Morphometry of the extremely thin pulmonary blood-gas barrier in the chicken lung

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Watson RR, Fu Z, West JB. Morphometry of the extremely thin pulmonary blood-gas barrier in the chicken lung. Am J Physiol Lung Cell Mol Physiol 292: L769–L777, 2007. First published November 17, 2006; doi:10.1152/ajplung.00355.2006.—The gas exchanging region in the avian lung, although proportionally smaller than that of the mammalian lung, efficiently manages respiration to meet the high energetic requirements of flapping flight. Gas exchange in the bird lung is enhanced, in part, by an extremely thin blood-gas barrier (BGB). We measured the arithmetic mean thickness of the different components (endothelium, interstitium, and epithelium) of the BGB in the domestic chicken lung and compared the results with three mammals. Morphometric analysis showed that the total BGB of the chicken lung was significantly thinner than that of the rabbit, dog, and horse (54, 66, and 70% thinner, respectively) and that all layers of the BGB were significantly thinner in the chicken compared with the mammals. The interstitial layer was strikingly thin in the chicken lung (~86% thinner than the dog and horse, and 75% thinner than rabbit) which is a paradox because the strength of the BGB is believed to come from the interstitium. In addition, the thickness of the interstitium was remarkably uniform, unlike the mammalian interstitium. The uniformity of the interstitial layer in the chicken is attributable to a lack of the supportive type I collagen cable that is found in mammalian alveolar lungs. We propose that the surrounding air capillaries provide additional structural support for the pulmonary capillaries in the bird lung, thus allowing the barrier to be both very thin and extremely uniform. The net result is to improve gas exchanging efficiency.

Capillary stress failure; lung morphology; bird respiratory physiology; air capillary; extracellular matrix; interstitium

IN THE VERTEBRATE LUNG, OPTIMAL thickness of the pulmonary blood-gas barrier (BGB) is affected by opposing selective pressures (65). The barrier thickness is minimized to allow efficient exchange of oxygen and carbon dioxide, yet the capillary walls must also be strong enough to withstand high pulmonary capillary pressures that are incurred during extreme physical exertion. The BGB is composed of three layers: capillary endothelium, an interstitial layer or extracellular matrix, and an epithelial layer (Fig. 1). Combined, these cellular layers are relatively thin, usually measuring <2 μm in total thickness under normal conditions (5, 33, 36). This three-ply ultrastructure of the BGB appears to be evolutionally well conserved over a variety of vertebrate taxa, indicating that its basic structure is efficient and relatively immalleable (18, 25, 27, 31, 33). The strength of the pulmonary capillary walls is likely related to the morphometry of the BGB, where the thickness of the interstitium determines the fragility of the capillaries (5). Where the BGB is thinnest, the interstitial layer is composed of only the fused basement membranes of the endothelium and epithelium layers. There is indirect evidence that the basement membrane determines the mechanical properties (e.g., wall strength) of the pulmonary capillaries (46). For example, in isolated perfused renal tubules of rabbits, the relationship between tubule diameter and applied transmural pressure was identical for the intact tubule and for a tubule consisting of only the basement membranes (62). These findings are consistent with the observation that single alveolar walls that are stripped of their endothelial and epithelial cellular constituents by a detergent do not show a reduction in the force required for extension (45).

Despite the apparent strength of the BGB, under certain conditions the barrier may fail and allow fluid to leak into the lungs. Virtually all horses bred and trained for competitive racing show evidence of exercise-induced pulmonary hemorrhage (EIPH), which manifests as bleeding from the nose after exertion or the presence of red blood cells or hemosiderophages in the airways (3, 6, 21, 39, 47, 53, 58, 67). The causative mechanism of EIPH was demonstrated experimentally when it was shown that high pulmonary capillary pressures can cause discrete breaks in the endothelium, epithelium, or the entire BGB in mammals (10, 50, 64, 66). Incidences of suspected EIPH (usually presenting as hemoptysis) have been documented in humans as well (15, 34, 35, 61), and evidence that maximal exercise can impair the integrity of the BGB and change the permeability of the capillary membrane was demonstrated by Hopkins et al. (16). However, submaximal exercise does not appear to affect the integrity of the BGB in humans or horses (17, 48). Stress failure also plays a role in the hospital setting, where overinflation of the lung via mechanical ventilation causes ventilator-induced lung injury.

