The thickness of the avian blood–gas barrier: qualitative and quantitative observations

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INTRODUCTION

Gas exchange in the avian lung takes place across a blood–gas barrier formed, as in other air-breathing vertebrates, by three essential components, an epithelial cell, a basal lamina, and an endothelial cell. The thickness of the barrier is of functional importance because it constitutes an integral determinant of both the pulmonary diffusing capacity and the tissue metabolism of the lung (Weibel & Knight, 1964; Weibel, 1970/71). Barrier thickness can be expressed either as the harmonic mean thickness which is a measure of the resistance to gas diffusion, or as the arithmetic mean thickness which expresses the tissue density of the barrier and hence its oxygen consumption (Weibel & Knight, 1964; Weibel, 1970/71; Weibel, 1973).

The avian lung is regarded as the outstandingly efficient gas exchange system among the air breathing vertebrates (Duncker, 1971; Lasiewski & Calder, 1971; Weibel, 1973; Scheid, 1979). Flight requires an unusually large expenditure of energy (Berger & Hart, 1974). For example, a budgerigar (*Melopsittacus undulatus*) flying at sea level uses oxygen at almost twice the rate of a mouse of similar weight running hard in a wheel (Tucker, 1968*a*). In a hypobaric chamber, mice become comatose but house sparrows (*Passer domesticus*) remain active at a simulated altitude of 6100 m (Tucker, 1968*b*). The ability of some birds to perform the strenuous work of flapping flight at high altitude is exceptional by mammalian standards (Tucker, 1972; Berger, 1974).

Attenuation of the blood–gas barrier without loss of its structural stability could be an evolutionary adaptation in the avian lung favouring a high oxygen transfer from air to blood. A number of authors (King, 1966; Duncker, 1970; King & Molony, 1971; Bouverot, 1978; Meban, 1980) have remarked on the thinness of the avian blood–gas barrier. However, few actual measurements have been published, the only available data being approximate estimates by Schulz (1962), Policard, Collet & Martin (1962) and King & Molony (1971), and a preliminary report of the present work by Maina (1981).

Our primary objective is to establish the thickness of the blood–gas barrier, the relative volume densities of its three components, and its ultrastructural characteristics, in several orders of birds. We have also formulated regression equations and looked for a correlation between barrier thickness and three other parameters, i.e. body weight, lung volume, and the surface area of the blood–gas barrier.

Table 1. *Mean thicknesses of blood–gas barrier of the avian lungs*

Order	Species	No. of birds	Harmonic mean $\tau h \pm$ S.E. μm	Minimum harmonic mean $\pm$ S.E. μm	Arithmetic mean $\tau \pm$ S.E. μm	$\tau : \tau h$
Passeriformes	Common Starling (*Sturnus vulgaris*)	10	0.141 ± 0.003	0.065 ± 0.002	1.240 ± 0.030	8.8
	House Sparrow (*Passer domesticus*)	5	0.096 ± 0.003	0.052 ± 0.003	1.030 ± 0.038	10.7
	Redwing (*Turdus iliacus*)	1	0.120	0.060	1.010	8.4
Psittaciformes	Budgerigar (*Melopsittacus undulatus*)	6	0.117 ± 0.007	0.068 ± 0.004	0.980 ± 0.040	8.4
Falconiformes	Common Kestrel (*Falco tinnunculus*)	1	0.210	0.099	1.660	7.9
Charadriiformes	Blackheaded Gull (*Larus ridibundus*)	5	0.146 ± 0.014	0.071 ± 0.007	0.925 ± 0.102	6.3
	Herring Gull (*Larus argentatus*)	2	0.153 ± 0.012	0.075 ± 0.003	1.280 ± 0.255	8.4
	Common Gull (*Larus canus*)	1	0.116	0.081	0.684	5.9
Anseriformes	Graylag Goose (*Anser anser*)	5	0.112 ± 0.003	0.050 ± 0.003	0.890 ± 0.090	7.9
	Mallard (*Anas platyrhynchos*)	5	0.133 ± 0.006	0.062 ± 0.003	0.903 ± 0.060	6.8

The minimum harmonic mean thickness for a given species was obtained by taking the 50 minimum measurements for each specimen of that species, and then calculating their mean.

