The morphology of the lung of the black mamba *Dendroaspis polylepis* (Reptilia: Ophidia: Elapidae). A scanning and transmission electron microscopic study

J. N. MAINA*

Department of Anatomy, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

INTRODUCTION

The black mamba (*Dendroaspis polylepis*) belongs to the family Elapidae that also includes the cobras, kraits and coral snakes. Generally, these snakes are extremely venomous and occur in the warmer parts of the world (Parker, 1977). They belong to the infraorder Caenophidia, which includes the most highly developed snakes (Webb, Wallwork & Elgood, 1978). The black mamba is a diurnal and only sparingly arboreal snake (Skinner, 1973). It is the fastest land snake, attaining speeds of 16–19 km/hr for short bursts (Wood, 1980). The structure of the ophidian lung is remarkably different from that of the other reptiles (Luchtel & Kardong, 1981) and the variation in the nature of the pulmonary morphology in this reptile group is remarkable (Porter, 1972; Duncker, 1978). This feature is clearly illustrated in the family Boidae where in the subfamily Boinae the left lung is well developed with the right lung being smaller, while in Tropidophinae only the right lung is developed; both lungs are equally well developed in Bolyeriinae (Goin & Goin, 1962). Further, in the family Colubridae the left lung is drastically reduced in size, whereas in Viperidae the left lung is largely absent, the right lung being extremely elongated (Cope, 1894; Butler, 1895). These variations show well the notable adaptive radiation this reptilian group has undergone (Brongersma, 1949, 1951; Underwood, 1967, 1976; Pohunkova & Hughes, 1985).

The morphological variations in the pulmonary anatomy of the Ophidia have been used to evaluate the systematic relationships within this reptilian taxon (Cope, 1894, 1900; Underwood, 1967, 1976). In this respect, extensive and detailed comparative studies on the ophidian lungs are essential for any meaningful systematic use of this differing morphology.

The study of the ophidian lung covers a long period of time (Cope, 1894, 1900; Butler, 1895; Marcus, 1937; Varde, 1951; George & Shah, 1956). These elegant early studies, however, are largely descriptive of gross anatomy or are histological. Apart from the detailed ultrastructural account of the lung of the rattlesnake (Luchtel & Kardong, 1981) and the garter snake (Pohunkova & Hughes, 1985), apparently only brief accounts of the ultrastructure of the snake lung are available (Okada *et al.* 1962; Nagaishi, Okada, Ishika & Daido, 1964; Brooks, 1970). Stinner (1982) gave an excellent account of the morphometry of lung of the colubrid snake *Pituophis melanoleucus*. The present study was intended to supplement these investigations by examining the lung of the black mamba.

* Usual address to which reprint requests should be sent: Department of Veterinary Anatomy, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

MATERIALS AND METHODS

Specimens of apparently mature black mamba *Dendroaspis polylepis* (Gunther) were captured by a commissioned, licensed animal trapper in the Kitui District, a semi-arid, open savanna grassland part of Kenya. The specimens were brought to the laboratory where they were anaesthetised with chloroform in an airtight chamber. A ventral median incision was made with the animal in a supine position and the lungs examined *in situ*.

Tissue samples were taken from the exchange tissue (the anterior part) region of the lung and immersed in 2·3% glutaraldehyde buffered with sodium phosphate, total osmolarity 350 mosm and pH 7·2. For transmission electron microscopy, some of these tissue samples were diced into small pieces (about 1 mm^3) which were postfixed in 2% osmium tetroxide for 2 hours, block stained in 2% uranyl acetate in maleic acid, dehydrated in ethanol, starting at 50% through absolute and acetone, before infiltration and embedding in TAAB resin. Ultrathin sections were counterstained with lead citrate before viewing in a Hitachi 900 transmission electron microscope.

The tissues for scanning electron microscopy were dehydrated in five daily changes of absolute ethanol, critical point dried in liquid carbon dioxide, sputter coated with gold–palladium complex and viewed on a Philips PSEM 275 scanning electron microscope at 7·2 to 15 kV.

RESULTS

The pulmonary system in the black mamba comprises a single lung, the right lung, that extends the whole length of the pleuro-peritoneal cavity, terminating close to the vent. The trachea runs from the pharyngeal cavity and enters the lung at the hilus. Due to the absence of the left lung, and hence the bifurcation of the trachea, it is not possible to distinguish the trachea from the extrapulmonary part of the bronchus. The trachea contains incomplete cartilages that are bridged on the dorsal aspect by a membrane. Close to the lung, the membrane becomes extensive and vascularised and bears shallow faveoli. The tracheal cartilages are continued into the lung as the bronchial cartilages which are located only on the ventral aspect of the single bronchus. On the dorsal aspect the bronchus, which runs about 50% of the length of the lung, opens into the faveoli. On the luminal aspect the trachea is lined by an epithelium composed of ciliated cells interspersed with secretory cells and tracks of smooth squamous cells (Figs. 1, 2). This heterogenous epithelium extends to the primary septa and also sparsely to the secondary septa.