In the present study, we quantified the thicknesses of the different components of the BGB in the chicken lung to compare with measurements collected in the same manner from rabbits, dogs, and horses. We selected the lung of a bird for our studies because of the paradoxical observation that the avian lung BGB is particularly thin despite the extremely high aerobic capacities and maximal oxygen consumptions found in some birds. Here, by measuring the individual components of the BGB, we wish to determine which layers are attenuated in birds compared with mammals with the ultimate goal of elucidating the mechanisms whereby the avian lung is superior in efficiency to the mammalian lung.

MATERIALS AND METHODS

Tissue sampling. The animal protocol for this experiment was approved by the Animal Subjects Committees of the University of California, San Diego. Animal surgery, perfusion, dissection, and

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sample preparation were similar to procedures previously performed in our laboratory (50). Chicken lungs were fixed using two methods, intravascular perfusion and tracheal instillation. For perfusion, five white leghorn chickens (Gallus gallus domesticus) were anesthetized with intravenous pentobarbital sodium (40 mg/kg) and the chest was opened to insert catheters into the main pulmonary artery and the left atrium. Mean pulmonary arterial pressure of 25 cmH2O and left atrial pressure of 20 cmH2O were maintained during vascular perfusion with heparinized saline/dextran solution (11.1 g NaCl, 350 mOsm; 3% T-70 dextran and 10 IU/ml heparin). Following wash-out, the pulmonary vasculature was perfusion fixed for 10 min with 2.5% glutaraldehyde and 3% T-70 dextran in 0.1 M phosphate buffer (total osmolarity 500 mOsm, pH 7.4), during which time the pulmonary artery and left atrial pressures were maintained constant. The lungs were then dissected free and stored in 2.5% gluteraldehyde at 4°C for 3 days. Intratracheal instillation was performed on one chicken, and the resultant samples were used for qualitative analysis and illustrative purposes only. During the intratracheal instillation procedure, the chicken was placed in a supine position after anesthesia and the lungs were infused with the fixation solution at a pressure of 20 cmH2O for 15 min. The lungs were removed, sampled as in the preceding procedure for perfusion, and all further procedures were identical.

Lung samples were taken from the paleopulmo portion of the lung from each chicken at about one-third the distance from the most caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from the entire width of each lung transverse to the cranial-caudal axis. This section was then systematically cut into 10 vertical slices from the cranial to the caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from each chicken at about one-third the distance from the most caudal aspect of the lung. The lungs were removed, sampled as in the preceding procedure for perfusion, and all further procedures were identical.

Lung samples were taken from the paleopulmo portion of the lung from each chicken at about one-third the distance from the most caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from the entire width of each lung transverse to the cranial-caudal axis. This section was then systematically cut into 10 vertical slices from the cranial to the caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from each chicken at about one-third the distance from the most caudal aspect of the lung. The lungs were removed, sampled as in the preceding procedure for perfusion, and all further procedures were identical.

Lung samples were taken from the paleopulmo portion of the lung from each chicken at about one-third the distance from the most caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from the entire width of each lung transverse to the cranial-caudal axis. This section was then systematically cut into 10 vertical slices from the cranial to the caudal aspect of the lung. A section of tissue 0.5-cm thick was excised from each chicken at about one-third the distance from the most caudal aspect of the lung. The lungs were removed, sampled as in the preceding procedure for perfusion, and all further procedures were identical.

**Electron microscopy.** Blocks were rinsed overnight in 0.1 M phosphate buffer (350 mOsm, pH 7.4) and postfixed for 2 h in osmium tetroxide (1% osmium tetroxide in 0.125 sodium cacodylate buffer; 400 mOsm, pH 7.4). The samples were then passed through stepwise dehydration in increasing concentrations of ethanol (50–100%), rinsed with 100% propylene oxide, and embedded in Araldite.

Two blocks from each animal were randomly selected for analysis. One-micrometer-thick sections were cut from each block with an LKB Ultratome III, stained with 0.1% toluidine blue aqueous solution, and examined by light microscopy to verify fixation quality. If fixation was inadequate in a selected block, it was excluded from analysis and another block from the same individual was randomly selected. The blocks were then cut into ultrathin sections (50–70 nm) and contrast stained with saturated uranyl acetate and bismuth subnitrate. Sections were examined at an accelerating voltage of 60 kV using a Zeiss EM10C transmission electron microscope. For each block, a total of 60 micrographs were taken on film plates at a magnification of ×2,500 (30 micrographs taken by systematic random sampling from a single ultrathin section from each of 2 blocks). Micrographs of a carbon grating replica were taken with each film for calibration and to confirm that the measured magnification was within 5% of nominal magnification. Negatives were converted to high resolution (1,200 dpi) digital images with a Microtek Scannaker 4 flatbed scanner (Microtek USA, Carson, CA) and saved on compact discs.