MATERIALS AND METHODS

Altogether, 41 birds were investigated representing 10 species from 5 orders, i.e. Passeriformes, Psittaciformes, Falconiformes, Charadriiformes and Anseriformes (Table 1). At least five specimens were examined from 6 of these species; only one or two examples of the other species were obtained, but these are included because of the comparative rarity of such material in conditions suitable for electron microscopy. All the birds were believed to be mature. The passeriforms, charadriiforms, kestrel, and mallards were wild and the Graylag geese were feral, whilst the psittaciforms were raised in captivity. The charadriiforms, kestrel and redwing, had suffered incurable wing damage. No pathological lesions were found in the lower respiratory tract. All the birds were killed by injecting Euthatal (May & Baker) intravenously, or, in the small species, intraperitoneally.

The birds were weighed. The lungs were usually perfused *in situ* with 2·3 % glutaraldehyde buffered with sodium cacodylate to a pH of 7·4, via a tracheal cannula and funnel held about 25 cm above the body which was in a supine position. When the fixative stopped flowing an incision was made caudal to the sternum and the fixative was allowed to pass freely through the lungs and escape from the air sacs into the body cavities. The trachea was then ligated and the respiratory organs were left *in situ*, covered in fixative, for about 20 minutes. The lungs were removed and immersed overnight in 2·3 % glutaraldehyde in a vacuum embedder to enhance the infiltration of the fixative into the small airways. This procedure was successful in large species, but in small species better results were obtained by initial perfusion with a combination of 1·5 % glutaraldehyde and 0·8 % formaldehyde (the half strength solution of Karnovsky, 1965) buffered in cacodylate. The volume of one

fixed lung from each bird was determined by water displacement (Scherle, 1970).

The right lung was cut transversely along the costal sulci into five slices. Each slice was cut in half just dorsal to the primary bronchus, thus creating a dorsal half and a ventral half from each slice. Each half was diced and the resulting pieces (about 2 mm³) were post-fixed in 2% osmium tetroxide, block stained in 2% uranyl acetate with maleic acid, and dehydrated in graded ethanol starting at 50% and acetone or propylene oxide before infiltration and embedding in Taab resin. This gave 4 to 10 resin blocks from each half.

One block was selected from each slice. The selection was random except that the dorsal and ventral halves were equally represented. The block was trimmed to eliminate all but exchange tissue. The sections were cut at about 90 nm thickness, stained with lead citrate, and examined electron microscopically. From the first technically adequate section, five electron micrographs were taken from predetermined corners of the grid squares. This stratified sampling procedure was essentially similar to that described by Weibel (1970/71) for the much larger rat lung. Thus a total of at least 25 micrographs was obtained from the lung of each bird. Initially 35 micrographs were examined (5 micrographs from 7 blocks) but summation average graphs (Dunnill, 1964) showed that 25 micrographs were sufficient.

A diffraction grating was included in each batch of micrographs to calibrate the magnification. The primary magnification was about × 3000. The negatives were printed with a superimposed quadratic lattice test grid. The secondary magnification was × 2·5, giving a final magnification of about × 7500, which was just enough to enable the three layers of the blood–gas barrier to be distinguished, and the requisite measurements to be made. Some electron micrographs were prepared at higher magnifications for studying the components of the barrier.

The harmonic and arithmetic mean thicknesses of the barrier were estimated on each electron micrograph using the methods of Weibel & Knight (1964). The relative volume density of each of the three main components of the barrier was determined by point-counting morphometry (Weibel, 1963; Dunnill, 1968); the measurement of the very thin epithelium included its osmiophilic surface lining. In some areas this lining was absent, presumably being lost during fixation. The surface area of the blood–gas barrier was determined by the stereological method of intersection counting (Weibel, 1970/71). The quadratic lattice grid used in the intercept length measurement for the harmonic mean thickness was utilised. The data acquired were correlated with the harmonic mean thickness.

The terminology for the components of the lung used in this study are those adopted by the International Committee on Avian Anatomical Nomenclature and published in *Nomina Anatomica Avium* (Baumel *et al.* 1979).

