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# Thickness of the air–blood barriers in vertebrate lungs

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## INTRODUCTION

In the respiratory portion of the vertebrate lung, air and blood are separated from each other by a thin wall of tissue which is commonly referred to as the 'air–blood barrier'. Studies using the electron microscope have contributed much information on the structure of the barriers in many species of mammals (for review, see Kilburn, 1974); in contrast, much less is known about the microstructure of the barriers in birds, reptiles and amphibians.

The thickness of the air–blood barrier is of particular interest as it is one of the factors determining the rate of gas exchange in the organism (Hills, 1974). This paper presents the results of a morphometric study of the air–blood barriers in the lungs of several different species of mammals, reptiles and amphibians.

## MATERIALS AND METHODS

Mature animals were obtained from commercial suppliers and other sources. The species used are listed in Tables 2–4.

The larger mammals were killed by stunning and bleeding from the abdominal aorta while the smaller mammals and reptiles were given an overdose of sodium thiopentone before being bled. The amphibians were pithed and then bled in a similar manner.

In each of the larger animals, a self-retaining catheter was placed in the trachea and a solution of 5 % glutaraldehyde in 0·1 M phosphate buffer (pH 7·3; 4 °C) was introduced to expand the lungs to a volume equal to that obtained at full inspiration. After 10 minutes, the right lung of each animal was cut into slices (approximately 50) and these were fixed for a further 110 minutes in the same solution. The slices were then placed on a numbered grid and four were selected from each lung (using numbers obtained from random sample tables) for further processing. Small blocks of tissue (1–2 $mm^3$) were cut from the slices, washed for 18 hours in 0·17 M sucrose in phosphate buffer, and then post-fixed in buffered 1 % osmium tetroxide solution for 1 hour. The blocks were washed in buffered sucrose solution, dehydrated in ethanol (60, 80, 100 % vol/vol; 15 minutes, 15 minutes and 120 minutes, respectively) and embedded in Durcupan. In the smaller animals with saccular lungs, the fixative was introduced through a 21 gauge needle. The lung of each animal was fixed for 2 hours and then cut into small blocks. Four blocks were selected at random and processed by the method outlined above. In the case of the alligator, blocks of tissue were removed from the lung and immersed directly in fixative. After prolonged fixation (9 weeks), these blocks were processed in the standard way.

