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The lung of the emu, *Dromaius novaehollandiae*: a microscopic and morphometric study*

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INTRODUCTION

The emu (*Dromaius novaehollandiae*) is the only extant member of the family Dromaiidae and is the most widespread of Australian flightless birds (Cameron & Harrison, 1978). After the African ostrich (*Struthio camelus*), it is the world's second largest living bird. The respiratory and cardiovascular physiology of large flightless birds has been investigated by Crawford & Schmidt-Nielsen (1967), Crawford & Lasiewski (1968), Schmidt-Nielsen *et al.* (1969), Calder & Dawson (1978), Jones, Grubb & Schmidt-Nielsen (1983) and Grubb, Jorgensen & Conner (1983). The ratites are generally considered to be phylogenetically among the most primitive of the extant groups of birds (Storer, 1971). They have a lower body temperature (about 38 °C) than carinate birds (Calder & Dawson, 1978; Jones *et al.* 1983). Furthermore the ostrich (Schmidt-Nielsen *et al.* 1969) and the emu (Jones *et al.* 1983), when heat stressed, are exceptionally unsusceptible to the respiratory alkalosis that overtakes most other birds after prolonged panting; this has been attributed to unknown structural and functional pulmonary adaptations (Jones, 1982*a*, *b*).

Gross investigations of the lungs of the emu and the kiwi have categorised them as primitive because they lack the neopulmonic system of parabronchi that characterises species which are supposed to be phylogenetically advanced (Duncker, 1971). Morphometric studies indicating the potential of the lung for gas exchange are evidently lacking for ratite birds.

An apparently mature and healthy adult emu, surplus stock from an Australian zoo, became available to us. In view of the rarity of such material, especially under conditions allowing fixation for electron microscopy, we decided to investigate the general microscopic and morphometric characteristics of its lungs. It is hoped that this would further elucidate the emerging picture (Maina, 1988) that, as in the terrestrial mammals, the pulmonary design of a bird reflects the oxygen demand upon it and that this depends on factors such as phylogenetic status, body mass and mode of life.

MATERIALS AND METHODS

The bird weighed 30 kg and was killed by an intravenous injection of Euthatal (MB^{R}). The lungs were fixed by intratracheal instillation of 2·3% glutaraldehyde buffered with sodium cacodylate (total osmolarity 350 m-osmol, pH 7·4) at a pressure head of 25 cm until no more fixative flowed down the trachea. After remaining

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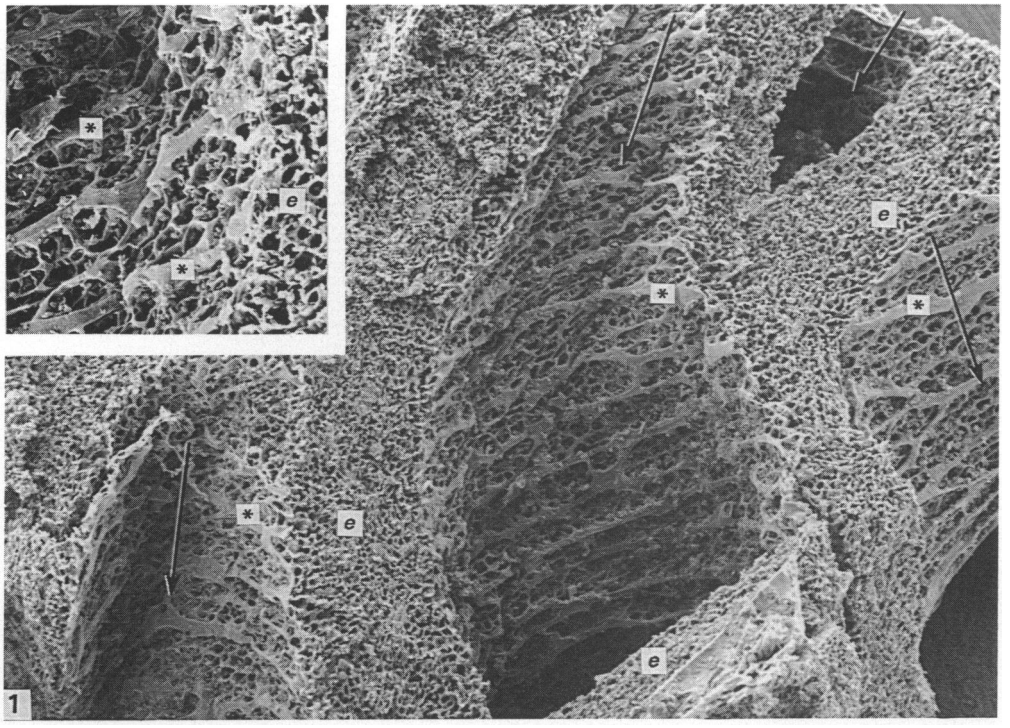
overnight *in situ* the lungs were removed and their fixed volume was estimated by the water displacement method of Scherle (1970).