**Analysis.** Digital images were optimized for on-screen viewing by adjusting the contrast and brightness of the image using Adobe Photoshop 7.0.1 loaded onto a PC. The sites for measuring the thickness of the layers of the BGB were determined at random by intersection of the barrier with fixed test line intersections (up to 5 intersections per micrograph). During all digital manipulations, care was taken to maintain the aspect ratio of the image to eliminate distortion. Measurements of the thicknesses of the components of the BGB were performed using MatLab 5.3. A calibration was performed with a digitized image of the carbon replica grating each time the program was launched. The thicknesses of the endothelial cell layer,

**Fig. 1.** High-magnification micrograph of the blood-gas barrier (BGB) of a chicken lung, showing the constituent layers of the BGB. Ac, air capillary; bc, lumen of blood capillary; s, surface layer or surfactant layer; epi, epithelial cell layer; int, interstitial space or extracellular matrix; end, endothelial cell layer; rbc, red blood cell; n, nucleus of red blood cell; pv, pinocytic vesicle or inclusion. Bar = 0.5 μm.
the interstitial layer, the epithelial cell layer, and the total BGB were measured orthogonal to the BGB.

Thickness data were tested for normality using a Kolmogorov-Smirnov test in SigmaStat for Windows 2.03. All sections of the barrier (endothelium, interstitium, epithelium, and total BGB) failed the normality test, so we used the nonparametric Kruskal-Wallis one-way ANOVA on Ranks test and Dunn’s method (post hoc) to identify intraspecific differences among individuals. We also used these nonparametric tests for interspecies comparisons. In all cases, \( P < 0.05 \) was required for statistical significance.

**RESULTS**

*Qualitative observations.* Light microscopy and ultrastructure of the perfusion- and tracheal instillation-fixed chicken lungs are shown in Figs. 1, 2, and 3 (2, 9, 42). Tightly-packed air capillaries were dispersed among blood capillaries, both of which were of small diameter compared with the diameter of mammalian alveoli (Fig. 2). Sections containing endothelial cell nuclei were common, while cuboidal-shaped epithelial cell nuclei were less frequent. The gas-exchanging tissue (BGB) consisted primarily of the thin cytoplasmic extensions of these cells. Fibroblasts, type I collagen fibers, and free macrophages were infrequent in the gas-exchanging region. The three layers of the BGB (endothelium, interstitium, and epithelium; Fig. 1) were usually distinct (Fig. 3). The epithelium was extremely attenuated in some areas (Fig. 1), and the transversely sectioned endothelium possessed a corrugated appearance (Figs. 1 and 3). Pinocytotic vesicles or inclusions were sometimes visible in the endothelium and epithelium. The vesicles were larger and more common in the endothelium (Fig. 1). Compared with the mammalian BGB, the bird BGB appeared to be of uniform thickness around the circumference of the capillary except near adjacent blood capillaries; in these areas, the extracellular matrix (and total BGB) was markedly thickened (Figs. 3, 4, 5). The tissue that separated air capillaries from one another was composed of abutting epithelial cell extensions (from different cells) that appeared to be separated by a thin basement membrane, although an extracellular space was not always visible (Fig. 5). The epithelium was usually thickened at the base of either side of the “epithelial bridge” where it joined the blood capillaries to form a pedicle-type structure (Figs. 3 and 5). An osmiophilic surface layer was sometimes observed on the epithelium (4, 29) (Fig. 1), but it was usually absent, most likely due to the fixation process (54).

**Fig. 3.** Electron micrograph of the chicken lung. The pulmonary capillaries (identified by the presence of red blood cells) are supported by struts of epithelial tissue (arrowheads) which allow a relatively uniform and thin BGB (inset). leu, Leukocyte; n, endothelial cell nucleus. Bar = 2 \( \mu \)m.

**Fig. 4.** Electron micrograph of a dog lung. The mammalian lung is characterized by pulmonary capillaries that appear “polarized” when orthogonally transected. The arrows point to the “thick” side of the capillary that is inefficient at gas exchange relative to the “thin” side. C, capillary; a, alveolus. Bar = 2 \( \mu \)m.