The lung displays two anatomically distinct regions. The anterior faveolar region, which contains the profusely compartmented gas exchange tissue, and a posterior capacious saccular smooth region devoid of respiratory tissue. The faveolar region consists of a central air duct which opens radially to the respiratory tissue between well developed primary septa (Figs. 3, 4). From the primary septa arise the smaller secondary septa that in turn give rise to the yet smaller and more deeply situated tertiary septa that define the openings to the faveoli and are continued as the

Fig. 1. A scanning electron micrograph of the bronchial epithelium of the black mamba: ciliated cells (*c*), secretory cells covered by microvilli (*m*) and tracts of smooth squamous cells (*s*). Scale bars: 10 μm.

Fig. 2. A high power view of the epithelial cells of the bronchus: *s*, smooth squamous cells: *m*, secretory cells with microvilli, and ciliated cells (*c*). Scale bars: 1 μm.
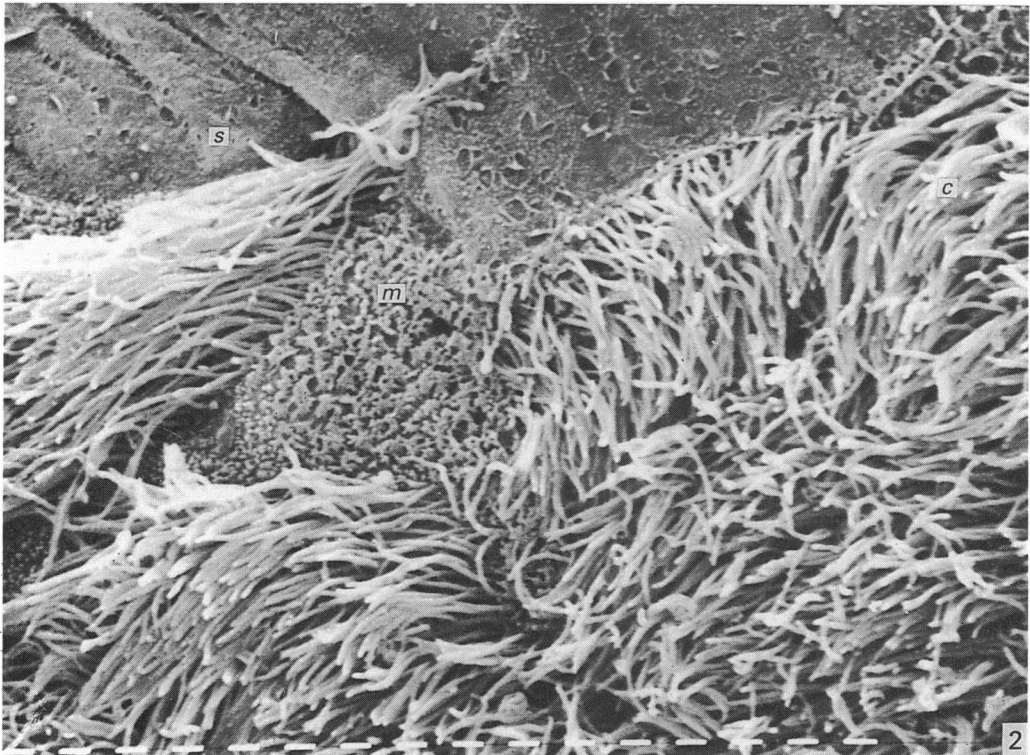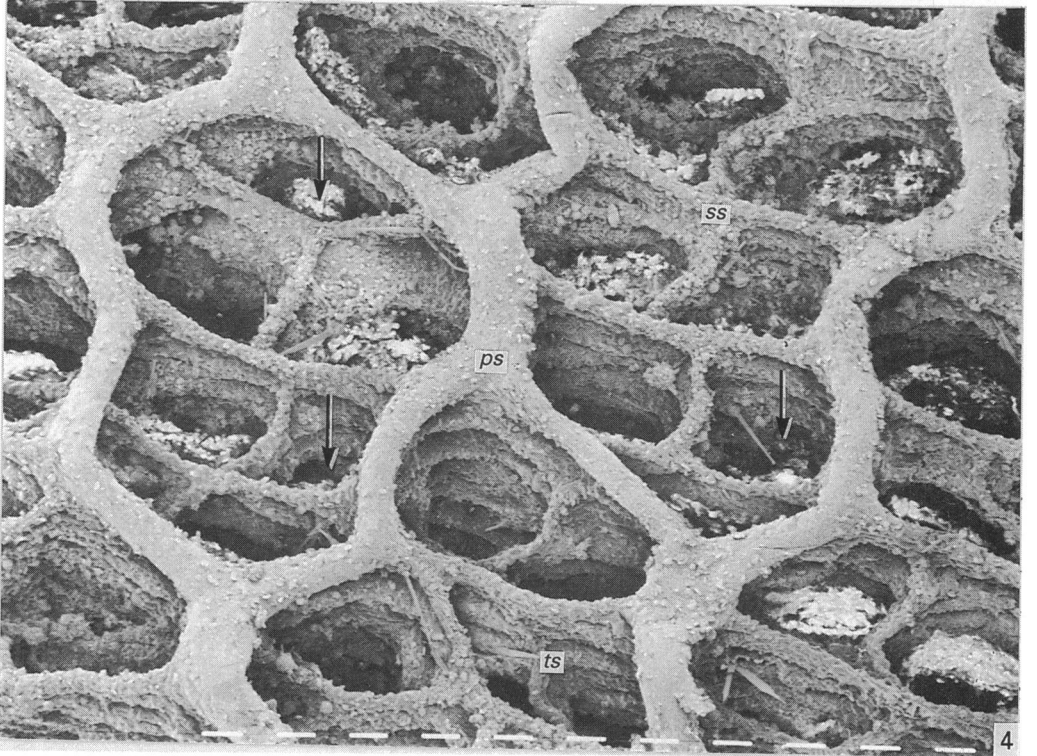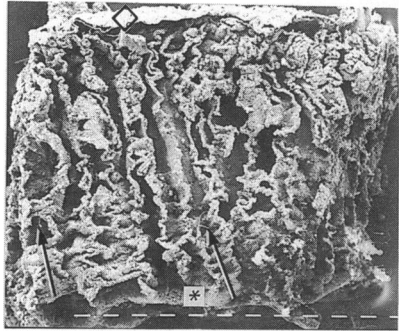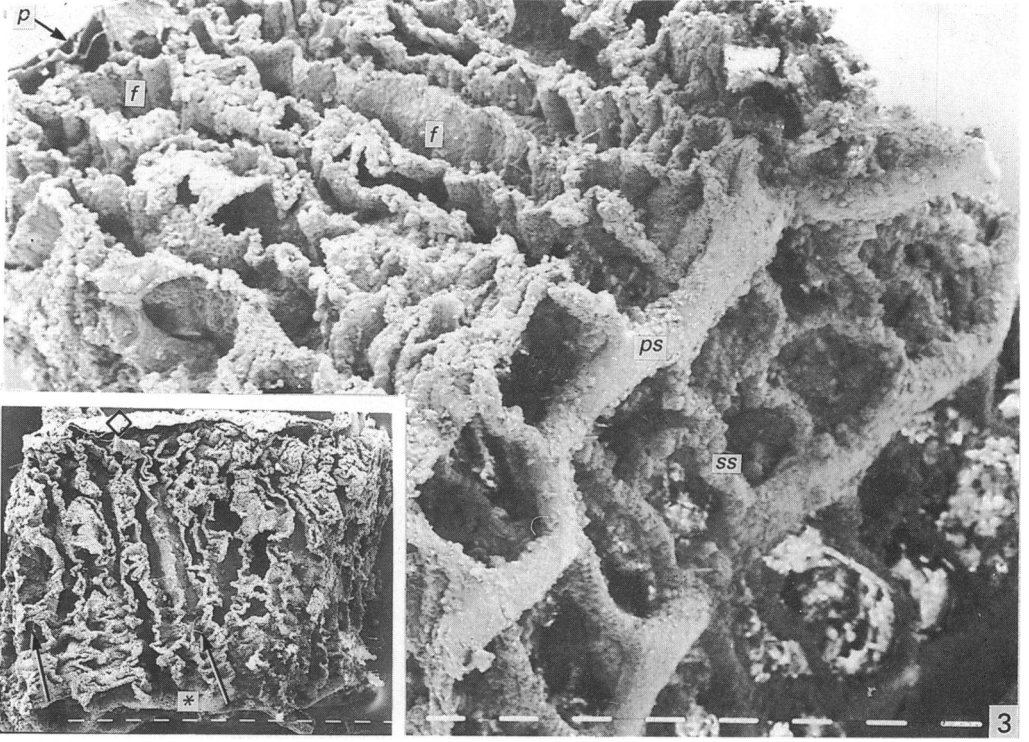
interfaveolar septa. These third-level septa are continued to the pleural surface of the lung as the capillary-bearing partitions, the interfaveolar septa (Figs. 3, 4, 5). The faveoli run parallel to each other maintaining a rather constant diameter. They are deepest at the rostral end of the lung and decrease caudally. The interfaveolar septum consists of profuse anastomosing blood capillaries located on both sides (Figs. 5, 6, 7) of the partitions. The capillaries are supported on a connective tissue band that consists mainly of fibrocytes, collagen and elastic tissue (Figs. 9, 11). They bulge into the lumen of the faveoli (Figs. 3, 8, 10). On the primary and secondary septa, the blood capillaries are exposed to air largely on one side, the septa being notably thick (Figs. 8, 9). They exhibit a double capillary system. At the faveolar level, the blood capillaries are to a varying extent exposed to air on both sides, although even here a thin and a thick side of the septum is clearly evident (Figs. 10, 11).