The sampling, tissue processing and subsequent hierarchical analysis of the lung by point counting were carried out using light and electron microscopy, as described by Abdalla *et al.* (1982), Maina & King (1982*a*, *b*), Maina, Abdalla & King (1982) and Maina (1987). The data were modelled (Weibel, 1970/71) and the individual diffusing capacities of the components of the air–haemoglobin pathway, and the total morphometric diffusing capacity of the lung, were estimated. Recently, the oxygen association factor (Θ_{O_2}) has become available for the red blood cells of the mature domestic form of the muscovy duck (*Cairina moschata*) and the domestic fowl (*Gallus gallus*) (Nguyen Phu, Yamaguchi, Scheid & Piiper, 1986). We have used the average value for these two birds, i.e. $2{\cdot}7 \times 10^{-2}$ ml O_2/sec/mbar, the value for the emu presently not being available. This avoids the use of mammalian constants adjusted for the nucleus of the avian red blood cell (see Abdalla *et al.* 1982; Maina & King, 1982*b*), a procedure which now turns out to give a substantial underestimate of the total morphometric pulmonary diffusing capacity of the avian lung.

RESULTS

The mantles of adjacent parabronchi merged with each other (Fig. 1), interparabronchial septa being absent in this species; interparabronchial blood vessels indicated the approximate boundaries between adjacent parabronchi. From their luminal surface, the parabronchi gave rise to atria delineated by well-developed interatrial muscles (Fig. 2). Each atrium opened into 2–4 infundibula, which led into the air capillaries. The exchange tissue consisted of interdigitating systems of air and blood capillaries, the former generally being of larger diameter than the latter (Fig. 2). The blood capillaries were lined by non-fenestrated endothelial cells (Figs. 3–6). The common basal membrane between the endothelial and the squamous epithelial cell was accompanied by a few collagen fibrils and fibrocytes (Fig. 3). The squamous epithelial cells were extremely thin and covered by a surface lining (Fig. 6); microvilli were more numerous on their free surface than in birds generally (Fig. 5). Long cytoplasmic extensions of granular epithelial cells containing osmiophilic bodies were frequently observed on the luminal surface of the air capillaries (Fig. 4).

The combined volume of the left and right lungs (V_L) was 1100 cm^3. The volume densities of the main components of the lung were: exchange tissue, 17·76%; lumina of the parabronchi and secondary bronchi including the atria, 49·11%; blood vessels larger than capillaries, 28·57%; and the primary bronchus, 4·56%. Of the volume of the exchange tissue (V_x), the air capillaries (V_a) and the blood capillaries (V_c) comprised 79·15 and 14·2%, while the blood–gas (tissue) barrier and the tissue not involved in gas exchange constituted 3·4 and 3·25%, respectively. The surface area of the blood–gas tissue barrier (S_t) was 16·28 m^2, the air capillaries (S_a) 22·56 m^2, the endothelium (S_c) 19·69 m^2, and the red blood cells (S_e) 23·16 m^2. The harmonic mean thickness of the tissue barrier (τ_{ht}) was 0·232 μm and that of the plasma was 0·103 μm. The diffusing capacity of the tissue barrier (Dt_{O_2}) was 2·88 ml O_2/sec/mbar, whilst that of the plasma and red blood cell were 8·21 and 0·504 ml O_2/sec/mbar respectively; the mean total morphometric pulmonary diffusing capacity (DL_{O_2}) was 0·407 ml O_2/sec/mbar. Further quantitative results are summarised in Table 1 where they are normalised with body mass (W) for comparison with other species of bird of different sizes and diverse life-styles.