Thin sections (50–70 nm) were cut from each tissue block and stained with uranyl

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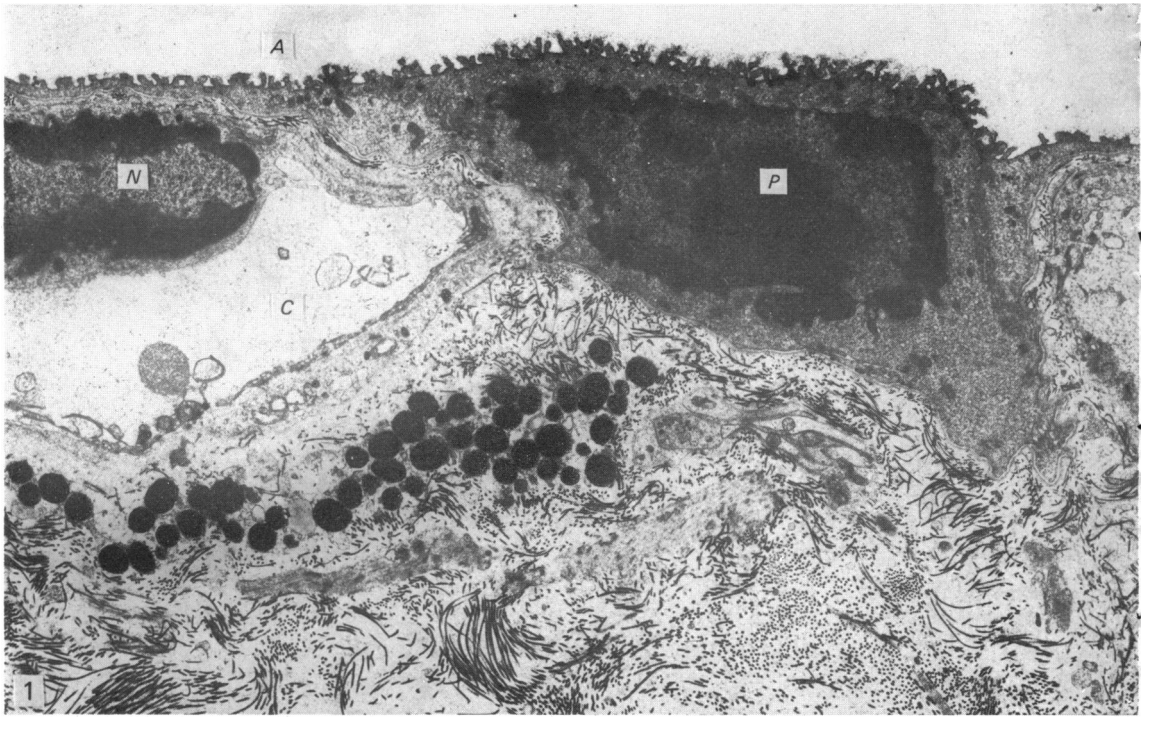
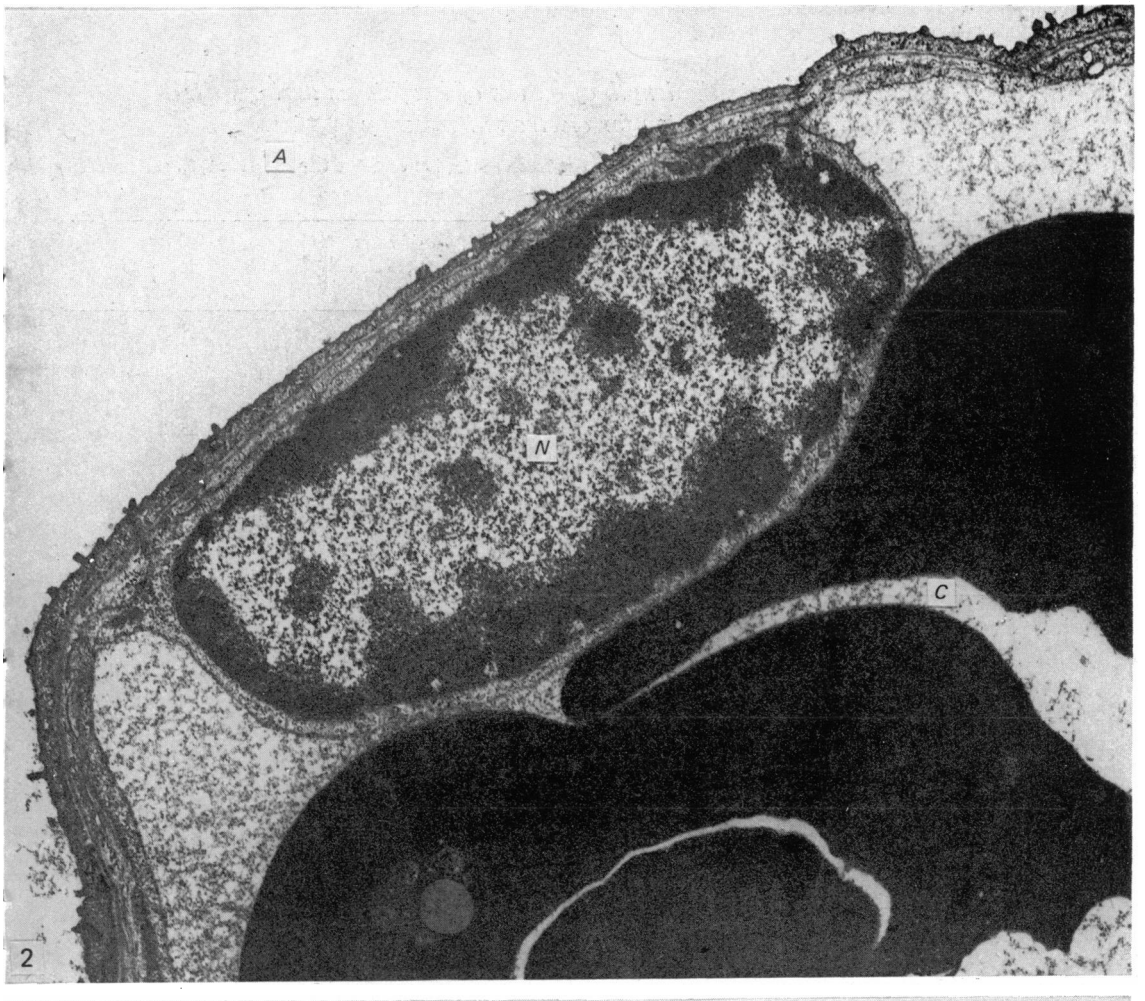