**Fig. 5.** Electron micrographs of the fine structure of the epithelial “bridge.” A: complete epithelial bridge spanning two blood capillaries. B: ultrastructure of an epithelial bridge showing the “tributary” of extracellular matrix that is continuous with the blood capillary and passes between abutting epithelial cells to an adjacent blood capillary. Bars in both images = 0.5 \( \mu \)m.

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Thickness of total BGB. We pooled all individual chicken data to make the comparisons between our bird data and the mammalian lung data previously collected in our lab. All data sets showed distribution profiles that were heavily skewed to the right (Table 1) (5, 12). However, in our chicken data, nonparametric statistical analysis showed no significant differences in thickness between individuals for total thickness or for each layer, with a few exceptions involving chicken 5 (Table 1).

The average total thickness of the BGB in the chicken lungs was 0.266 ± 0.09 μm, which was, as expected, significantly thinner than the total thickness of the rabbit, dog, and horse (0.593 ± 0.40, 0.796 ± 0.86, and 0.921 ± 1.00 μm, respectively; Tables 1 and 2 and Fig. 6). Thus the BGB in the chicken lung was 54% thinner than the BGB in the rabbit, 66% thinner than that in the dog, and 70% thinner than that in the horse. On average, the tissue thickness of the avian BGB is known to be thinner than that of mammals, and our data show that this holds true for weak flyers such as the domestic chicken (order: Galliformes). Quantitative analysis of harmonic mean thickness data appears to show a slight positive correlation with body mass in mammals and birds (26, 30, 56). To test for a similar allometric effect in our arithmetic mean thickness data, we fitted a least squares regression line to logarithmically transformed published data for the arithmetic mean thicknesses of the total BGB of a variety of mammalian and avian species ranging in body mass from 5 g to 500 kg. There was a slight correlation between body mass and BGB thickness in birds and mammals over a large range of masses (mammals, n = 40, F = 5.1, P = 0.03, r² = 0.12; birds n = 36, F = 1.9, P = 0.18, r² = 0.05); however, it appears that most of the variation is attributable to other, unknown, variables.

Thickness of components of BGB. The thicknesses of the endothelium, interstitium, and epithelium layers in the chicken lung averaged 0.135 ± 0.06, 0.045 ± 0.02, and 0.086 ± 0.05 μm, respectively (Table 1 and Fig. 6). All individual layers were significantly thinner in the chicken compared with that of the horse, dog, and rabbit, as demonstrated in the relative frequency histogram (Fig. 7). The much smaller dispersion of the data in the chicken compared with the mammals also qualitatively indicates that there was not a substantial amount of thickness variation in any of the BGB layers measured in the chicken. The cumulative frequency histogram is a different graphical view of the same data (Fig. 8). It shows the accumulated percentage of data points for each thickness category, as defined by the relative frequency histogram. In the cumulative frequency histogram, the area of the sigmoidal curve with the steepest slope indicates that the highest percentage of data points fall within those thickness categories. The cumulative frequency histograms for the different layers of the BGB of the four different species show that 1) the chicken lung has a higher proportion of thin measurements in all layers compared with that of the mammals and that 2) the interstitial layer of the chicken has a much higher percentage of very thin measurements in very few thickness categories; that is, the interstitial layer in the chicken reaches nearly 100% faster than any of the mammals (Fig. 8). Thus the interstitial layer possessed an especially high proportion (~72%) of thin measurements (<0.05 μm) in the chicken lung compared with the mammalian lungs (Figs. 6–8).

Uniformity of BGB. Images of the avian lung ultrastructure show that the BGB surrounding the blood capillaries in the chicken lung are remarkably uniform, so that the walls of transverse sections of avian blood capillaries appear nearly radially symmetric (Fig. 3, inset). This qualitative observation is in contrast to mammalian pulmonary capillaries, which, when transversely sectioned, have walls with a bilaterally symmetric appearance (Fig. 4). To quantitatively compare the dispersion of the thicknesses of the different layers in the mammals and the birds, we generated a coefficient of variation table to isolate the variation in the different measurements. The coefficients of variation showed that the variation in measurements for all layers in the chicken was substantially smaller compared with the variation found in the mammalian measurements (Fig. 9). Thus the appearance of a virtually uniform BGB is reflected in the relatively small dispersion of values in the chicken. Although there was little variation in the thicknesses of all of the layers in the chicken, the interstitium displayed a particularly low coefficient of variation, indicating