The blood capillaries are lined on average by two adjoining endothelial cells that exhibit sporadic attenuation of the cytoplasmic extensions (Fig. 11), but no fenestrations are evident. In most cases the endothelial cells overlap and are joined through junctional complexes (Figs. 10, 15). The nuclei of the endothelial cells bulge into the lumen of the blood capillaries and their cytoplasmic flanges contain numerous micropinocytotic vesicles (Figs. 12, 14). Overlying the endothelial cells on the immediate abluminal border are occasional pericytes (Fig. 11). The interstitium contains smooth muscle cells, collagen, fibrocytes and occasional tissue macrophages (Figs. 11, 14).

Two populations of pneumocytes are observed on the faveolar surface, the Type II cells, or granular pneumocytes, and the Type I cells, or squamous pneumocytes. The nuclei of both these cells are located in the intercapillary spaces (Figs. 6, 9, 10). The Type II cells display numerous microvilli, a nucleus situated at the base, osmiophilic lamellated bodies in the apical region of the cell and numerous mitochondria and Golgi complexes (Figs. 9, 12). The Type I cells have a large ovoid nucleus and extensive cytoplasmic arborisations covering most of the faveolar surface (Fig. 10). As in the Type II cells, these cytoplasmic extensions contain numerous micropinocytotic vesicles (Figs. 13, 14). Microvilli are absent from the surface of these cells. Free macrophages are not observed on the faveolar surface. Unmyelinated axons are occasionally observed in the interstitial space of the primary septa (Fig. 13). In such areas the epithelial cells send basal cytoplasmic extensions into the intercapillary spaces (Fig. 13). In the area where the blood capillaries are directly exposed to air, the blood–gas barrier is made up of the epithelial cell, a common basal lamina and the endothelial cell (Fig. 15). The thickness of the blood–gas barrier in such attenuated areas of about 0·55 μm is notably small.

DISCUSSION

The developmental and topographic variability of the organs in snakes is attributed to the restrictive tubular nature of the snake's body (Marcus, 1937; Klauber, 1956;

Fig. 3. Gas exchange tissue of the lung of the black mamba. The air duct is lined by the primary septa (*ps*) that give rise to the secondary septa (*ss*) which in turn give rise to the tertiary septa that form the openings into the faveoli (*f*). The faveoli run parallel to each other from the luminal aspect (asterisk) and the pleural aspect (diamond) of the lung boundaries across which the faveoli (arrows) traverse. *p*, pleura. Scale bars: 100 μm; inset: 100 μm.

Fig. 4. A view of the luminal aspect of the lung of the black mamba showing the primary septa (*ps*), the secondary septa (*ss*) and the tertiary septa (*ts*) that form the mouths of the faveoli (arrows). Scale bars: 100 μm.
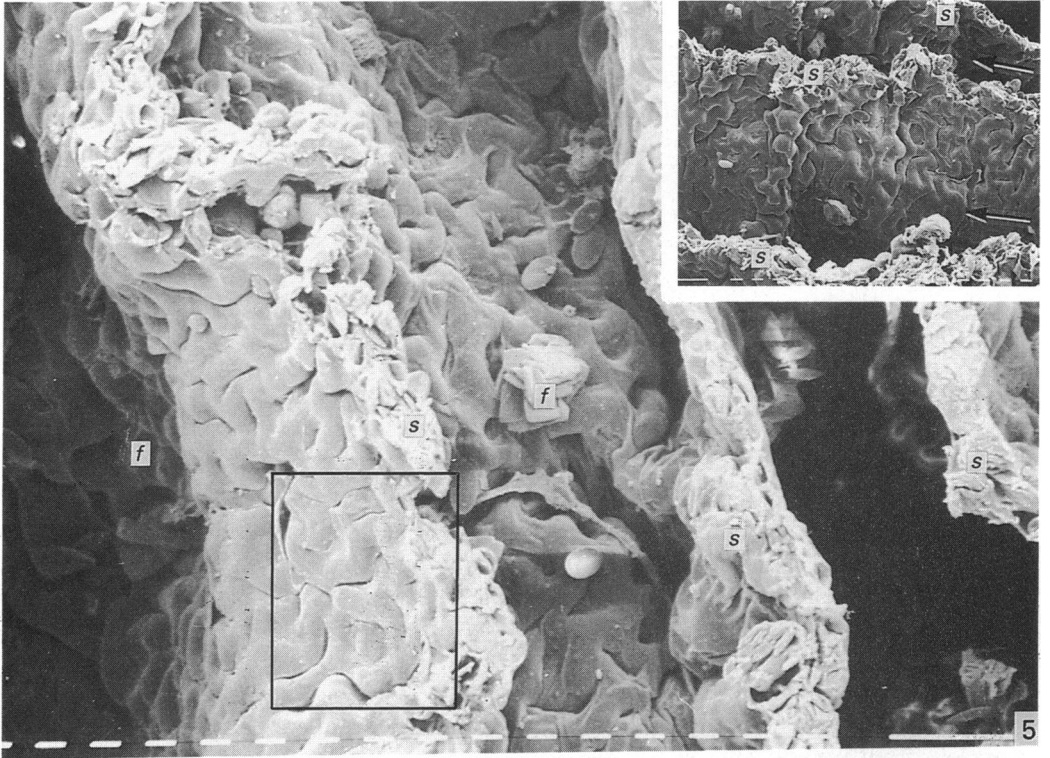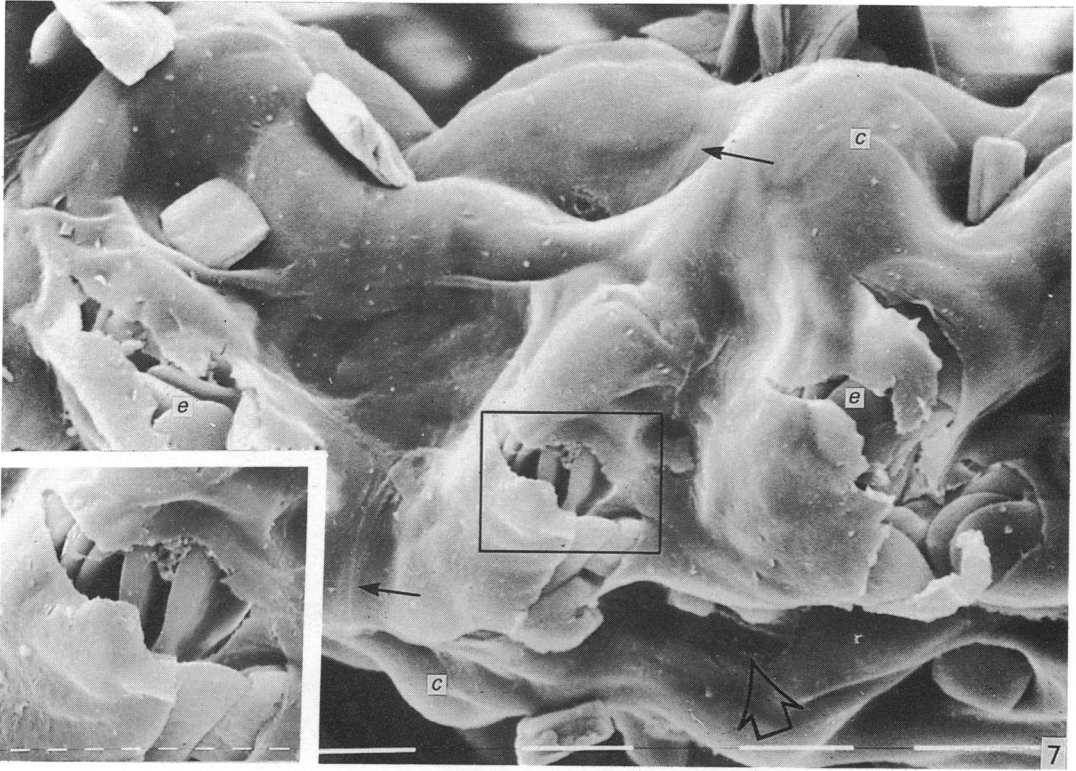
Fig. 7. A close up of blood capillaries (*c*) lining the interfaveolar septum. The red blood cells (*e*) in the capillaries are clearly visible. The small arrows show the intercellular junctions of the Type I cells and the open arrow a Type II cell. The enclosed area in the Figure is enlarged in the inset. Scale bars: 10 μm; inset: 2 μm.