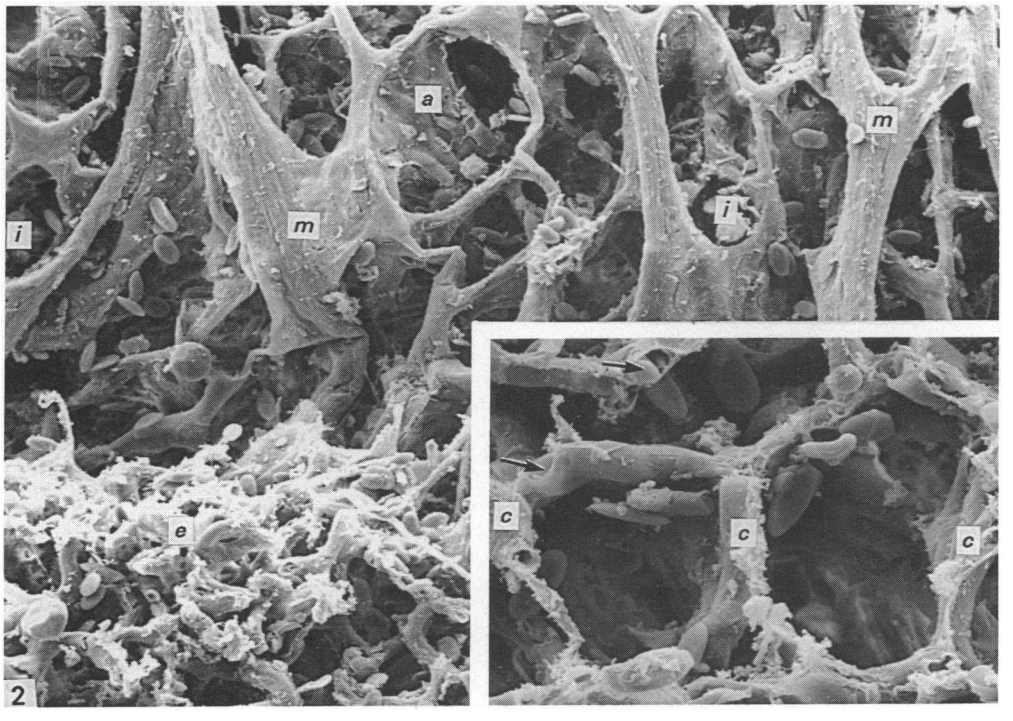
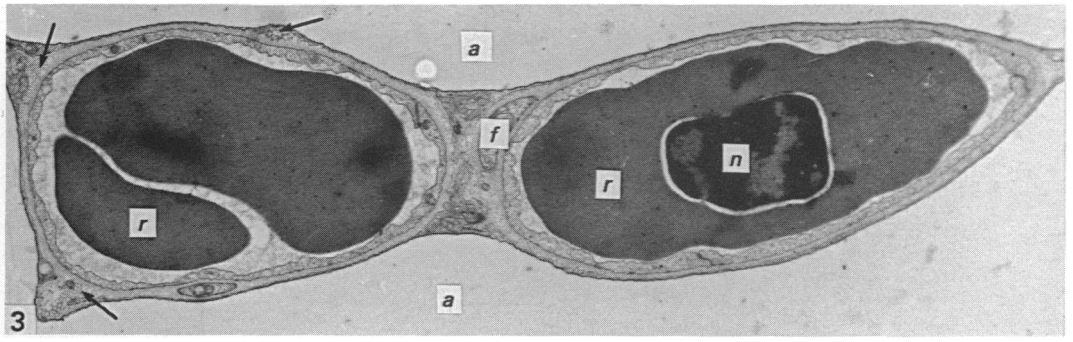