Table 1. Morphometric data of the chicken lungs

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of Measurements</th>
<th>Endothelium</th>
<th>Interstitium</th>
<th>Epithelium</th>
<th>Total BGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>295</td>
<td>0.143±0.06*</td>
<td>0.047±0.02*</td>
<td>0.086±0.05</td>
<td>0.276±0.09*</td>
</tr>
<tr>
<td>2</td>
<td>285</td>
<td>0.147±0.06*</td>
<td>0.046±0.02*</td>
<td>0.091±0.06</td>
<td>0.265±0.09*</td>
</tr>
<tr>
<td>3</td>
<td>299</td>
<td>0.140±0.07*</td>
<td>0.045±0.02*</td>
<td>0.084±0.05</td>
<td>0.269±0.10*</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>0.133±0.05*</td>
<td>0.044±0.01</td>
<td>0.087±0.04</td>
<td>0.264±0.07*</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>0.114±0.05</td>
<td>0.041±0.01</td>
<td>0.084±0.04</td>
<td>0.239±0.07</td>
</tr>
<tr>
<td>Mean (5)</td>
<td>1,479</td>
<td>0.135±0.06</td>
<td>0.045±0.02</td>
<td>0.086±0.05</td>
<td>0.266±0.09</td>
</tr>
</tbody>
</table>

Values are averages ± SD in μm. *Significantly greater than animal 5 (P < 0.05). BGB, blood-gas barrier.

Table 2. Morphometric data of the chicken, horse, dog, and rabbit lungs

<table>
<thead>
<tr>
<th>Animal (n)</th>
<th>No. of Measurements</th>
<th>Endothelium</th>
<th>Interstitium</th>
<th>Epithelium</th>
<th>Total BGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken (5)</td>
<td>1,479</td>
<td>0.135±0.06</td>
<td>0.045±0.02</td>
<td>0.086±0.05</td>
<td>0.266±0.09</td>
</tr>
<tr>
<td>Horse (2)</td>
<td>662</td>
<td>0.248±0.13</td>
<td>0.386±0.64</td>
<td>0.287±0.23</td>
<td>0.921±1.00</td>
</tr>
<tr>
<td>Dog (3)</td>
<td>651</td>
<td>0.248±0.25</td>
<td>0.319±0.51</td>
<td>0.230±0.12</td>
<td>0.796±0.86</td>
</tr>
<tr>
<td>Rabbit (3)</td>
<td>714</td>
<td>0.216±0.14</td>
<td>0.174±0.23</td>
<td>0.202±0.17</td>
<td>0.593±0.40</td>
</tr>
</tbody>
</table>

Values are averages ± SD in μm and are averaged for all individuals of the same species.
a remarkably uniform thin interstitial layer in the blood capillaries of the bird.

As stated above, a transverse section of the pulmonary capillaries of the mammalian lung is characteristically polarized in appearance. One side of the capillary wall is attenuated to minimize resistance to diffusion during gas exchange, while the other side is thickened with connective tissue (Fig. 4). For example, in the human lung ~50% of the BGB is thin and 50% thick (13). The thick portion of the capillary wall tends to have a larger extracellular space due, in part, to the prevalence of type I collagen fibrils; there may also be intervening fibroblasts. This collagen cable is thought to maintain the integrity of the large alveolar walls, which are suspended in the air spaces of the lung (54). The thickened section of the pulmonary capillary of the mammalian lung appears to play a necessary “supportive” role and is therefore relatively resistant to diffusion, thus effectively reducing the area of the capillary that is available for gas exchange. The occurrence of thick and thin sections of the interstitium increases the variation of thickness measurements for both the interstitium and the total BGB, causing the high coefficient of variation in the mammals (Fig. 9). In contrast, the chickens’ blood capillaries do not possess a thin and thick side. Their capillaries have uniformly thin walls with subsequent low

![Fig. 6. Bar graphs of average thicknesses ± SD of all layers of the BGB measured in the lungs of the chicken, horse, dog, and rabbit. Statistical symbols: a, significantly different from chicken; b, significantly different from horse; c, significantly different from dog; d, significantly different from rabbit.](image)

![Fig. 7. Relative frequency histograms of the various layers of the BGB in the chicken, horse, dog, and rabbit. Chicken curves are shifted to the left indicating a higher proportion of thin measurements.](image)
variability and low coefficients of variation in the thickness measurements of both the interstitium and total BGB. Functionally, the uniformly thin barrier translates into a higher proportion of surface area that is maximized for gas exchange compared with the mammal. Images of the mammalian pulmonary compared with the blood capillary of the bird illustrate the differences (Figs. 3, inset, and 4).