Fig. 1. Section through the edge of a septum in the lung of a salamander. *A*, air space; *C*, lumen of pulmonary capillary; *N*. nucleus of an endothelial cell; *P*, nucleus of a pneumonocyte. × 5000.

acetate and lead citrate. The sections were examined in an AEI 801 electron microscope and photographed at a magnification of × 12800. A replica of a diffraction grating with 2160 lines/mm was used to verify the magnification of the micrographs.

The thickness of the air–blood barriers was estimated using the stereological method developed by Weibel & Knight (1964). A transparent sheet marked with a system of test lines was placed over each micrograph and the following measurements were made: (1) a count of the number of intersections of the test lines with the internal and external surfaces of the barrier; (2) a count of the number of end-points of the test lines lying over the barrier; and (3) the intercept length of the test lines crossing the barrier. These data were used to compute the arithmetic mean thickness and the harmonic mean thickness of the barriers. In addition, the thickness of each barrier was measured at its most attenuated point. The relative volumes of the major components of the barriers were estimated in some species by differential point counting using a transparent sheet with a point pattern of the type described by Dunnill (1962).

The effect of the fixatives, dehydrating agents and embedding medium on the dimensions of the tissues was assessed in the following way: ten rectangular blocks of mouse lung were prepared for electron microscopy by the standard methods; the length of each block was measured at the times indicated in Table 1 and the ratio of mean block length after processing (Lp) to the mean block length in the fresh state (Lo) was computed.



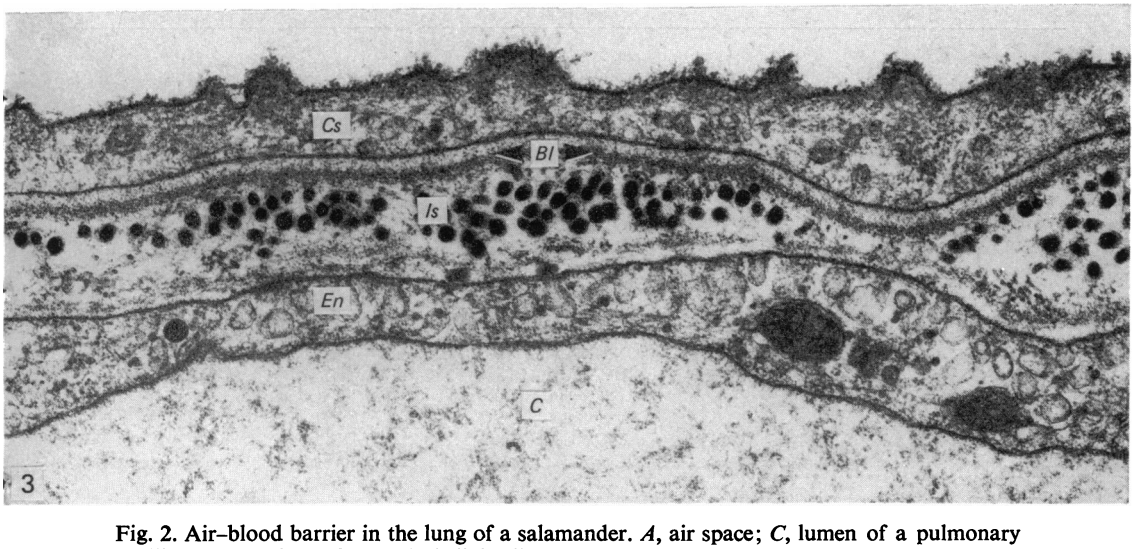




Fig. 2. Air–blood barrier in the lung of a salamander. *A*, air space; *C*, lumen of a pulmonary capillary; *N*, nucleus of an endothelial cell. × 10500.