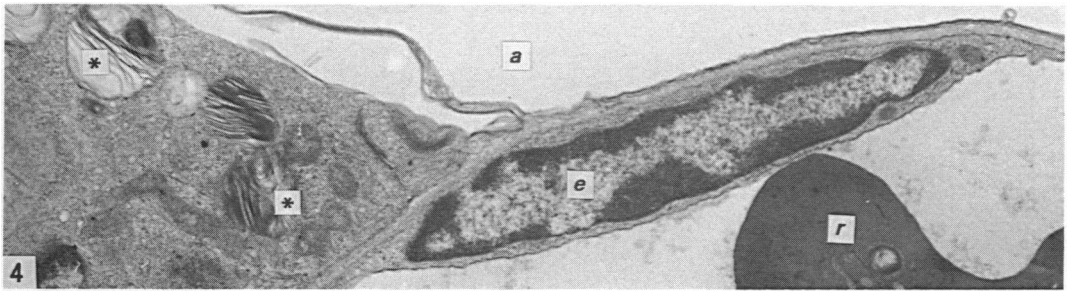
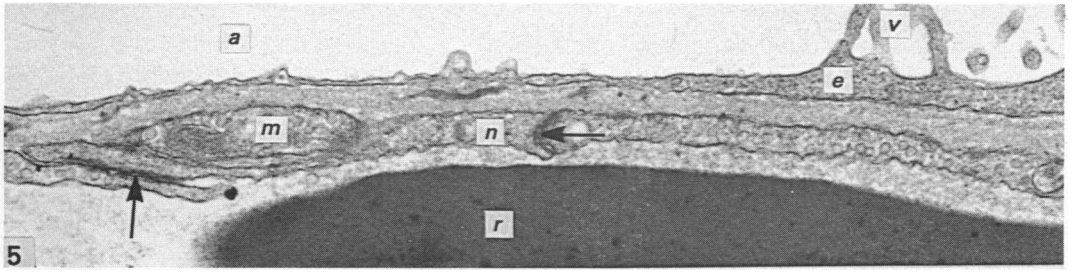


