), which continued for 20 min (total volume 120 liters) to wash out the blood. The lungs were then fixed by perfusing for 50 min (total volume 120 liters) with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (total osmolarity 500 mosM, pH 7.4). As in previous studies (8, 13, 18, 22), all three perfusions were done at the same preset Ptm.

All pressures were measured with reference to midlung. Pulmonary arterial pressures were adjusted to 38, 63, 88, 113, or 163 ± 1 mmHg, and the pulmonary venous (i.e., left atrial) pressures were maintained 4 ± 1 mmHg lower than the arterial pressures. Capillary hydrostatic pressures were therefore 36, 61, 86, 111, or 161 ± 2 mmHg. Because airway pressure was a constant 11 mmHg, midlung capillary Ptm values were 25, 50, 75, 100, or 150 ± 2 mmHg. The preparations were randomly assigned to one of the pressure protocols, and pressures were monitored by transducers (Statham P23Db) attached to each of the cannulas. Perfusion solutions were continuously pumped to a container suspended by a pulley above the lung preparation. The container had an overflow standpipe that maintained a constant level in the container as the pump inflow rate exceeded the flow directed to the cannula in the pulmonary artery. The height of the container was adjusted to maintain the preset pulmonary arterial perfusion pressure. Left atrial pressure was maintained similarly by adjusting the height of the outflow line from the left atrium. After 50 min of perfusion fixation, the pulmonary venous pressure was raised to match the arterial pressure, and the lung was kept at that pressure overnight.

EXERCISE-INDUCED pulmonary hemorrhage (EIPH) occurs in all Thoroughbred racehorses during severe exercise (15, 23). We have shown that this hemorrhage results from stress failure of pulmonary capillaries, presumably caused by high capillary transmural pressure (Ptm) (21). It is known that pulmonary vascular pressures in exercising Thoroughbreds can be very high, with mean pulmonary arterial and left atrial pressures up to 120 and 70 mmHg, respectively (3, 7, 10, 11, 21). However, the actual pressures required to cause stress failure of pulmonary capillaries in racehorses are unknown. Previous studies in rabbits have shown that stress failure of the blood-gas barrier (BGB) consistently occurs when pulmonary capillaries are exposed to Ptm > 40 mmHg (8, 17). In dogs, a Ptm of ~70 mmHg was required to cause similar stress failure (13). Although we have shown that structural failure of pulmonary capillaries in Thoroughbreds occurs at the high pulmonary vascular pressures observed during severe exercise (21), it is important to determine the minimal pressures required for stress failure. The present study was designed to measure this value in Thoroughbred racehorses.
Tissue Sampling and Preparation

The lungs were removed from the water tank and dissected from the surrounding tissues, and a transverse slice ~1-cm thick was cut from the left lower lobe, perpendicular to the craniocaudal plane, at a distance from the caudalmost aspect of ~22% of total lung length. From these slices, two 1 × 1 × 1-cm blocks were taken along the dorsoventral midline of the cut surface of the tissue slices, each block including the whole thickness of the slice. One block was taken at one-third the distance from the dorsal edge of the lung to the top of the middle third of the slice (dorsal sample), and the other was cut at one-third the distance from the ventral edge of the lung to the bottom of the middle third of the slice (ventral sample). These sites were chosen to examine the dorsoventral region, which is known to be most frequently associated with EIPH in racehorses (12, 14), and compare it with the ventral aspect of the lung at the same level along the craniocaudal plane. The blocks were stored in the glutaraldehyde fixative at 4°C for 10–120 days.

Microscopy

The fixed samples were cut into smaller blocks, rinsed overnight in 0.1 M phosphate buffer (350 mosM, pH 7.4), and then postfixed for 2 h in 1% osmium tetroxide in 0.125 M sodium cacodylate buffer (total osmolarity 400 mosM, pH 7.4). They were dehydrated in increasing concentration (70–100%) of ethanol, rinsed in propylene oxide, and embedded in Araldite. Sections (1 µm) were cut from each sample, stained with 0.1% toluidine blue aqueous solution, and examined by light microscopy. For electron microscopy, ultrathin sections (50–70 nm) were contrasted with uranyl acetate and bismuth oxinitrate (16) and examined with a Zeiss 10 electron microscope. Micrographs for morphometry were taken on 70-mm films, and micrographs of a carbon grating replica (E. F. Fullam, Schenectady, NY) were recorded for calibration on each film.