RESULTS

The measurements of barrier thickness are summarized in Table 1. The thinnest barrier (harmonic mean thickness) was found in the passeriform house sparrow, but the barrier in the common starling was thicker than in the anseriform and psittaciform species. When considered as a group, the charadriiforms had a relatively thick barrier. By far the thickest barrier was found in the single available example of Falconiformes. The minimum thickness (minimum harmonic mean) was always extremely small, although the value was considerably higher in the falconiform than

Table 2. *Regression functions relating the harmonic mean thickness of the barrier to body weight, volume of the lungs, and surface area of the blood–gas barrier*

Y	=	a	X^b	r
τh	=	112·2	V$^{0·016}$	0·143
τh	=	114·8	W$^{0·018}$	0·165
τh	=	112·2	Sa$^{0·015}$	0·110

W, body weight in grams; V, volume of lung in mm^3; Sa, surface area of blood–gas barrier cm^2; τh, harmonic mean thickness of blood–gas barrier in nm; r, correlation coefficient.

in any other species. The mean of the minimum thickness in all species was 0·068 μm. In general, the minimum thickness was about 50 % that of the harmonic mean itself.

Of the arithmetic mean thicknesses, those of the anseriforms and psittaciforms showed the smallest values, while the largest were again found in the falconiform bird (Table 1). The values in the passeriforms and charadriiforms were intermediate. The ratio of the arithmetic to harmonic mean was high throughout the series, ranging from about 6:1 to nearly 11:1.

No correlation was found between the harmonic mean thickness of the barrier and the body weight, the lung volume, or the surface area of the blood–gas barrier. The regression equations of these parameters, and their correlation coefficients with the harmonic mean thickness of the barrier, are given in Table 2. The regression equations were calculated by fitting the least square regression line to the logarithmically transformed data (Lasiewski & Calder, 1971).

In the electron micrographs, the endothelial cell was readily distinguished from the other components of the barrier (Figs. 1–4). Its cytoplasm was relatively thick and contained numerous micropinocytotic vesicles. The nucleus was also relatively often seen (Fig. 1). A basal lamina was always conspicuous (Figs. 1–4). Interstitial tissue was observed very rarely, notably at the angles formed by the apposition of two blood capillaries where infolded osmiophilic laminae, collagen, and fibrocyte profiles were sometimes encountered. In many places, the epithelial cell was so thin that it was scarcely detectable at the low magnifications used for intercept measurements. At higher magnifications, its cytoplasm was just recognisable, being not much thicker than the trilaminar unit membrane itself (Figs. 2–4). The epithelial cell nucleus was seldom seen. The osmiophilic surface layer was usually visible as a black line (Fig. 3). In all the species examined the epithelial cell apparently lacked a basal lamina, because none was ever seen where two epithelial cells lay adjacent to each other, a relationship which is found at the rather uncommon sites where