Fig. 3. Detail of the air–blood barrier in the lung of a salamander. *Bl*, basal lamina of pneumonocyte; *C*, lumen of capillary; *Cs*, cytoplasmic sheet of a pneumonocyte; *En*, cytoplasm of an endothelial cell; *Is*, interstitial space containing collagen fibres. × 39500.

Table 1. *Change in the length of blocks of lung tissue during their preparation for electron microscopy*

(Lo denotes the mean length of the blocks in the fresh state; Lp denotes the mean length of the blocks at a particular stage of preparation.)

| Stage of preparation | Length of lung blocks (mean in mm ± S.E.) | Ratio Lp/Lo |
|---|---|---|
| Fresh | 0·41 ± 0·12 | 1·00 |
| After fixation in glutaraldehyde solution | 0·38 ± 0·11 | 0·93 |
| After washing in sucrose-buffer solution | 0·41 ± 0·09 | 1·00 |
| After post-fixation in osmium tetroxide solution | 0·40 ± 0·12 | 0·98 |
| After dehydration in ethanol | 0·40 ± 0·10 | 0·98 |
| After embedding in Durcupan | 0·39 ± 0·09 | 0·95 |

Table 2. *Thickness of the air–blood barriers in the lungs of mammals*

| Species | Number of animals | Arithmetic mean thickness of barrier ± S.E. (μm) | Harmonic mean thickness of barrier ± S.E. (μm) | Minimum thickness of barrier (μm) |
|---|---|---|---|---|
| Pig | 4 | 1·90 ± 0·21 | 0·72 ± 0·04 | 0·19 |
| Sheep | 5 | 1·87 ± 0·18 | 0·68 ± 0·03 | 0·20 |
| Dog | 4 | 1·78 ± 0·19 | 0·67 ± 0·03 | 0·22 |
| Rhesus monkey | 2 | 1·80 ± 0·31 | 0·65 ± 0·07 | 0·19 |
| Rabbit | 5 | 1·40 ± 0·15 | 0·65 ± 0·03 | 0·24 |
| Hamster | 7 | 1·49 ± 0·12 | 0·56 ± 0·03 | 0·19 |
| Gerbil | 4 | 1·34 ± 0·14 | 0·51 ± 0·04 | 0·20 |
| Mouse | 5 | 1·27 ± 0·13 | 0·44 ± 0·02 | 0·19 |

## RESULTS

In each of the animals studied the pulmonary air–blood barrier was composed of an epithelial layer, an interstitial layer and a layer of capillary endothelium. The characteristic features of the barrier in a salamander (*Salamandra salamandra*) are illustrated in Figures 1–3.

The changes in the dimensions of blocks of lung tissue at different stages of the processing schedule are shown in Table 1. Overall, the various procedures caused a slight reduction in the mean length of the tissue blocks (Lp/Lo = 0·95). The measurements obtained from the micrographs were therefore adjusted to compensate for this shrinkage.

Table 2 presents the data on the lungs of eight different species of mammals. The arithmetic mean thickness of the air–blood barriers ranged from 1·90 μm in the pig to only 1·27 μm in the mouse. The harmonic mean thickness ($t_H$; in micrometres) was related to the body weight (W; in grams) in the following way:

$$t_H = 4{\cdot}1OW^{0{\cdot}05}.$$

The relationship is represented in graphic form in Figure 4. The minimum thickness of the barriers (i.e. the lowest value obtained from any animal of the species concerned) ranged from 0·19 $\mu$m to 0·24 $\mu$m. This parameter bore no obvious relationship to either the arithmetic mean thickness or the harmonic mean thickness of the barrier.