Morphometry

A total of 60 electron micrographs (12 micrographs taken by systematic random sampling from each of 5 blocks) were taken from each sample. A Videometric 150 image analyzer (American Innovision) was used to obtain electronic positive reversal of the 70-mm negative electron-micrograph films and quantify the frequency of endothelial and epithelial disruptions as well as the thickness of the BGF at a final magnification of ×11,000. Prints of each micrograph were available for positive identification of structures, in particular to distinguish basement membranes or identify small disruptions on the video monitor of the image analyzer, as needed during measurement. Quantification of the frequency of disruptions of the BGF was achieved by tracing the contour of the alveolar (outer) and capillary (inner) boundaries in each field of view and recording the number and length of disruptions in each segment. At each disruption site, the presence or absence of red blood cells (RBCs) and continuous basement membrane were also noted, as well as the number of RBCs located extravascularly, either in the interstitium or alveoli. Measurements of the thickness of the various layers of the barrier were made at right angles to the barrier at the sites determined at random by intersection of the barrier with fixed test lines generated on the image analyzer (up to 5 intersections per micrograph). As in all our previous studies, the reader of the electron-microscopic morphometry was blinded to the pressure group.

Statistics

Data are expressed as means ± SE. The equation for the SE of ratio (5) was used to compute the SE of the estimates of the number of breaks per unit boundary length. Data from all micrographs (n = 60) from a single sample were pooled for these estimates, so the computed SE represents the variability between micrographs at that site. We also examined the variability between blocks (n = 5) in each site for the same total number of 60 micrographs, by pooling the data of the 12 micrographs within each block. Means were compared by using analysis of variance. A P value of < 0.05 was considered statistically significant. When P < 0.05, Tukey's post hoc test was used to identify those means that were significantly different. Group mean comparisons between dorsal and ventral sites at a given pressure were done by paired Student's t-test.

RESULTS

Macroscopic and Microscopic Findings

For all lungs perfused at the lowest capillary Ptm of 25 mmHg, no obvious changes were observed at the lung surface, and no fluid was apparent in the tracheal cannula. During perfusion at Ptm of 50 and 75 mmHg, fluid droplets appeared on the lung surface and clear, frothy fluid was noted in the tracheal cannula. At Ptm = 100 mmHg, the fluid at the lung surface and within the trachea was red tinged and greatly increased in volume. Tracheal fluid was first observed ~31 min after the start of perfusion at 50 mmHg Ptm, ~15 min at 75 mmHg, ~8 min at 100 mmHg, and 2.5 min at 150 mmHg Ptm. Figure 1 shows light micrographs of the appearance of the lung parenchyma in dorsal (A-E) and ventral samples (F-J) at each Ptm. At the lower pressures (25–75 mmHg), capillaries appeared more distended in ventral than in dorsal samples, for the same perfusion pressure. With increasing Ptm (100 and 150 mmHg), several capillaries did not appear more distended than at lower Ptm, both in dorsal and ventral regions, possibly because disruption of the endothelium (see Endothelial Breaks) occurred in the area and released the high pressure in the capillaries. RBCs were found in some capillaries and, occasionally, in alveolar spaces of lungs perfused at Ptm of 75 mmHg and below. At higher pressures, the number of RBCs in alveolar spaces greatly increased and interstitial edema was apparent. Figure 1, E and J, shows dense packing of RBCs in capillaries, RBCs in interstitial and alveolar space, and evidence of interstitial edema in both dorsal and ventral sites after perfusion at 150 mmHg Ptm.