**Epithelial struts.** Although birds have a semirigid lung (8, 9), in the absence of the structural support of a thick interstitium, the tissue in the avian lung is potentially susceptible to mechanical damages sustained from elevated transmural pressures that occur with exercise and subsequent increased oxygen consumption and elevated cardiac output. Support to the small blood capillaries in the avian lung may be afforded by the aforementioned epithelial “crossbraces” that are an anatomical feature unique to the bird lung (Figs. 2 and 3) (20, 29, 43). These epithelial struts are composed of abutting cytoplasmic appendages from adjacent epithelial cells. They are extremely attenuated at the midsection and then thicken at either end where the epithelial pedicle joins to the blood capillary (Figs. 3 and 5) (20). While Maina (28) asserts that the air capillaries are not themselves “rigid” and proposes that they are therefore of little structural significance for support to the parabronchial unit, we maintain that they serve a supportive roll in the tightly packed air and blood capillary network within the fine structure of the parabronchii (20, 43).

The ultrastructure of the epithelial crossbrace has not been previously described in any great detail. Maina and King (29) and others (20, 43) recognized the presence of this unique structure in all bird species studied to date and observed that the “rather uncommon sites” where two air capillaries share a common wall are made of adjoining epithelial cell extensions. Klika et al. (20) describes the crossbrace as being composed of “paired retinacula”; however, the provided images display evidence of edema and artifactual thickening. Our images of the epithelial bridges from tracheal-fixed chickens show very closely abutting epithelial cell extensions that are connected via an interstitial space, but it is not always visible (Fig. 5). For this reason, there has been some conjecture regarding the presence or absence of an interstitial space between the two extensions and how the cells would otherwise be joined if an interstitium were lacking (20, 29). The epithelial extensions are thickened where they attach at the blood capillary, and in the area of thickening, it appears as if a portion of the blood capillary’s interstitium diverges to form a smaller “tributary” that runs the length of the epithelial bridge to join the interstitium of another blood capillary (Fig. 5). Although several authors have stated that a basal lamina appears to be lacking in

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Fig. 8. Cumulative frequency plots constructed from relative frequency histograms for all layers of the BGB measured in the chicken, horse, dog, and rabbit. A steeper curve indicates less variability in thickness measurements.
energetic hummingbird (*Colibri coruscans*), allometrically optimized (33). Instead, variations in BGB arithmetic mean thickness among avian species have been attributed to variations in energetic demand. For example, a highly energetic hummingbird (*Colibri coruscans*) has an average arithmetic mean thickness of 0.183 μm, compared with the relatively inactive guinea fowl (*Numida meleagris*), whose average thickness is 1.12 μm (1, 7). The parallel metabolic correlation among mammalian species appears not to exist; for example, the highly aerobic dog and bat have arithmetic mean thickness values of 1.78 and 1.39 μm, respectively, compared with the naked mole rat and baboon (1.09 and 1.12 μm, respectively) (24, 30, 32, 36). Most avian species have a thinner BGB compared with all but the smallest mammals (14, 33). A notable exception exists in the common kestrel (*Falco tinnunculus*), whose BGB arithmetic mean thickness averaged 1.66 μm (30).

Although the thickness of the total BGB from ~30 species of birds has been reported, to our knowledge there has been only one previous study that has measured the thicknesses of the different components of the BGB in an avian lung. Weidner et al. (59) used a tracheal instillation fixation method and a line-intercept counting technique to estimate endothelial cell and septal interstitial thickness in volume-loaded chickens. Their control (nonloaded) chickens measured endothelium and interstitium values of 0.16 ± 0.04 and 1.70 ± 0.49 μm, respectively (59). The much higher value for interstitial thickness is possibly explained by some edema formation during lung fixation. Interestingly, although unquantified, the basal lamina of the emperor penguin (*Aptenodytes forsteri*) was observed to be unusually thick and possessed numerous collagen fibrils. The added support to the interstitium was thought to be an adaptation to protect the BGB from compression stress during the high hydrostatic pressures incurred during deep diving in these marine birds (63).