Fig. 1. Scanning electron micrograph of parabronchi in the emu lung. The parabronchial lumen is designated with arrows, and *e* indicates the exchange tissue. The atria are delineated by well-developed atrial muscles (asterisks). These are shown at a higher magnification in the inset. × 53; inset × 133.

Fig. 2. Scanning electron micrographs to show the atrial muscles (*m*) lining the atria (*a*). The atria lead into the infundibula (*i*). The air capillaries and blood capillaries constitute the bulk of the exchange tissue (*e*). The inset is an enlargement of the exchange tissue showing air capillaries surrounded by blood capillaries (*c*). Arrows indicate red blood cells emerging from cut capillaries. × 467; inset × 857.







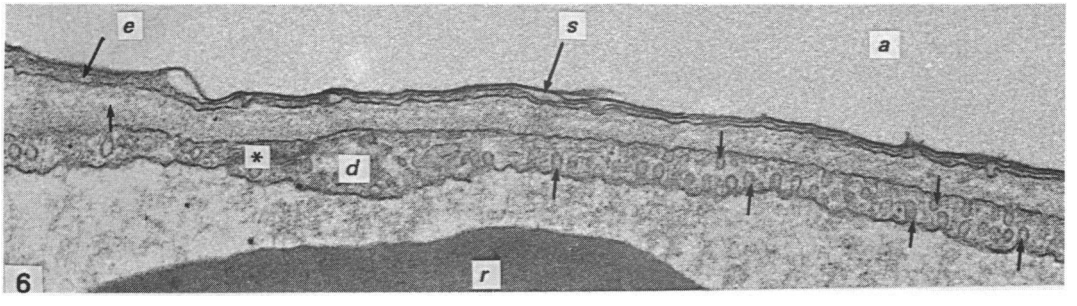


Fig. 3. Transmission electron micrograph showing air capillaries (*a*) and blood capillaries containing red blood cells (*r*). A common basal membrane lies between the endothelial and squamous epithelial cell, and is accompanied by clusters of collagen fibrils (arrows) and occasional fibrocytes (*f*); *n*, nucleus of red blood cell. × 7333.

Fig. 4. Transmission electron micrograph of the blood–gas barrier showing a granular epithelial cell with osmiophilic bodies (asterisks); *a*, lumen of air capillary; *e*, nucleus of endothelial cell; *r*, red blood cell. × 11400.

Fig. 5. Transmission electron micrograph of the blood–gas barrier. *e*, squamous epithelial cell with microvilli (*V*). Adjacent endothelial cells (*n*) fuse at tight junctions (arrows). *m*, mitochondrion; *a*, lumen of air capillary; *r*, red blood cell. × 19500.

Fig. 6. Transmission electron micrograph of the blood–gas barrier showing a squamous epithelial cell (*e*) and an endothelial cell (*d*). Between the two cells is a common basal membrane. *s*, layer of surfactant; arrows, micropinocytotic vesicles; *r*, red blood cell; asterisk, tight junction. × 30000.

Table 1. *Comparison of pulmonary morphometric parameters in species of birds exhibiting different modes of life**

English name	Latin name	V_L/W (cm^3/kg)	S_t/W (cm^2/g)	S_t/V_x (mm^2/mm^3)	V_c/W (cm^3/kg)	V_a/W (cm^3/kg)	τ_{ht} (μm)	Dt_{O_2}/W ($ml O_2/sec/mbar/kg$)	DL_{O_2}/W ($ml O_2/sec/mbar/kg$)
Emu	*Dromaius novaehollandiae*[a]	36·7	5·4	83	0·93	5·2	0·232	0·096	0·014
Domestic fowl	*Gallus gallus*[b]	12·6	8·7	172	1·63	3·6	0·318	0·113	0·029
Humboldt penguin	*Spheniscus humboldti*[c]	30·4	18·1	116	8·0	5·4	0·530	0·141	0·074
Black-headed gull	*Larus ridibundus*[d]	26·9	23·5	238	2·8	5·3	0·138	0·736	0·061
Mallard	*Anas platyrhynchos*[e]	29·5	28·6	240	3·9	7·0	0·133	0·900	0·077
Common starling	*Sturnus vulgaris*[f]	27·8	49·3	342	4·7	7·4	0·141	1·450	0·105
Violet-headed hummingbird	*Colibri coruscans*[g]	42·9	87·1	389	6·3	9·3	0·099	3·500	—

* The symbols are defined in the text.
Sources of the data: [a] This study. [b] Abdalla *et al.* (1982). [c] Maina & King (1987). [d] Maina (1987). [e] Maina & King (1982). [f] Maina (1984). [g] Dubach (1981).
All the values for DL_{O_2} were calculated using the values for avian θ_{O_2} reported by Nguyen Phu *et al.* (1986).

DISCUSSION

Several structural characteristics indicate that the emu lung is less well adapted for gas exchange than that of some 28 species from 11 avian orders examined by Maina (1988). In these latter species, the granular epithelial cells were restricted mainly to the atria of the parabronchi and this appears to be an adaptation to achieve a thin blood–gas barrier and reduce oxygen consumption by the cellular components of the lung (Maina, 1988). In the emu, however, granular epithelial cells are widespread in the exchange tissue. Moreover, the presence of relatively abundant microvilli on the squamous epithelial cells is believed to indicate a poor differentiation of the two main populations of pneumocytes, a feature that characterises amphibians (Meban, 1973; Goniakowska-Witalinska, 1978) and Dipnoi (Maina & Maloiy, 1985).