Electron-Microscopic Findings

General appearance. Figure 2 shows examples of the ultrastructural appearance of the pulmonary capillaries perfusion fixed at low (25 mmHg) and high (100 mmHg) Ptm. At low Ptm, the integrity of the endothelial layer was generally preserved. A typical example of a capillary perfused at Ptm of 25 mmHg is shown in Figure 2A. At Ptm ~ 100 mmHg, a number of disruptions of the endothelial layer were found (Fig. 2B), resulting in the presence of RBCs within the intersti-
Fig. 1. Light micrographs of portions of parenchyma in dorsal (A-E) and ventral samples (F-J) of horse lungs perfused at capillary transmural pressure (Ptm) values of 25 (A, F), 50 (B, G), 75 (C, H), 100 (D, I), and 150 mmHg (E, J). Note red blood cells (RBCs) in interstitium (solid arrow), widening of blood-gas barrier by interstitial edema (open arrow), and RBCs in alveoli (arrowhead) at 150 mmHg. All micrographs are at same magnification and, for each pressure, micrographs of dorsal and ventral samples are from same lung. Bar, 20 µm.
tium (Fig. 2C), in the alveoli (Fig. 2B), and frequently protruding through the area of disruption (Fig. 2B). For reasons addressed in DISCUSSION, unexpected disruptions in the alveolar epithelial layer were observed in a number of samples. A typical example of an epithelial disruption is shown in Fig. 2D.

Discontinuities in BGB. Tables 1 and 2 show the average frequencies and lengths of disruptions of the capillary endothelium and alveolar epithelium in dorsal and ventral samples, respectively, from horse lungs perfusion fixed at each Ptm.

Endothelial Breaks

Break frequencies and lengths for capillary endothelium are plotted in Fig. 3. A few endothelial breaks were found in dorsal (Table 1) and ventral sites (Table 2) of some animals at Ptm ≤ 75 mmHg. Break frequencies of <1, 1.5, and 2.9/mm in those animals correspond to the finding of 1, 3, and 5 endothelial disruptions, respectively, out of 55–60 micrographs examined in each site from each animal. The number of endothelial breaks per millimeter was significantly greater (P < 0.05) at Ptm of 100 and 150 mmHg than for Ptm of 25, 50, and 75 mmHg both in dorsal and ventral samples. Overall, the endothelial breaks tended to be systematically more frequent in dorsal than ventral samples (Fig. 3A), but the differences were not statistically significant because of large intragroup variability.

To further investigate the large intragroup variability in the frequency of disruptions, we compared the coefficient of variation (CV = SD/mean) between micrographs (total 55–60 from 5 blocks) with that between the 5 blocks (n = 5) in each sample. At each pressure, CV between blocks for endothelial and epithelial breaks (mean for both sites and all animals 1.46 ± 0.10; n = 42) was substantially lower than between micrographs (4.78 ± 0.30). As previously found in rabbits (8) and dogs (13), this indicated that the source of variability in
Table 1. Morphometric measurements of endothelial and epithelial breaks per millimeter and average break length at each capillary transmural pressure in dorsal samples