Parker, 1977). This apparently also holds true in those amphibians like the caecilians that have thin, elongated bodies, notably with respect to the lung (Pattle, Schock, Creasey & Hughes, 1977; Maina & Maloiy, 1988). It is generally maintained that a great degree of pulmonary asymmetry indicates a high degree of deviation from the primitive condition (Underwood, 1976; Parker, 1977). For example, in general the snakes in the family Colubridae have developed left and right lungs, while in the Elapidae the left lung is only rudimentarily present, whereas in Viperidae (the most advanced snakes) the left lung has completely regressed (Thompson, 1913, 1914; Parker, 1977). It is plausible that, to compensate for the reduction or absence of the left lung, the right lung would, in most morphological aspects, be more elaborate in the higher and generally more agile snakes. This feature was established by Stinner (1982) who found that the morphometric characteristics of the lung of the colubrid snake *Pituophis melanoleucus* were comparable with those of other reptiles with a paired lung.

Fig. 5. A high power view of the faveoli (*f*). The faveoli are separated by interfaveolar septa (*s*). The faveoli are covered by a profuse anastomosing system of blood capillaries. The enclosed area is magnified in Figure 6. The inset is a side view of the faveoli (arrows). They are separated by the interfaveolar septa (*s*) that are continuations of the tertiary septa. Scale bars: 100 μm; inset: 10 μm.