Figs. 1–4. Electron micrographs of the blood–gas barrier of the blackheaded gull (*Larus ridibundus*), fixed *in situ* by intratracheal perfusion. The endothelial cell and basal lamina are conspicuous in all the Figures but the epithelial cell is clearly visible only at the higher magnifications (Figs. 2–4). Areas of thickening occur in the endothelial cell in all the Figures. Fig. 1 is a general view of the barrier at a relatively low magnification to show areas of extreme thinness of the barrier (e.g. between the arrows), and the beginning of a major thickening caused by the nucleus of an endothelial cell at the bottom of the picture ($\times$ 37000); Fig. 2 shows two adjacent blood capillaries, the epithelial cell being thickened in the angle between the two blood capillaries ($\times$ 50000); Fig. 3 shows a relatively distinct osmiophilic surface layer and epithelial cell, the latter exhibiting a thickened area at the bottom of the Figure ($\times$ 50000); Fig. 4 shows the characteristic micropinocytotic vesicles of the endothelial cell, the epithelial cell and the osmiophilic surface layer also being visible ($\times$ 75000). *AC*, air capillary; *BC*, blood capillary; *BL*, basal lamina; *En*, endothelium; *Ep*, epithelium; *N*, nucleus; *P* plasma; *R*, red blood cell; *SL*, surface lining; W, white blood cell.
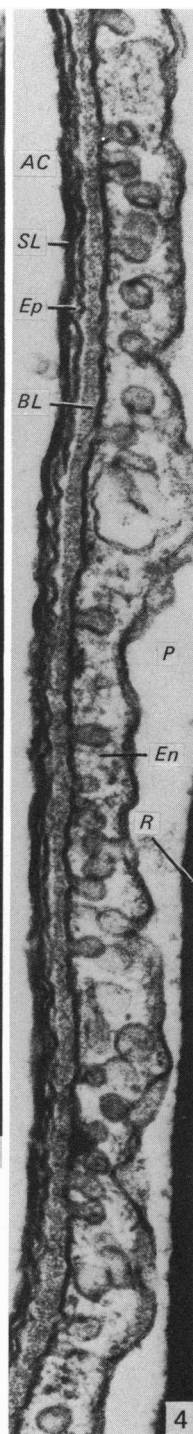
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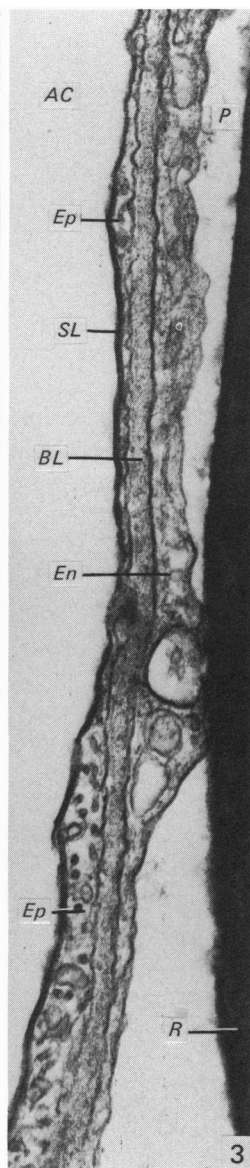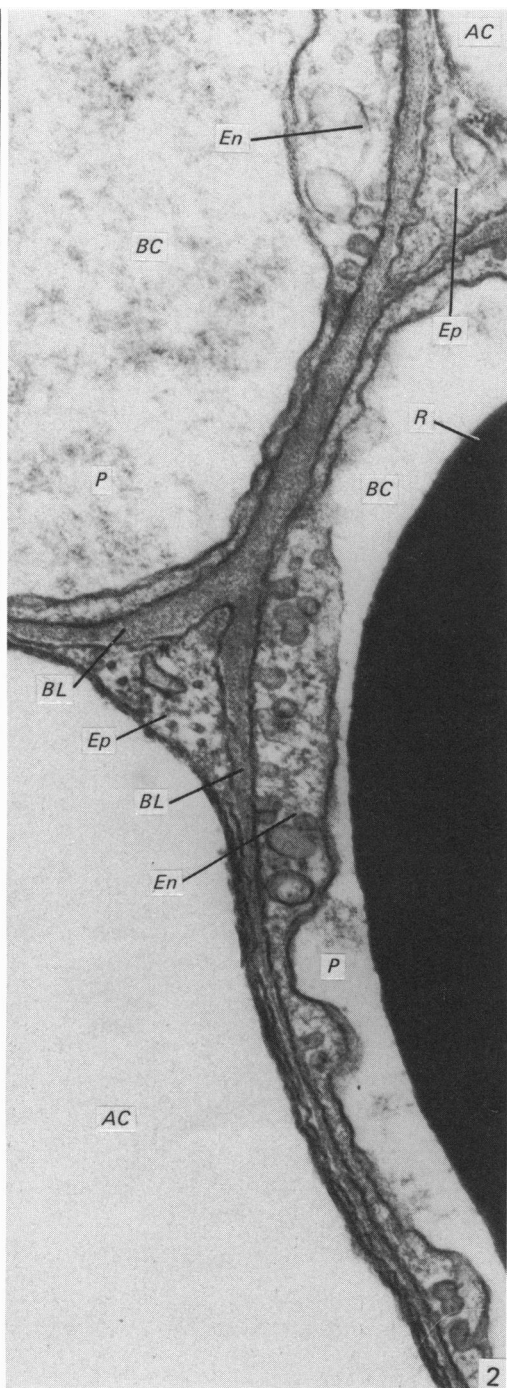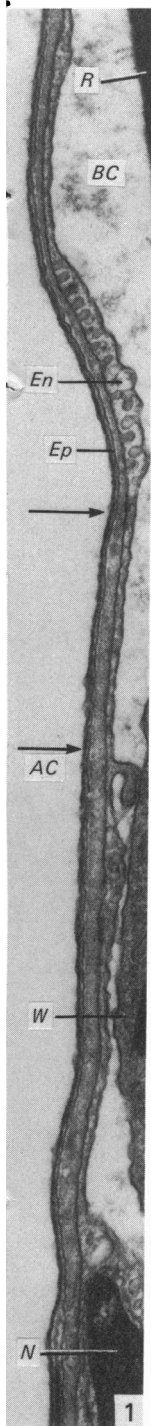Table 3. *Relative volume densities of the three main components of the blood–gas barrier*