<table>
<thead>
<tr>
<th>Capillary Transmural Pressure, mmHg</th>
<th>Animal No.</th>
<th>Animal Wt, kg</th>
<th>Sex</th>
<th>Endothelial Breaks/mm</th>
<th>Endothelial Break Length, µm</th>
<th>Epithelial Breaks/mm</th>
<th>Epithelial Break Length, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1</td>
<td>433</td>
<td>G</td>
<td>0</td>
<td>4.7 ± 3.2</td>
<td>1.62 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>432</td>
<td>G</td>
<td>0</td>
<td>22.5 ± 8.8</td>
<td>2.28 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>581</td>
<td>G</td>
<td>0</td>
<td>0</td>
<td>0.54 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>461 ± 41</td>
<td></td>
<td>0.2 ± 0.2</td>
<td>0.68 ± 0.05</td>
<td>7.3 ± 5.1</td>
<td>1.48 ± 0.51</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>458</td>
<td>G</td>
<td>0.4 ± 0.4</td>
<td>0.62 ± 0.04</td>
<td>8.0 ± 3.3</td>
<td>5.36 ± 1.14</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>390</td>
<td>M</td>
<td>0.8 ± 0.8</td>
<td>1.27 ± 0.2</td>
<td>9.2 ± 6.0</td>
<td>2.56 ± 1.22</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>400</td>
<td>M</td>
<td>0.5 ± 0.5</td>
<td>2.49 ± 0.1</td>
<td>7.0 ± 2.7</td>
<td>2.22 ± 0.37</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>416 ± 21</td>
<td></td>
<td>0.6 ± 0.1</td>
<td>1.46 ± 0.55</td>
<td>8.1 ± 0.6</td>
<td>3.38 ± 0.99</td>
</tr>
<tr>
<td>75</td>
<td>8</td>
<td>470</td>
<td>M</td>
<td>0</td>
<td>1.9 ± 0.9</td>
<td>1.21 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>9</td>
<td>491</td>
<td>G</td>
<td>2.9 ± 1.5</td>
<td>0.74 ± 0.17</td>
<td>3.4 ± 1.9</td>
<td>1.23 ± 0.19</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
<td>395</td>
<td>M</td>
<td>1.5 ± 0.8</td>
<td>0.68 ± 0.4</td>
<td>3.2 ± 1.0</td>
<td>2.20 ± 0.29</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>452 ± 29</td>
<td></td>
<td>1.5 ± 0.8</td>
<td>0.71 ± 0.03</td>
<td>12.5 ± 9.9</td>
<td>1.55 ± 0.32</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>539</td>
<td>M</td>
<td>9.8 ± 3.3</td>
<td>4.14 ± 1.52</td>
<td>2.6 ± 0.2</td>
<td>4.34 ± 1.99</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>406</td>
<td>G</td>
<td>3.3 ± 2.9</td>
<td>0.90 ± 0.31</td>
<td>1.4 ± 0.8</td>
<td>3.18 ± 0.39</td>
</tr>
<tr>
<td>100</td>
<td>13</td>
<td>485</td>
<td>M</td>
<td>2.9 ± 1.7</td>
<td>1.44 ± 0.44</td>
<td>7.2 ± 2.3</td>
<td>6.82 ± 2.67</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>477 ± 39</td>
<td></td>
<td>5.3 ± 2.2</td>
<td>2.16 ± 1.00</td>
<td>3.7 ± 1.8</td>
<td>4.78 ± 1.07</td>
</tr>
<tr>
<td>150</td>
<td>14</td>
<td>369</td>
<td>M</td>
<td>5.0 ± 2.1</td>
<td>1.58 ± 0.30</td>
<td>6.5 ± 2.0</td>
<td>2.04 ± 0.35</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>425</td>
<td>M</td>
<td>3.6 ± 1.3</td>
<td>1.38 ± 0.46</td>
<td>17.1 ± 4.6</td>
<td>3.01 ± 0.43</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>397 ± 28</td>
<td></td>
<td>4.3 ± 0.7</td>
<td>1.48 ± 0.1</td>
<td>11.8 ± 5.3</td>
<td>2.53 ± 0.49</td>
</tr>
</tbody>
</table>

Values are means ± SE of data from 5 blocks (total 55–60 micrographs). SE between micrographs (breaks/mm) or individual measurements (break length) in each animal is shown. G, gelding; M, mare.

The frequency of endothelial and epithelial disruptions was at the level of the micrographs (i.e., individual capillaries and alveoli), rather than regional (between blocks) in each site in any given animal. We expect to find such a high variability in a study in which the end point is stress failure, i.e., rupture of the weakest point of the system (8, 13).

Endothelial break length did not differ significantly between dorsal and ventral sites or with increasing Ptm (Fig. 3B), and the majority (60–100%) of the

Fig. 3. Group means ± SE of number of endothelial breaks per millimeter endothelial boundary length (A) and average length of endothelial discontinuities (B) in dorsal (○) and ventral samples (●) at each Ptm.
endothelial breaks were associated with a continuous basement membrane. As we have previously reported in rabbits (8, 17) and in dog lungs (13) exposed to increased Ptm, RBCs were often found at the sites of endothelial discontinuities. The percentage of endothelial breaks associated with RBCs also increased with Ptm, reaching values of 50–70% at Ptm \( \geq 100 \) mmHg.

Epithelial Breaks

No significant differences were apparent in either the number or lengths of breaks per millimeter boundary length of alveolar epithelium at the different Ptm in dorsal (Table 1) or ventral samples (Table 2). Surprisingly, even at the lowest Ptm, a large number of epithelial breaks were seen in both sites. A comparison of the number or length of breaks in dorsal vs. ventral samples revealed no significant difference at any Ptm, except at 50 mmHg, where the number of epithelial disruptions was significantly greater in dorsal than ventral sample. As seen with the capillary endothelium, the majority (70–100%) of the alveolar epithelial breaks were associated with a continuous basement membrane.