Flapping flight is an energetic form of locomotion that has an intermediate cost-of-transport between vertebrate animals that swim (most efficient) and those that run (least efficient) (44, 51, 52). However, flying does require a high rate of energy expenditure per unit time despite the behavioral adaptations that make volant locomotion extremely efficient in many birds (38). Exercising birds likely have elevated pulmonary capillary pressures in a manner similar to exercising mammals, although pulmonary vascular pressures have not been measured in flying birds. Although the domestic chicken is a weak flyer, we found that it has a total BGB thickness that is comparable to (or thinner than) that of other bird species. If pulmonary capillary pressures in exercising birds are similar or higher than that found in exercising mammals of similar mass and the total BGB thickness in birds is similar to or thinner than that found in mammals, there is the potential for birds to suffer from pulmonary stress failure as mammals do. Moreover, in mammals there appears to be a trend where the thinner the interstitium layer of the BGB, the lower the pressure required to induce pulmonary stress failure (5). However, to the best of our knowledge, stress failure has not been reported in birds (although there has been a report of the presence of erythrocytes in the air spaces of bird lungs), and the evolutionary success of this diverse taxon indicates that it is unlikely a normal occurrence. We therefore suspect that the avian BGB possesses additional structural support in the microanatomy of the lung parenchyma that may not have a mammalian analog.

The avian respiratory system is structurally and functionally different from the mammalian respiratory system and arguably more efficient. The small, flow-through lungs experience unidirectional air flow during inspiration and expiration. Ventilation is separated from gas exchange and is performed by paired air sacs located throughout the thoracic and abdominal cavities. The blood capillaries and air capillaries are intricately intertwined and form a meshwork of parenchyma tissue separated by numerous atria in the parabronchi (8, 9, 23). The diameter of the air capillaries in an avian lung is a fraction of the size of the mammalian alveoli; the resultant surface tension forces in

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**Fig. 9.** Coefficient of variation bar graph calculated from thickness measurements of the various layers of the BGB in the chicken, dog, rabbit, and horse. The coefficient of variation of the interstitial layer in the chicken is considerably lower than that of the mammals.

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**DISCUSSION**

We found that all of the layers of the BGB were thinner in the chicken compared with that in the lungs of the horse, dog, and rabbit, including the interstitial layer. In addition, our results show that the circumferential thickness of the interstitial layer is uniform in the chicken compared with that of the mammal. Finally, the allometric scaling effect on the BGB appears to be negligible in both mammals and birds. We are able to make these comparisons because despite the considerable anatomical differences between mammalian and avian lungs, the structural design of the BGB is essentially the same.

In the avian lung, there has been a considerable amount of harmonic mean thickness data published. However, while this measurement is appropriate for estimating the diffusing capacity of the lung, in the context of tissue strength, the arithmetic mean thickness is a better measurement (33, 55, 57). In measurements of arithmetic and harmonic mean thickness over a range of body masses, there is little or no correlation of body mass to barrier thickness in either birds or mammals. This lack of correlation suggests that the thickness of the BGB has been allometrically optimized (33). Instead, variations in BGB arithmetic mean thickness among avian species have been attributed to variations in energetic demand. For example, a highly energetic hummingbird (*Colibri coruscans*) has an average arithmetic mean thickness of 0.183 μm, compared with the relatively inactive guinea fowl (*Numida meleagris*), whose average thickness is 1.12 μm (1, 7). The parallel metabolic correlation among mammalian species appears not to exist; for example, the highly aerobic dog and bat have arithmetic mean thickness values of 1.78 and 1.39 μm, respectively, compared with the naked mole rat and baboon (1.09 and 1.12 μm, respectively) (24, 30, 32, 36). Most avian species have a thinner BGB compared with all but the smallest mammals (14, 33). A notable exception exists in the common kestrel (*Falco tinnunculus*), whose BGB arithmetic mean thickness averaged 1.66 μm (30).

Although the thickness of the total BGB from ~30 species of birds has been reported, to our knowledge there has been only one previous study that has measured the thicknesses of the different components of the BGB in an avian lung. Weidner et al. (59) used a tracheal instillation fixation method and a line-intercept counting technique to estimate endothelial cell and septal interstitial thickness in volume-loaded chickens. Their control (nonloaded) chickens measured endothelium and interstitium values of 0.16 ± 0.04 and 1.70 ± 0.49 μm, respectively (59). The much higher value for interstitial thickness is possibly explained by some edema formation during lung fixation. Interestingly, although unquantified, the basal lamina of the emperor penguin (*Aptenodytes forsteri*) was observed to be unusually thick and possessed numerous collagen fibrils. The added support to the interstitium was thought to be an adaptation to protect the BGB from compression stress during the high hydrostatic pressures incurred during deep diving in these marine birds (63).