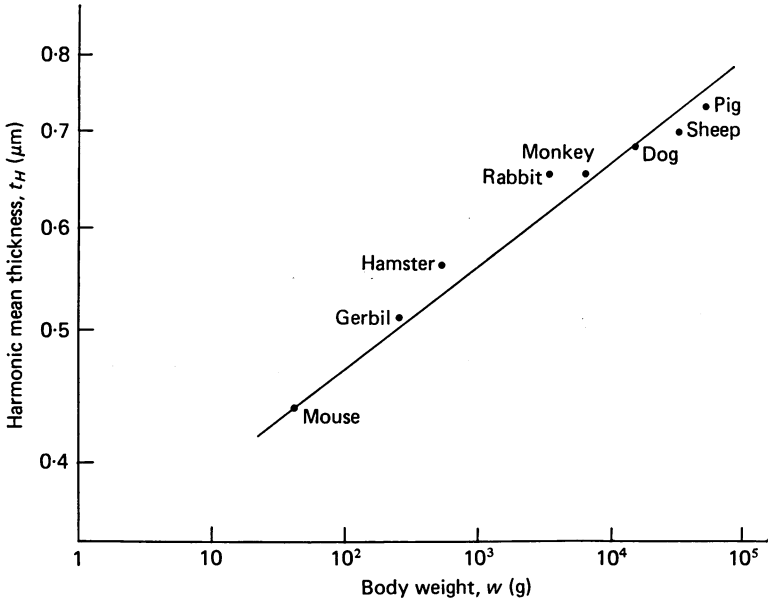



Fig. 4. Logarithmic plot of the harmonic mean thickness of the air–blood barrier against body weight in mammals. The regression equation was computed using the individual estimates in each species. Only the mean value for each species is plotted. The equation was $t_H = 4.1OW^{0.05}$ and the correlation coefficient was 0·74.

Table 3. *Thickness of the air–blood barriers in the lungs of reptiles*

| Species | Number of animals | Arithmetic mean thickness of barrier ± S.E. ($\mu$m) | Harmonic mean thickness of barrier ± S.E. ($\mu$m) | Minimum thickness of barrier ($\mu$m) |
|---|---|---|---|---|
| Green lizard (*Lacerta viridis*) | 6 | 1·96 ± 0·17 | 0·90 ± 0·03 | 0·24 |
| Wall lizard (*Lacerta muralis*) | 4 | 1·69 ± 0·17 | 0·84 ± 0·03 | 0·21 |
| Garden lizard (*Calotes nemoricola*) | 4 | 1·99 ± 0·18 | 1·03 ± 0·04 | 0·25 |
| Blind slow-worm (*Anguis fragilis*) | 6 | 2·06 ± 0·18 | 0·90 ± 0·03 | 0·20 |
| European chameleon (*Chamaeleo chamaeleon*) | 3 | 1·68 ± 0·22 | 0·73 ± 0·04 | 0·18 |
| Grass snake (*Natrix natrix*) | 4 | 2·42 ± 0·24 | 1·34 ± 0·07 | 0·20 |
| Smooth snake (*Coronella austriaca*) | 4 | 2·31 ± 0·23 | 1·02 ± 0·04 | 0·25 |
| American alligator (*Alligator mississippiensis*) | 1 | 1·65 | 0·73 | 0·20 |
| European tortoise (*Testudo graeca*) | 5 | 2·49 ± 0·24 | 1·38 ± 0·06 | 0·23 |
| Red-eared turtle (*Pseudemys scripta*) | 5 | 2·10 ± 0·21 | 1·19 ± 0·05 | 0·23 |

Table 4. *Thickness of the air–blood barriers in the lungs of amphibians*

| Species | Number of animals | Arithmetic mean thickness of barrier ± S.E. (μm) | Harmonic mean thickness of barrier ± S.E. (μm) | Minimum thickness of barrier (μm) |
|---|---|---|---|---|
| South African clawed toad (*Xenopus laevis*) | 5 | 1·71 ± 0·18 | 1·21 ± 0·05 | 0·24 |
| Nigerian clawed toad (*Xenopus tropicalis*) | 4 | 1·75 ± 0·20 | 1·23 ± 0·05 | 0·19 |
| Common frog (*Rana temporaria*) | 4 | 2·06 ± 0·21 | 1·50 ± 0·07 | 0·21 |
| Bullfrog (*Rana catesbeiana*) | 3 | 1·95 ± 0·25 | 1·72 ± 0·10 | 0·20 |
| Northern leopard frog (*Rana pipiens*) | 5 | 2·04 ± 0·19 | 1·53 ± 0·04 | 0·20 |
| Common toad (*Bufo bufo*) | 5 | 1·85 ± 0·17 | 1·67 ± 0·04 | 0·19 |
| European spotted salamander (*Salamandra salamandra*) | 3 | 2·30 ± 0·28 | 1·78 ± 0·10 | 0·25 |
| Fire-bellied newt (*Paramesotriton hongkongensis*) | 5 | 2·60 ± 0·22 | 1·81 ± 0·09 | 0·19 |
| Common newt (*Triturus vulgaris*) | 5 | 2·63 ± 0·21 | 2·34 ± 0·10 | 0·25 |
| Italian crested newt (*Triturus cristatus*) | 4 | 2·81 ± 0·24 | 2·20 ± 0·10 | 0·20 |