Thickness of BGB. Figure 4 summarizes data for the average thickness of each of the layers of the BGB and total barrier thickness, with the group means for each layer plotted against Ptm in each site. There were no significant differences in the thicknesses of any layers with increased Ptm in either site. Although barrier thickness tended to be systematically greater in the ventral than dorsal site, none of the differences was significant for any layer at any pressure.

DISCUSSION

The present study provides the first direct measurements of the pulmonary Ptm required to cause stress failure of the BGB in Thoroughbred racehorses. A previous report (4) using morphological measurements predicted that this pressure would be greater than those found to cause similar disruptions in rabbits (8, 17) and dogs (13). The results reported here confirm this prediction. In addition, we have now shown that the pressures required to cause stress failure are lower than those that exist in the galloping animal, confirming that the pathogenesis of EIPH in racehorses is stress failure of pulmonary capillaries caused by very high pulmonary Ptm (21).

The appearance of fluid droplets on the surface of the perfused lungs and, more importantly, the apparent increase in volume and RBC content of the fluid in the airways at the higher Ptm, indicate edema formation. The relationship of stress failure to this type of edema formation has been discussed in detail (17, 18, 21, 22). In the present study, fluid was seen within the tracheal catheter at Ptm \( \geq 50 \) mmHg, with the time of first observance being inversely related to Ptm (i.e., earlier during the perfusion protocol at higher Ptm). Similar results were found in perfused rabbit lungs (17), except that no fluid was observed at the outer surface of the lungs at any Ptm. Additionally, similar macroscopic findings were observed in perfused dog lungs (13). Although this perfusion-fixation technique has been successfully utilized to preserve lungs of several species for electron microscopy (1, 13, 22), there have been, to our knowledge, no other reports on the macroscopic appearance of lungs perfused at the high pulmonary vascular pressures associated with high-intensity exercise in Thoroughbred racehorses.

The ultrastructural findings (Fig. 2, A–D) are similar to those of a number of reports in which elevated Ptm was a feature (13, 17, 22). Consistent findings at high Ptm include 1) disruptions of either capillary endothelium, alveolar epithelium, or both; 2) RBCs within the interstitium or in the alveoli; and 3) blood cell elements associated with the basement membrane in areas of endothelial discontinuity. In the present study, significant disruption of the capillary endothelium was found at Ptm \( \geq 100 \) mmHg in dorsal samples. That this Ptm is physiologically relevant is supported by recent reports of pulmonary arterial pressures ranging from 85 to 130 mmHg in racehorses during exercise and pulmonary venous pressures of 60–70 mmHg (3, 7, 10, 11, 21). Thus the Ptm of 100 mmHg found in this study to cause significant disruptions of the pulmonary capillary endothelium is consistent with measured pulmonary vascular pressures associated with clinical evidence of EIPH (21). It is \( \approx 30 \) mmHg higher than that shown to cause similar changes in dog lungs (13) and \( \geq 60 \) mmHg higher than that for rabbit lungs (8, 17, 22).

We recently provided an analysis of the structural elements responsible for the strength of the BGB and predicted that stress failure would occur at a low Ptm in rabbits, an intermediate value in dogs, and the highest value in horses (4). Those predictions were supported by direct experimental data in rabbits (17, 22), dogs (13), and horses in the present study. However, as also found in dogs (13), the actual Ptm required to cause significant stress failure of the BGB in horses was greater than predicted from morphological measurements, with the assumption of equal tissue strength in the three species (4). In addition, interstitial thickness at low Ptm in horse (0.3 µm; Fig. 4) did not differ significantly from that measured in dogs (13), whereas
a greater Ptm was required to cause failure of the BGB in horses. Both observations suggest that differences in the strength of structural elements within the BGB, possibly a thicker or molecularly stronger basement membrane, may contribute to the greater resistance to stress failure of horses compared with dog and rabbit capillaries, in addition to differences in tissue dimensions. The mechanisms responsible for the stress failure of pulmonary capillaries have been presented in detail elsewhere (4, 19, 22). They indicate that EIPH in horses can be explained by high pulmonary vascular Ptm leading to stress failure of the BGB and that the BGB in racehorses is extremely strong.