Fig. 6. A high power view of the surface of the interfaveolar septum showing the anastomosing blood capillaries (*c*). The nuclei of the pneumocytes are located in intercapillary spaces. *g*, nucleus of a granular (Type II) pneumocyte; *s*, nucleus of a smooth (Type I) pneumocyte. Scale bar: 10 μm.
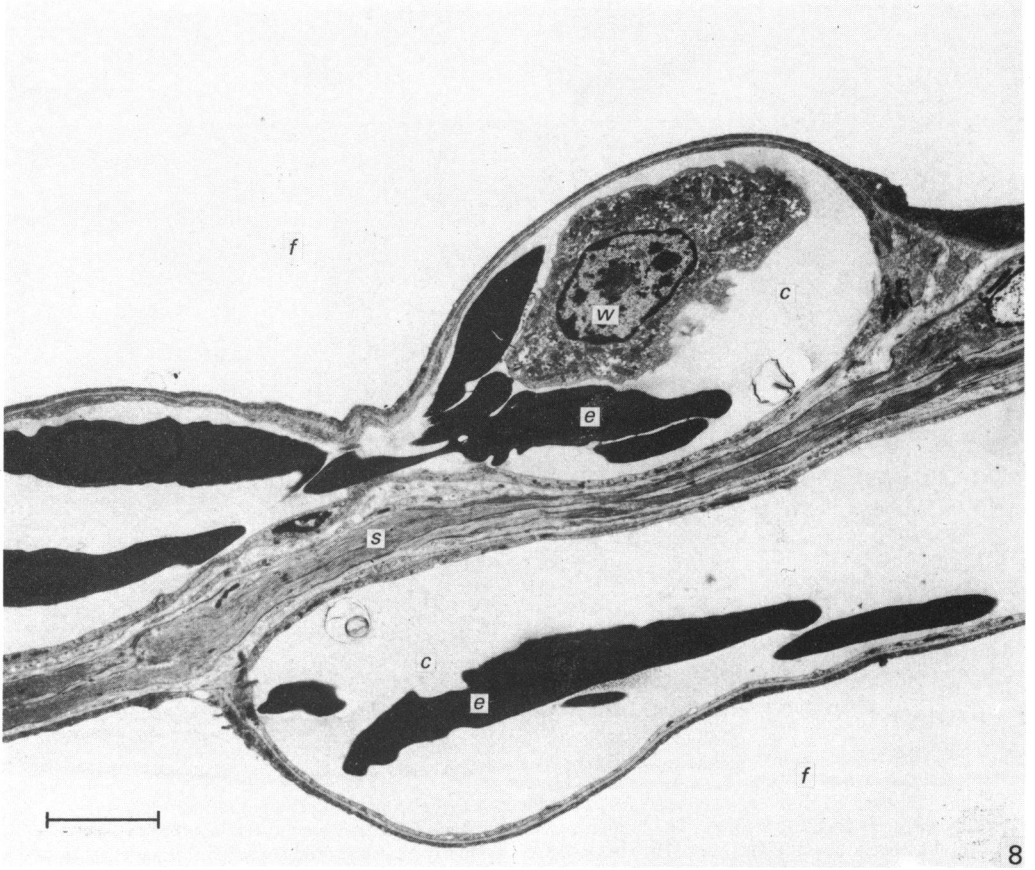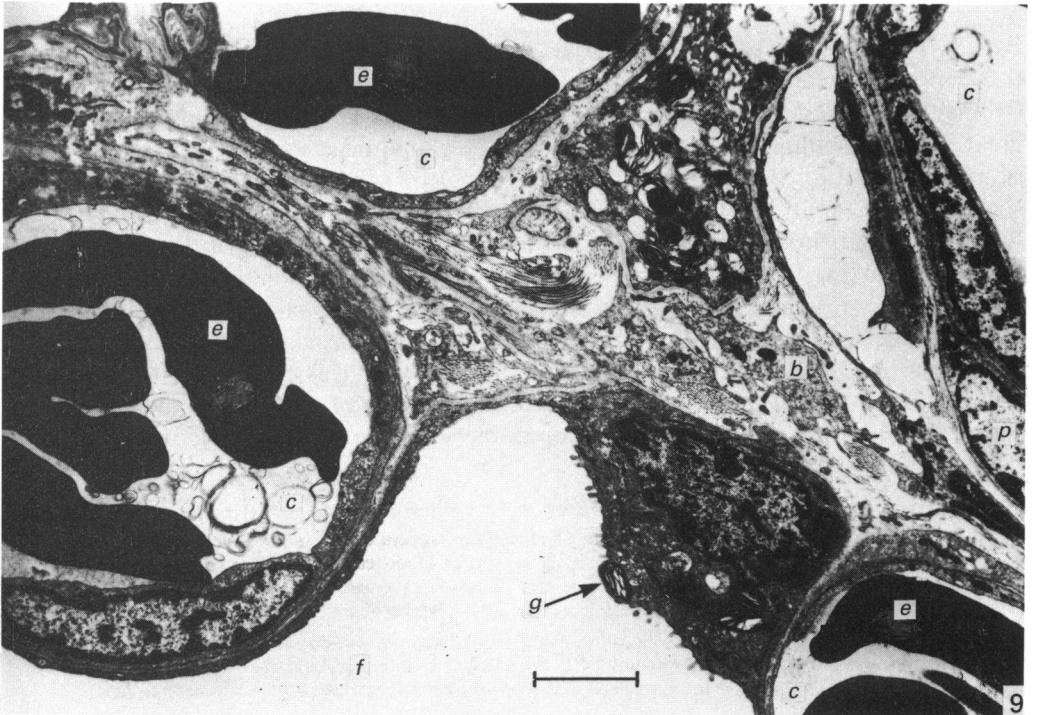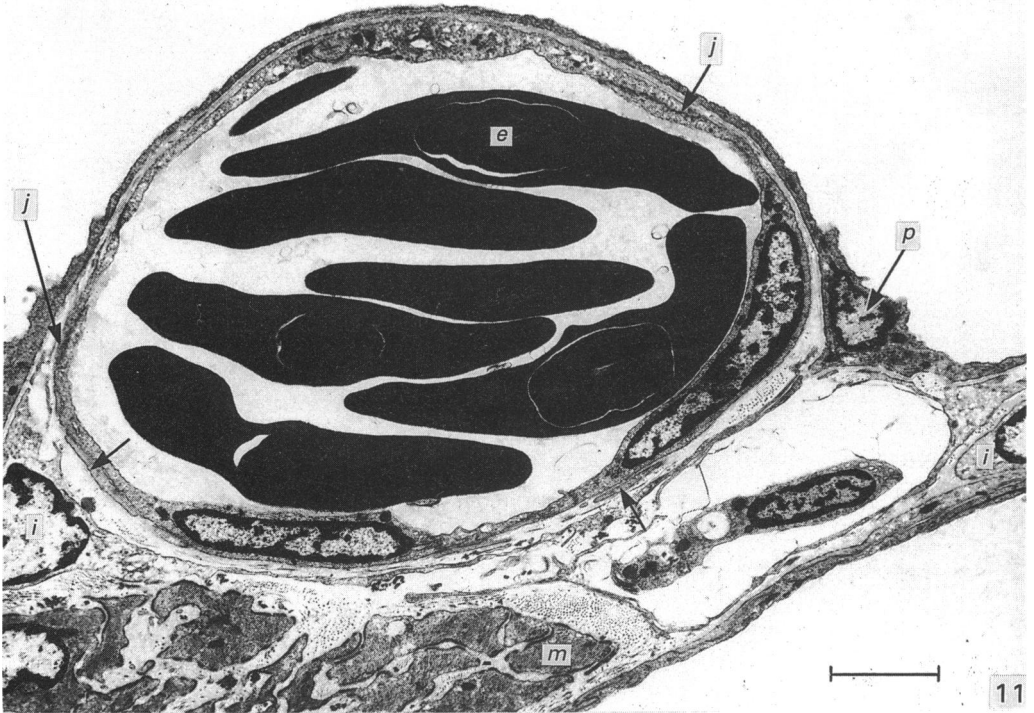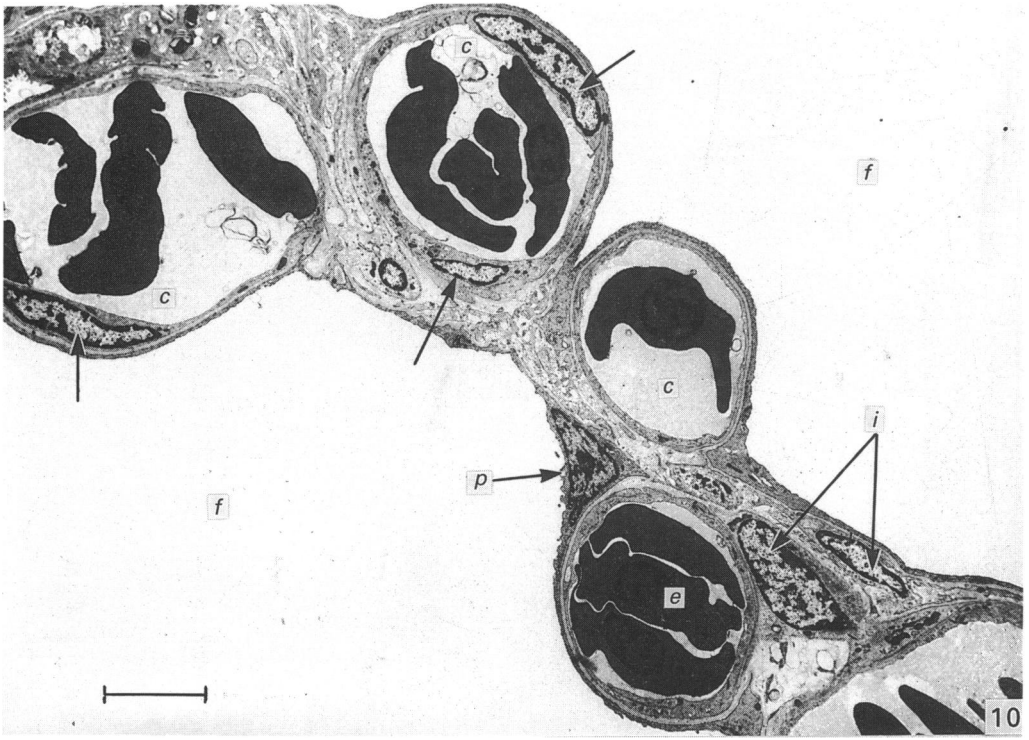
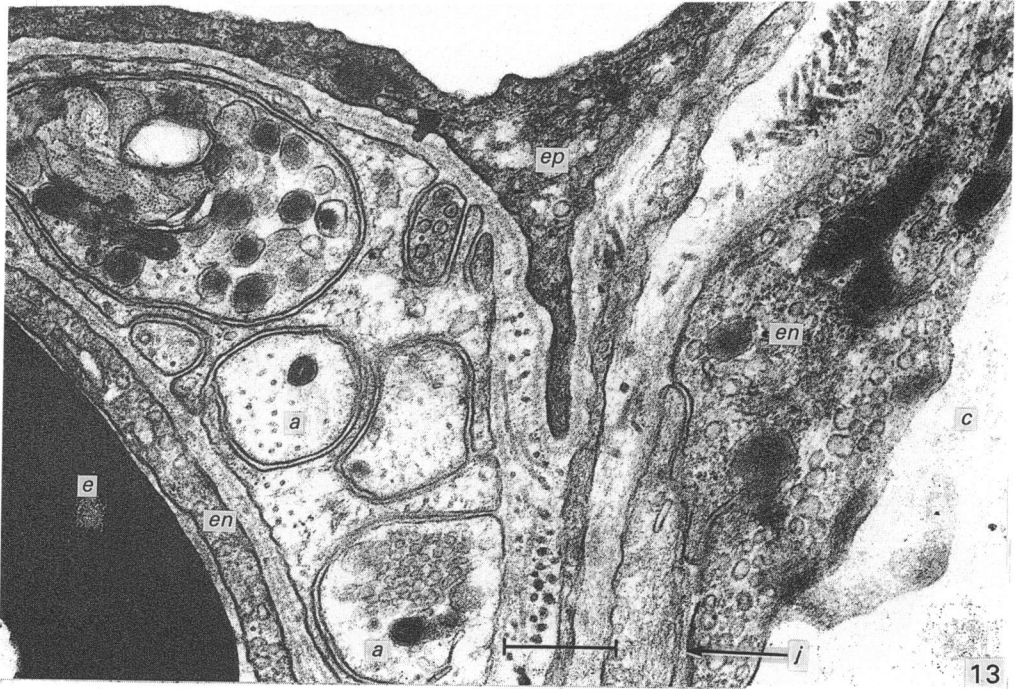Figs. 8–9. For legends see p. 41.