Order	No. of birds	Endothelium %	Basal lamina %	Epithelium plus osmio- philic surface lining %
Passeriformes	12	68·95	18·30	12·75
Psittaciformes	4	73·95	16·74	9·31
Falconiformes	1	63·88	23·11	13·01
Charadriiformes	5	56·48	29·09	14·43
Anseriformes	6	70·38	17·74	11·88
Mean		66·73	21·00	12·28

The number of birds examined per species is less than in Table 1, as this analysis was done only on the birds which showed very good fixation.

the walls of two air capillaries adjoin each other. Therefore the conspicuous basal lamina in the blood–gas barrier itself was interpreted as belonging strictly to the endothelial cell.

Measurements of the relative volume densities of the three main components of the barrier (Table 3) confirmed that the endothelium formed the great majority (67%) of the barrier, and that the epithelial cells (12%) constituted a smaller proportion than the basal lamina (21%). In some quite extensive sites the barrier was extremely thin, and in such places the basal lamina was thicker than the other two components together (Fig. 1). At many other points the barrier was locally thickened, the thickest areas being caused by the presence of a nucleus and perinuclear cytoplasm of an endothelial cell (Fig. 1). Thick areas were also caused by the nucleus and associated cytoplasm of an epithelial cell, but these were rare. There were many lesser bulges in the barrier where there were no nuclei but small irregular accumulations of cytoplasm; these accumulations occurred mainly in endothelial cells (Figs. 1–4), but sometimes also in epithelial cells (Figs. 2, 3).

DISCUSSION

All the surfaces of the avian lung are firmly held by pleural attachments to the horizontal septum, vertebrae and to the ribs which are deeply embedded in the costal sulci. Therefore, when the lung is fixed *in situ* the reduction in its external volume is negligible. There is almost certain to be some shrinkage during processing for electron microscopy and this would affect the linear measurements of the blood–gas barrier, but such shrinkage has been shown to be very small in other tissues. Thus Weibel & Knight (1964) observed only 5% shrinkage in the liver and kidney of the rat, and Meban (1980) also found it to be 5% in the lung of the mouse; in subsequent work on the mammalian lung Weibel (1970/71) noted that his procedure "should not cause any appreciable dimensional changes in the tissue". Their fixation and processing differed from ours only in detail, and therefore we have disregarded shrinkage.

The harmonic mean thickness of the barrier varied in the different orders of birds (Table 1). Despite the higher standard metabolic rate per unit weight which characterizes passerine birds (Lasiewski & Dawson, 1967), extreme thinness of the blood–gas barrier was not a consistent feature in our passerine species. The thinnest

Table 4. *Comparison of thicknesses of blood–gas barrier* in vertebrates*

Class	Source of data	Mean harmonic mean thickness (τh) μm	Minimum harmonic mean thickness μm	Mean arithmetic mean thickness (τ) μm	$\tau : \tau h$
Birds	This study	0·134	0·068	1·060	7·95:1
Mammals	Weibel (1972; 1973) Meban (1980) Gehr & Erni (1980)	0·53†	0·20†	1·50†	2·8:1†
Reptiles	Meban (1980)	1·01†	0·22†	2·02†	2·0:1†
Amphibians	Meban (1980)	1·70†	0·21†	2·22†	1·3:1†
Fish (lungfish)	Hughes & Weibel (1976)	0·865	—	3·76	4·35:1
Fish (tench)	Hughes (1972)	2·473	—	—	—