The number of endothelial breaks per millimeter capillary boundary length observed at 100 mmHg Ptm in horse lung (Table 1) was approximately the same as that found in dogs (13) and rabbits (17), at the Ptm necessary to cause stress failure in those species (~70 and 40 mmHg, respectively). Also, average endothelial break length did not differ across species. These findings suggest that once the critical Ptm is reached, the amount of damage to the BGB is similar regardless of the species or absolute Ptm value responsible for stress failure. This is consistent with the notion that the BGB, which needs to be as thin as possible in each species to maintain its maximal efficiency for gas exchange, may have evolved toward the minimal barrier consistent with enough strength to sustain the most challenging physiological conditions encountered (20), beyond which similar damages to the BGB are seen across species. The similar frequencies and break lengths at the Ptm necessary to cause stress failure in the three species are also consistent with a similar behavior of the basement membrane under tension.

Continuous basement membranes were commonly found at sites of endothelial disruptions, as also found in rabbits (17) and dogs (13). Both the short length of the breaks and their frequent presence along a continuous basement membrane are consistent with a rapid reversibility of the breaks once the pressure is reduced (6). Once ruptures have occurred in a given capillary segment, the pressure is released, and this can possibly allow the reversibility of some of the breaks during the longer perfusion durations in the horse (this study) and dog preparations (13) compared with that of rabbit (17). This can possibly explain why the sharp increase in break frequencies with increasing pressure above the threshold for stress failure seen in rabbits (17) was not found in horses or dogs above their respective threshold. It may also explain the paucity of open disruptions we had observed in lungs of horses fixed ~1 h after the animals galloped at intensities that induced documented EIPH (21). This and the frequent presence of RBCs at the sites of disruption are also consistent with the notion of rapid closure or obstruction of the openings and with the fact that the hemorrhage usually stops, once the pressure is reduced.

Another interesting finding was that endothelial breaks tended to be systematically less frequent in ventral than dorsal caudal region (Fig. 3). This is also consistent with the pathology of EIPH in racehorses, in which lesions are known to be most abundant in the dorsocaudal region (12, 14). Several functional differences could explain this observation, including greater blood flow, lower transient alveolar pressure, and/or greater alveolar size in the dorsal region during maximal galloping (2, 9, 21). The present study shows that the ventral region appears less vulnerable to stress failure of the pulmonary capillaries than the dorsal part of the lung, despite the higher Ptm (due to the vertical hydrostatic gradient) in the ventral region. Because the lungs were floated before inflation, we could not accurately measure lung heights at the time of the experiment. However, we estimate a 40- to 50-cm vertical lung height in the caudal slice (22% of total lung length) from which samples were taken. With pressures referenced to midlung, this represents differences of ±13 ± 1 mmHg in the ventral and dorsal sites in which samples were examined, respectively. According to the Laplace relationship, capillary wall stress is proportional to Ptm times radius of curvature divided by wall thickness (22). It is not known whether stronger structural elements within the BGB or smaller radius of curvature of capillaries in ventral site also contributes to its apparent greater resistance to stress failure. Also, distortion due to the weight of the lung makes it probable that the alveoli in the dorsal region are larger than those of more ventral areas. Interestingly, light-microscopic examination showed that capillaries appeared less distended in dorsal than ventral samples at lower pressures (25–75 mmHg), suggesting greater alveolar inflation in dorsal samples. As we have shown, increasing alveolar volume greatly increases the incidence of stress failure of pulmonary capillaries (8).