Flapping flight is an energetic form of locomotion that has an intermediate cost-of-transport between vertebrate animals that swim (most efficient) and those that run (least efficient) (44, 51, 52). However, flying does require a high rate of energy expenditure per unit time despite the behavioral adaptations that make volant locomotion extremely efficient in many birds (38). Exercising birds likely have elevated pulmonary capillary pressures in a manner similar to exercising mammals, although pulmonary vascular pressures have not been measured in flying birds. Although the domestic chicken is a weak flyer, we found that it has a total BGB thickness that is comparable to (or thinner than) that of other bird species. If pulmonary capillary pressures in exercising birds are similar or higher than that found in exercising mammals of similar mass and the total BGB thickness in birds is similar to or thinner than that found in mammals, there is the potential for birds to suffer from pulmonary stress failure as mammals do. Moreover, in mammals there appears to be a trend where the thinner the interstitium layer of the BGB, the lower the pressure required to induce pulmonary stress failure (5). However, to the best of our knowledge, stress failure has not been reported in birds (although there has been a report of the presence of erythrocytes in the air spaces of bird lungs), and the evolutionary success of this diverse taxon indicates that it is unlikely a normal occurrence. We therefore suspect that the avian BGB possesses additional structural support in the microanatomy of the lung parenchyma that may not have a mammalian analog.

The avian respiratory system is structurally and functionally different from the mammalian respiratory system and arguably more efficient. The small, flow-through lungs experience unidirectional air flow during inspiration and expiration. Ventilation is separated from gas exchange and is performed by paired air sacs located throughout the thoracic and abdominal cavities. The blood capillaries and air capillaries are intricately intertwined and form a meshwork of parenchyma tissue separated by numerous atria in the parabronchi (8, 9, 23). The diameter of the air capillaries in an avian lung is a fraction of the size of the mammalian alveoli; the resultant surface tension forces in
the air capillaries may be very strong and patency is ensured by the presence of a unique surfactant-like material (4, 8, 9). Because of the physiologically impossible forces required to reinflate the air capillaries upon collapse, it was long held that the avian lung was completely rigid and noncompliant (40). We now know that this is not entirely true; during one respiration cycle in a resting bird, lung volume changes 1.4% (19) and the parabronchi themselves show limited compliance (22). Of interest in the latter study was the observation that the fragile air capillaries did not show evidence of collapse upon compression of the respiratory system; the authors remarked that possibly “unknown factors serve to confer a remarkable stability on these fine structures” (22). Coupled with empirical data by Powell et al. (41) demonstrating a lack of recruitment and distention of blood capillaries in the avian lung during temporary unilateral pulmonary arterial occlusion, these studies provide further evidence that additional structural support in the avian lung affords it remarkable integrity despite the thin interstitial layer.

It has been proposed that the epithelial crossbraces of the avian lung formed a spiderweb-like system of air capillaries that functioned to anchor and support the air capillaries to the lung parenchyma (20, 43). This theory was suggested because of the noticeable lack of significant extracellular space and supportive connective tissue surrounding the blood capillaries (20, 29). However, the provided transmission electron microscope images of the crossbraces show considerable edema. We agree that the epithelial crossbraces provide support to the gas exchange tissue, and we further propose that the epithelial extensions serve to reduce or absorb the transmural stresses that occur within the blood capillaries upon exercise. The presence of these epithelial bridges creates a honeycomb-like structure in the bird lung upon cross-section of the parenchyma, and the honeycomb shape, much like a geodesic dome, efficiently transmits stress throughout the entire structure and has a high ratio of volume to weight, therefore requiring a minimum amount of tissue for metabolic maintenance. Additionally, our images show a continuous extracellular matrix from blood capillary to air capillary, which we believe provides evidence that the epithelial struts may play an important role in lung fluid balance. Finally, the epithelial bridges may themselves be afforded support by the anchoring system to the thicker blood capillaries. The anchoring of the air capillaries to the blood capillaries [as proposed by Klika et al. (20)] may stabilize the very thin epithelial extensions during the small in volume changes of the avian lung during respiration and aid in the maintenance of small airway patency.

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REFERENCES


46. Sweeney CR, Soma LR. Exercise-induced pulmonary haemorrhage in horses after different competitive exercises. In: *Equine Exercise Physiolo-