Table 5. *Relative volumes of the components of the air–blood barriers in the lungs of mammals, reptiles and amphibians*

| Species | Relative volumes of components (%) | | |
|---|---|---|---|
| | Epithelium | Interstitium | Endothelium |
| Mammals | | | |
| Pig | 33·3 | 41·7 | 25·0 |
| Dog | 31·9 | 36·2 | 31·9 |
| Mouse | 27·3 | 45·4 | 27·3 |
| Reptiles | | | |
| European chameleon | 29·4 | 44·1 | 26·5 |
| Green lizard | 31·0 | 43·0 | 26·0 |
| Blind slow-worm | 30·4 | 45·3 | 24·3 |
| European tortoise | 32·0 | 43·7 | 24·3 |
| Amphibians | | | |
| South African clawed toad | 31·3 | 39·6 | 29·1 |
| Common frog | 32·8 | 44·6 | 22·6 |
| European salamander | 30·5 | 41·3 | 28·2 |
| Crested newt | 30·9 | 43·9 | 25·2 |

In some reptiles (e.g. *Lacerta muralis*), the barrier thickness lay within the range found in mammals; in others (e.g. *Testudo graeca*), the barrier was much thicker (Table 3). The range of minimum barrier thicknesses in reptiles was similar to that in mammals. Neither the arithmetic mean thickness nor the harmonic mean thickness of the barrier was related to body weight.

The data relating to the amphibians are given in Table 4. The air–blood barrier was found to vary considerably in thickness, having an arithmetic mean thickness of 1·71 μm in *Xenopus laevis* and 2·81 μm in *Triturus cristatus*. The range of minimum

barrier thicknesses was similar to that in reptiles and mammals. Barrier thickness was not related to total body weight in the amphibians studied.

The relative volumes of the major components of the air–blood barriers of several species are given in Table 5. No significant differences were detected in the composition of the barriers in the amphibians, reptiles and mammals.

## DISCUSSION

The results of this investigation show that the air–blood barriers in the lungs of vertebrates are very thin. For example, the arithmetic mean thickness of the barriers ranged from 2·81 $\mu$m in the crested newt to only 1·27 $\mu$m in the mouse. Blood capillaries are not located so close to a free surface of any other organ in these animals. The attenuation of the pulmonary barrier is all the more remarkable when one remembers that the media in contact with it (air and blood) undergo continuous changes in pressure.

In all the animals studied, the air–blood barrier has a tripartite structure consisting of an outer epithelial layer (the cytoplasmic flanges of pneumonocytes), an intermediate layer of collagen fibres and basal laminae, and an inner layer of capillary endothelium. It is interesting that the volume proportions of the three layers are approximately equal in the animals investigated.

The harmonic mean thickness is a measure of the resistance that the air–blood barrier offers to gaseous diffusion (Weibel & Knight, 1964). In the mammals this parameter was found to be related to total body weight, the thickness increasing with weight to a power of about 0·05. A similar relationship was detected in data obtained from a different series of mammals by Weibel (1972). In contrast, no evidence was found in the present study of a correlation of barrier thickness and body weight in reptiles or amphibians. This is not surprising in view of the fact that these animals often exhibit nanoid growth patterns (Porter, 1972; Parker, 1977).

The minimum barrier thickness is of considerable interest as it indicates how far an animal can go in attenuating its respiratory tissues without jeopardizing their mechanical stability. In the different animals studied the minimum barrier thickness ranged from 0·18 $\mu$m to 0·25 $\mu$m. It is notable that there are no significant differences between the values from the amphibians, reptiles and mammals. Weibel, Burri & Claassen (1971) have observed a much thinner barrier in the lungs of the Etruscan shrew, the smallest extant mammal.