One difference between the present study and our previous reports of stress failure of the BGB (8, 13, 17) is the fact that, in these horses, there were no significant differences in the number of disruptions of the alveolar epithelial layer at the different Ptm. Although the average number of breaks observed at the two

Table 3. Morphometric measurements of endothelial and epithelial breaks per millimeter, average break length, and blood-gas barrier dimensions in horse lung perfusion fixed at low pressure with solutions containing dextran

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sample No.</th>
<th>Site</th>
<th>Endothelial Breaks/mm</th>
<th>Epithelial Breaks/mm</th>
<th>Epithelial Break Length, µm</th>
<th>Endothelial Thickness, µm</th>
<th>Interstitial Thickness, µm</th>
<th>Epithelial Thickness, µm</th>
<th>Total Thickness, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>222-1*</td>
<td>L15 s</td>
<td>Dorsal</td>
<td>0</td>
<td>0.3 ± 0.3</td>
<td>1.84 (n = 1)</td>
<td>0.18 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td>0.26 ± 0.01</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>222-1*</td>
<td>L15 u</td>
<td>Ventral</td>
<td>2.3 ± 1.5</td>
<td>0.95 ± 0.23</td>
<td>0.18 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.65 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of data from 5 blocks (total, 60 micrographs). SE between micrographs (breaks/mm) or individual measurements in each site is shown. Perfusions were with use of saline (10 min) and glutaraldehyde (16 min) at pulmonary arterial pressure of 45 cmH2O, pulmonary venous pressure of 10 cmH2O, and alveolar pressure of 10 cmH2O. *Experiment 1 (21).
highest Ptm (100 and 150 mmHg) tended to be greater than at lower pressures both in dorsal and ventral sites, there was a great deal of intra- and intergroup variability, making none of the differences statistically significant. Indeed, the average numbers of epithelial breaks at 100 mmHg Ptm (dorsal) and 75 mmHg Ptm (ventral) were actually less than those found at 25 mmHg Ptm in that region. It is also of interest that even at the low Ptm, many more breaks were seen than in similarly designed studies of rabbits (8, 17) and dogs (13).

One difference in the protocol of the present study that could possibly explain these observations is the fact that the saline and glutaraldehyde perfusates contained no dextran (because of the great expense), whereas dextran was used in both the rabbit and dog studies. In our previous study of horse lung (21), we found no morphological differences in pulmonary capillaries after perfusion with solutions containing dextran or not. However, that study included only a single animal at each treatment. Thus the lack of dextran could have produced alveolar epithelial damage unrelated to Ptm. In addition, the fact that, in the present study, samples were taken from the lung region most commonly involved in EIPH also raises the possibility that the alveolar epithelium may be particularly susceptible to stress failure in this region because of inherent physical or physiological features.

However, a substantial number of epithelial breaks were found at low Ptm in both dorsal and ventral sites (Tables 1 and 2), this without any endothelial disruption (Fig. 3A) or evidence of interstitial edema. In addition, morphometric measurements in a portion of the lung as close as possible to that examined in this study were performed in the lung perfusion fixed with solutions containing dextran in the previous study (experiment 1 in Ref. 21). The results (Table 3) show that epithelial breaks were also found in the tissues after perfusion with dextran, in this absence of evidence of endothelial disruption or interstitial edema and at a lower Ptm (=18 mmHg) than considered in this study. Also, more epithelial disruptions were found in the ventral portion of the slab in this particular animal. Thus neither the lack of dextran nor a greater susceptibility of the dorsal region to epithelial damage, independent of Ptm, appears to account for the large incidence of epithelial breaks found in this study.

In contrast, a common feature to all studies in horses is that the lungs were inflated to 25 cm H2O (21) or 25 mmHg (this study) with compressed air until all areas were aerated, before reduction of alveolar pressure to the constant values (10 cm H2O and 11 mmHg, respectively) maintained during the perfusion. It is possible that overdistension of some areas of the lungs occurred during this high inflation before perfusion, causing epithelial damage. In this context, it is interesting that none or very few endothelial breaks were found in samples showing substantial epithelial damage (Tables 1 and 2). This supports the notion that the cellular layer contributes little to the strength of the BGB (19).

In conclusion, this is the first study relating the frequency of stress failure of pulmonary capillaries to capillary Ptm in racehorses. The study supports previous observations that EIPH occurs at very high capillary Ptm, and it confirmed our earlier observation that the layers of the BGB of horses are thicker than those in rabbits but not in dogs. The Ptm necessary to cause significant disruptions of pulmonary capillary endothelium in the lung of racehorses is between 75 and 100 mmHg.

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