* In the tench, water–blood barrier.
† These are estimates calculated from the mean values reported by the authors for each of their species.

harmonic mean measurement (0·096 μm) did occur in one of the passeriforms, the house sparrow, but in the passerine common starling and redwing the barrier was thicker than in either the anseriform Graylag goose or the psittaciform budgerigar. The relative thinness of the barrier in the two anseriforms could perhaps be associated with an energetic mode of flight. The barrier was relatively thick in two of the charadriiform species and this may be related to the relatively low energetic cost of the soaring and gliding flight which these birds often employ, although the single specimen of a common gull had a relatively thin barrier. It was surprising to find by far the thickest barrier in the falconiform kestrel (0·210 μm), which is an energetic flyer capable of hovering but the data from this single individual may not be representative of the species. In two galliform species examined by Abdalla, Maina, King, King and Henry (unpublished observations), i.e. the domestic fowl (*Gallus gallus*) and domestic guinea-fowl (*Numida meleagris*), the harmonic mean thickness of the barrier was very much greater (0·30 and 0·32 μm respectively) than in any of the species examined here, and this may be correlated with the fact that these birds do not sustain flight for any length of time. Clearly the small number of species which we have examined necessitates caution in generalizing about the orders which they represent.

Table 4 shows that in all respects the blood–gas barrier of the avian lung is very thin compared to that of mammals, reptiles, and amphibians, and also to fish, so far as the data allow. The thinnest harmonic mean known in any mammal is 0·27 μm in the Etruscan shrew (*Suncus etruscus*) reported by Gehr *et al.* (1980), but even this is 2 times thicker than the mean value for the birds. Again, by far the thinnest *minimum* harmonic mean thickness found in any mammal is 0·1 μm in the Etruscan shrew (Weibel, 1973), but this, too, is 1·5 times thicker than the mean of the minimum thicknesses in our birds. The very low values for the *minimum* thickness of the avian barrier (Tables 1, 4) suggest that extreme sporadic attenuation of the blood–gas barrier occurs in birds, without endangering its mechanical stability; this would be consistent with the concept (Duncker, 1971; Perry & Duncker, 1980) that the avian lung undergoes minimal volume changes during the respiratory cycle, so that the air capillaries can be regarded as virtually rigid tubes which are not subjected to rhythmic inflation and deflation.

Electron micrographs of the avian exchange tissue generally show marked varia-

tions in the thickness of the blood–gas barrier (Figs. 1–4), which suggest that the barrier may be corrugated. It is claimed (Weibel, 1973; Meban, 1980) that such apparent qualitative corrugations of the barrier are reflected quantitatively in the ratio of the arithmetic to the harmonic mean thickness. A ratio of about 3:1 in mammals, as in Table 4, is held to indicate corrugation (Weibel, 1972, 1973; Meban, 1980). On the other hand, the lower ratios in reptiles and amphibians are thought to indicate less corrugation (Meban, 1980). Since the mean ratio in our birds was 7·95, this line of reasoning suggests that the avian barrier is much more corrugated than that of other air breathing vertebrates. Corrugation has been regarded as physiologically advantageous on the grounds that it preserves a reasonable degree of mechanical stability and at the same time offers a lower overall resistance to gaseous diffusion than a barrier of uniform thickness (Weibel, 1973; Meban, 1980). However, it is noteworthy that the Etruscan shrew, which is a minute and therefore metabolically extremely demanding mammal, has a ratio of only about 2; Weibel (1972) and Gehr et al. (1980) accounted for this low ratio by the opposite line of argument, i.e. that the low ratio is an extreme effort to reduce overall barrier thickness so that the arithmetic mean approaches the harmonic mean.

The mean of the arithmetic mean thicknesses in our birds was 1·060 μm, which was considerably less than that of mammals, reptiles, amphibians and the lungfish (Table 4). Since the arithmetic mean thickness reflects the mass of tissue constituting the barrier and consuming oxygen (Weibel & Knight, 1964) it would seem that the avian lung has achieved mechanical stability of the barrier at a low cost in the consumption of oxygen by the actual tissues of the barrier.