Morphometrically, the emu blood–gas barrier was remarkably thick (Table 1), being exceeded only by the domestic galliform species (Duncker, 1972; Abdalla & Maina, 1981) and the Humboldt penguin (Maina & King, 1987). The weight-specific total morphometric pulmonary diffusing capacity for oxygen (DL_{O_2}/W) was very low, and it is this parameter that most comprehensively defines the anatomical potential for pulmonary gas exchange. The low value in the emu arises from the combination of a relatively small surface area of the blood–gas barrier (S_t/W), relatively small volume of pulmonary capillary blood (V_c/W), and a relatively thick blood–gas barrier (τ_{ht}) (Table 1).

The emu has a comparatively low resting oxygen consumption (3·91 ml O_2/min/kg), whereas values of 5·28 ml O_2/min/kg were reported in the rhea (*Rhea americana*) by Crawford & Lasiewski (1968) and 4·72 ml O_2/min/kg in the ostrich by Schmidt-Nielsen *et al.* (1969). Furthermore, whilst the ostrich can run at 60 km/h the emu can only manage 30 km/h (Cameron & Harrison, 1978).

Wide ranging conclusions cannot safely be drawn from anatomical observations on a single specimen. However, these morphometric and physiological observations together suggest that the emu has a relatively low demand for oxygen, and this appears to be consistent with the evolution of a bird of relatively large body mass and low surface area, in a warm environment, and with few effective predators. It is hoped that these morphometric observations may stimulate further similar observations on this species and thus augment the increasingly abundant allometric data for the pulmonary anatomy of birds, which in recent years has begun to match that of mammals.

SUMMARY

Qualitative and quantitative characteristics suggest that the lung of the emu is poorly adapted for gas exchange when compared with that of other birds. The granular epithelial cells extend over the air capillaries, and the squamous epithelial cells have microvilli indicating a poor differentiation of the epithelium of the exchange tissue. The surface area of the blood–gas tissue barrier per unit body mass was only 5·4 cm^2/g, the volume of the pulmonary capillary blood per unit body mass was only 0·93 cm^3/kg, and the tissue barrier was unusually thick (0·232 μm). These parameters produce a relatively small total morphometric pulmonary diffusing capacity for oxygen of 0·014 ml O_2/sec/mbar/kg. The findings conform to the evolution of a very large flightless bird in a warm environment lacking effective predators.

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REFERENCES

ABDALLA, M. A. & MAINA, J. N. (1981). Quantitative analysis of the exchange tissue of the avian lung (Galliforms). *Journal of Anatomy* **133**, 677–680.

ABDALLA, M. A., MAINA, J. N., KING, A. S., KING, D. Z. & HENRY, J. (1982). Morphometrics of the avian lung. 1. The domestic fowl *Gallus gallus var. domesticus*. *Respiration Physiology* **47**, 267–278.

CALDER, W. A. & DAWSON, T. J. (1978). Resting metabolic rates of ratite birds: the kiwis and the emu. *Comparative Biochemistry and Physiology* **60A**, 479–481.

CAMERON, A. D. & HARRISON, C. J. O. (1978). *Bird Families of the World.* Oxford: Elsevier-Phaidon.

CRAWFORD, E. C. & SCHMIDT-NIELSEN, K. (1967). Temperature regulation and evaporative cooling in the Ostrich. *American Journal of Physiology* **212**, 317–353.

CRAWFORD, E. C. & LASIEWSKI, R. C. (1968). Oxygen consumption and respiratory evaporation of the Emu and Rhea. *Condor* **70**, 333–339.

DUBACH, M. (1981). Quantitative analysis of the respiratory system of the house sparrow, budgerigar and violet-eared hummingbird. *Respiration Physiology* **46**, 43–60.