Amphibians have more sites of gas exchange than any other type of vertebrate. At different times in their development they use the skin, oral mucosa, lungs and gills for breathing (Steen, 1971). There is considerable interspecies variation in the relative effectiveness of these different exchange sites. In general, however, both physiological measurements (Krogh, 1904; Foxon, 1964; Whitford & Hutchison, 1963) and morphometric data (Czopek, 1965) suggest that the lungs of urodeles are less important for gas exchange than those of anurans. The results of the present study support this conclusion in that the urodeles (species of *Triturus*, *Paramesotriton* and *Salamandra*) had thicker – and presumably less efficient – pulmonary barriers than those of the anurans (species of *Xenopus*, *Rana* and *Bufo*).

The harmonic mean thickness of the air–blood barrier is less than the arithmetic mean thickness in all the animals studied. This discrepancy is due to the fact that the barriers are not of constant thickness; rather, they consist of alternating thick

and thin regions. This non-uniformity is a highly desirable feature because, assuming a given mass of tissue is necessary for structural stability, a corrugated barrier will have a lower overall resistance to gaseous diffusion than a barrier of even thickness. In the mammals studied, the ratio of arithmetic mean thickness to harmonic mean thickness ranged from 2·6 to 3·7; the corresponding ranges in the reptiles and amphibians were 1·8–2·3 and 1·1–1·4, respectively. These data suggest that the process of optimization of lung architecture for gas exchange is most active in mammals.

It must be pointed out that the values estimated in this study do not represent the true distances that gas molecules travel in their passage from the air spaces to the blood. Apart from the tissue barrier, one must also take into account the fluid film (the pulmonary surfactant complex) that lines the alveolar surface during life, and the layer of plasma separating the erythrocytes from the inner border of the capillary endothelium. If these and several other parameters are estimated, it is possible to compute a value for lung diffusion capacity which is in fair agreement with the value obtained by physiological methods (Siegwart, Gehr, Gil & Weibel, 1971).

It is interesting to compare the dimensions of the pulmonary barriers in mammals, reptiles and amphibians with those in the gas-exchange sites of other vertebrates. Many investigators have remarked on the thinness of the air–blood barriers in the lungs of birds, but few have given measurements. However, Schulz (1962) has reported that the barrier thickness is only 0·10–0·14 $\mu$m in the pigeon. The gill barriers of fish are of variable thickness, ranging from 0·31 $\mu$m in the cichlid fish, *Haplochromis multicolor* (Schulz, 1962) to 20 $\mu$m in the climbing perch, *Anabas testudineus* (Hughes & Datta Munshi, 1973). Some species of fish use transcutaneous respiration as well as gill breathing; the length of the diffusion path across the skin may vary from 31–38 $\mu$m in the flounder to 263 $\mu$m in the eel (Jakubowski, 1963). Klika & Lelek (1967) have reported that the pulmonary barrier in the lungfish *Protopterus annectens* is about 0·5 $\mu$m, while Hughes & Datta Munshi (1973) have estimated that the diffusion distance in the accessory respiratory organs (suprabranchial chamber and labyrinthine plates) of the climbing perch is 0·12–0·30 $\mu$m. Jaskinski (1973) has also observed a very thin barrier ($\geqslant$ 0·188 $\mu$m) in the respiratory intestine of the pond loach, *Misgurnus fossalis*. Clearly, a short diffusion path is a feature common to the respiratory organs of a wide variety of vertebrates.

## SUMMARY

The thickness of the air–blood barriers in the lungs of a series of mammals, reptiles and amphibians was estimated using stereological methods. There was considerable variation in the measurements, the thickest barrier being that of the crested newt (2·81 $\mu$m) and the thinnest that of the mouse (1·27 $\mu$m). The arithmetic mean thickness of the barriers was related to body weight in mammals but not in reptiles or amphibians. There were no significant differences between the minimum thickness of the barriers nor in the volume proportions of their constituent layers in the three orders of animals. The ratio of the arithmetic mean thickness to the harmonic mean thickness of the barriers was highest in mammals. These data indicate that the process of optimization of lung architecture for gas exchange is most marked in mammals.

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