Though Weibel (1972) and Meban (1980) observed a strong positive correlation (of 0·843 and 0·74, respectively) between body weight and thickness of the barrier in mammals, no such correlation was found between these two parameters in our birds (nor was one found in reptiles and amphibians by Meban).

The endothelium, the intermediate layer (formed by the basal lamina), and the epithelium plus osmiophilic surface lining, constituted, respectively, 67, 21, and 12 % of the barrier in our birds. In most of those mammals, reptiles, and amphibians which have been investigated (Weibel, 1973; Meban, 1980), the intermediate layer constituted about 40 % of the barrier while the endothelium and epithelium each accounted for about 30 %. In man (Gehr, Bachofen & Weibel, 1978) the intermediate layer is even thicker, constituting 56 % of the barrier; this is because the intermediate layer includes fibroblasts, and many collagen and elastic fibres which form the "fibrous skeleton of lung parenchyma". In complete contrast, the intermediate layer in shrews (Gehr et al. 1980) constitutes only 26 %, and large parts of the barrier are devoid of fibres. In the avian barrier, the intermediate layer has been reduced virtually to a basal lamina and therefore almost completely lacks the 'fibrous skeleton' which characterizes mammals (except the smallest mammal of all). The absence of a fibrous skeleton makes it all the more remarkable that the avian air capillaries so successfully resist collapse (Macklem, Bouverot & Scheid, 1979).

In conclusion, we suggest that the avian lung has evolved a very much thinner blood–gas barrier than that of any other known air breathing vertebrate, but without sacrificing the structural stability of the barrier. This adaptation maximises the diffusing capacity of the lung, and minimises the cost in oxygen incurred by the metabolism of the barrier itself. Thus, birds have achieved perfection in barrier design, and this would contribute to their remarkable ability to expend energy during flight, especially at high altitude.

SUMMARY

The blood–gas barrier was investigated in 41 birds from 10 species representing Passeriformes, Psittaciformes, Falconiformes, Charadriiformes and Anseriformes. In all species, the barrier was reduced to a very thin epithelial cell, a conspicuous basal lamina, and a relatively thick endothelial cell, connective tissue elements being almost entirely absent.

A stratified sampling procedure was adopted for measurements. Linear shrinkage was ignored. The harmonic mean thickness ranged from 0·096–0·210 μm, the mean for all species being 0·134 μm. The thinnest barrier was observed in the house sparrow (0·096 μm), but extreme barrier thinness was not observed in all of the passerines. The anseriform and psittaciform barrier was also relatively thin. The charadriiforms as a group had a relatively thick barrier. The thickest barrier was found in the kestrel (0·21 μm). Possible relationships between these species variations and metabolic rate or physical activity are discussed. The harmonic mean thickness of the avian barrier is evidently much less than that of other vertebrates. Sporadic attenuation in the thickness of the avian barrier was observed. The *minimum* harmonic mean thickness ranged from 0·050–0·099 μm with a mean in all species of 0·068 μm, the smallest measurements being in the Graylag goose and the largest in the kestrel. These values are far lower than those of amphibians, reptiles and mammals.

The arithmetic mean thickness ranged from 0·684–1·66 μm with a mean of 1·060 μm. The smallest value occurred in the common gull and the largest in the kestrel. The mean ratio of arithmetic to harmonic mean thickness for all the species was 7·95. This value is much higher than in other air-breathing vertebrates, and may indicate marked corrugation of the barrier. Direct qualitative evidence for corrugation was seen on electron micrographs from all species, in the form of extensive areas of extreme thinness and zones of sporadic thickness.

Point-counting morphometry showed that the endothelium made up 67 % of the barrier, the basal lamina 21 %, and the epithelium 12 %. No correlation was found between the thickness of the barrier and the body weight, the volume of the lungs, and the surface area of the blood–gas barrier.

By evolving a very thin blood–gas barrier, birds have maximised the diffusing capacity of the lung and minimised the metabolic cost of the barrier itself, thereby achieving a perfect barrier design which facilitates the strenuous work of flight, especially at high altitude.

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