DUNCKER, H. R. (1971). The lung air sac system of birds. A contribution to the functional anatomy of the respiratory apparatus. *Ergebnisse der Anatomie und Entwicklungsgeschichte* **45**, 1–176.

DUNCKER, H. R. (1972). Structure of avian lungs. *Respiration Physiology* **14**, 44–63.

GONIAKOWSKA-WITALINSKA, L. (1978). Ultrastructural and morphometric study of the lung of the European Salamanda. *Cell and Tissue Research* **191**, 343–356.

GRUBB, B., JORGENSEN, D. D. & CONNER, M. (1983). Cardiovascular changes in the exercising Emu. *Journal of Experimental Biology* **104**, 193–201.

JONES, J. H. (1982*a*). Pulmonary blood flow distribution in panting ostriches. *Journal of Applied Physiology* **53**, 1411–1417.

JONES, J. H. (1982*b*). Hot ostriches: intraparabronchial blood flow changes during panting. *Federation Proceedings* **41**, 1096.

JONES, J. H., GRUBB, B. & SCHMIDT-NIELSEN, K. (1983). Panting in the Emu causes arterial hypoxemia. *Respiration Physiology* **54**, 189–195.

MAINA, J. N. (1984). Morphometrics of the avian lung. 3. The structural design of the passeriform lung. *Respiration Physiology* **55**, 291–307.

MAINA, J. N. (1987). Morphometrics of the avian lung. 4. The structural design of the charadriiform lung. *Respiration Physiology* **68**, 99–119.

MAINA, J. N. (1988). The morphometry of the avian lung. In *Form and Function in Birds*, vol. 4 (ed. A. S. King & J. McLelland), ch. 6. London: Academic Press (in the Press).

MAINA, J. N., ABDALLA, M. A. & KING, A. S. (1982). Light microscopic morphometry of the lung of 19 avian species. *Acta anatomica* **112**, 264–270.

MAINA, J. N. & KING, A. S. (1982*a*). The thickness of the avian blood–gas barrier: qualitative and quantitative observations. *Journal of Anatomy* **134**, 553–562.

MAINA, J. N. & KING, A. S. (1982*b*). Morphometrics of the avian lung. 2. The Wild Mallard (*Anas platyrhynchos*) and Graylag Goose (*Anser anser*). *Respiration Physiology* **50**, 299–310.

MAINA, J. N. & KING, A. S. (1987). A morphometric study of the lung of a Humboldt Penguin (*Spheniscus humboldti*). *Zentralblatt für Veterinärmedizin, Reihe C, Anatomia, Histologia, Embryologia* **16**, 293–297.

MAINA, J. N. & MALOIY, G. M. O. (1985). The morphometry of the lung of the African lungfish (*Protopterus aethiopicus*): its structural functional correlations. *Proceedings of the Royal Society of London, B* **224**, 399–420.

MEBAN, C. (1973). The pneumocytes in the lung of *Xenopus laevis*. *Journal of Anatomy* **114**, 235–244.

NGUYEN PHU, D., YAMAGUCHI, K., SCHEID, P. & PIIPER, J. (1986). Kinetics of oxygen uptake and release by red blood cells of chicken and duck. *Journal of Experimental Biology* **125**, 15–27.

SCHERLE, W. F. (1970). A simple method for volumetry of organs in quantitative stereology. *Mikroskopie* **26**, 57–60.

SCHMIDT-NIELSEN, K., KANWISHER, J., LASIEWSKI, R. C., COHN, J. E. & BRETZ, W. L. (1969). Temperature regulation and respiration in the Ostrich. *Condor* **71**, 341–352.

STORER, R. W. (1971). Adaptive radiation in birds. In *Avian Biology*, vol. 1 (ed. D. S. Farner & J. R. King), pp. 147–188. New York–London: Academic Press.

WEIBEL, E. R. (1970/71). Morphometric estimation of pulmonary diffusing capacity. I. Model and method. *Respiration Physiology* **11**, 54–75.