Figs. 10–11. For legends see p. 41.

The overall organisation of the lung of the black mamba was similar to that of other snakes. The lung could be divided into two major anatomical regions, an anterior part that was termed 'vascular region' by McDonald (1959), 'bronchial lung' by Klauber (1956) and 'tracheal lung' by Beddard (1903). On structural and functional grounds, we would prefer to call this area the 'faveolar gas exchange region,' a similar term to 'respiratory region' proposed by Luchtel & Kardong (1981). The posterior part of the ophidian lung has been referred to as 'air sac' by Brattstrom (1959) and Stinner (1982), 'avascular sac' by Cantor (1841) and 'mechanical zone' by Wolf (1933). The term 'air sac' was rather unfortunate as it is more appropriately used for the capacious and anatomically distinct air chambers that ventilate the avian lung. We concur with the descriptive term 'saccular region' used by Luchtel & Kardong (1981) to denote the posterior part of the ophidian lung.

The finer organisation of the faveolar region of the lung of the black mamba resembles that of other snakes like the rattlesnake (Luchtel & Kardong, 1981) and the garter snake (Pohunkova & Hughes, 1985) where similar investigations have been carried out. The exchange region is divided into the honeycomb-like units, the faveoli, by the hierarchically arranged stratified septa. This design greatly increases the surface area available for gas exchange over that which would be furnished by a simple saccular lung of similar size. In the only apparently available morphometric analysis of the ophidian lung, Stinner (1982) reported in *Pituophis melanoleucus* a total pulmonary air volume (in a 1 kg snake) of 97·5 ml, a respiratory surface area of 2000 cm^2, a surface density (surface area of the respiratory surface per unit volume of the lung) of 101 cm^2/cm^3, a harmonic mean thickness of the blood–gas barrier of 0.46×10^{-4} cm and an anatomical diffusion factor (ratio of the respiratory surface area to the diffusion distance) of 0.44×10^8 cm. The latter value which defines (in part) the lung's capacity for gas exchange was comparable to that reported in the red-eared turtle (*Pseudemys scripta* elegans) by Perry (1978). The thickness of the blood–gas barrier in the lung of the black mamba (0·55 μm) reported here is comparable to that estimated by Stinner (1982) in *Pituophis melanoleucus*. The surface density of the respiratory surface is a measure of the intensity of the compartmentation of the gas exchange region in a lung. The high value observed in *Pituophis* by Stinner (1982) of 101 cm^2/cm^3 far exceeds that of 18 cm^2/cm^3 estimated in the turtle by Perry (1978).

Fig. 8. Transmission electron micrograph of a secondary septum showing the blood capillaries (*c*) bulging on both sides of the septum (*s*) into the faveoli (*f*). Such capillaries are exposed to air only on one side, a design feature that has been termed 'double capillary system.' *e*, erythrocytes; *w*, white blood cell. Scale bar: 5 μm.

Fig. 9. High power view of the secondary septum showing the blood capillaries (*c*) containing the erythrocytes (*e*). Occasional granular pneumocytes (*g*) are found on the surface. The septum contains supportive tissue elements like fibrocytes (*b*). *p*, pericyte; *f*, faveolus. Scale bar: 2·5 μm.

Fig. 10. Interfaveolar septum from which the blood capillaries (*c*) protrude. At this level in the exchange tissue the capillaries are exposed to air on both sides but the thickening of the septum on one side is evident. The arrows indicate the endothelial cells. *e*, erythrocyte; *p*, smooth pneumocyte; *i*, interstitial cell; *f*. faveolus. Scale bar: 5 μm.

Fig. 11. A close up of a blood capillary containing red blood cells (*e*). The endothelial cells join at junctions (*j*). Smooth muscle cells (*m*) and interstitial cells (*i*) can be seen in the septum. *p*, Type I, smooth pneumocytes; the arrow shows the pericytic profiles. Scale bar: 2·5 μm.

Fig. 12. Type II pneumocyte. These cells have microvilli on their free surface and numerous intracytoplasmic organelles such as a Golgi apparatus (arrows), mitochondria (*m*) and rough and smooth endoplasmic reticulum. The characteristic organelle in this cell is the osmiophilic lamellated body (*o*). *c*, blood capillary; *e*, erythrocyte; *n*, nucleus. Scale bar: 0·5 μm.

Fig. 13. Interfaveolar septum showing unmyelinated axons (*a*), intercalated between the epithelial (*ep*) and endothelial (*en*) cells. *c*, blood capillary; *e*, erythrocyte; *j*, endothelial cell junction. Scale bar: 0·5 μm.
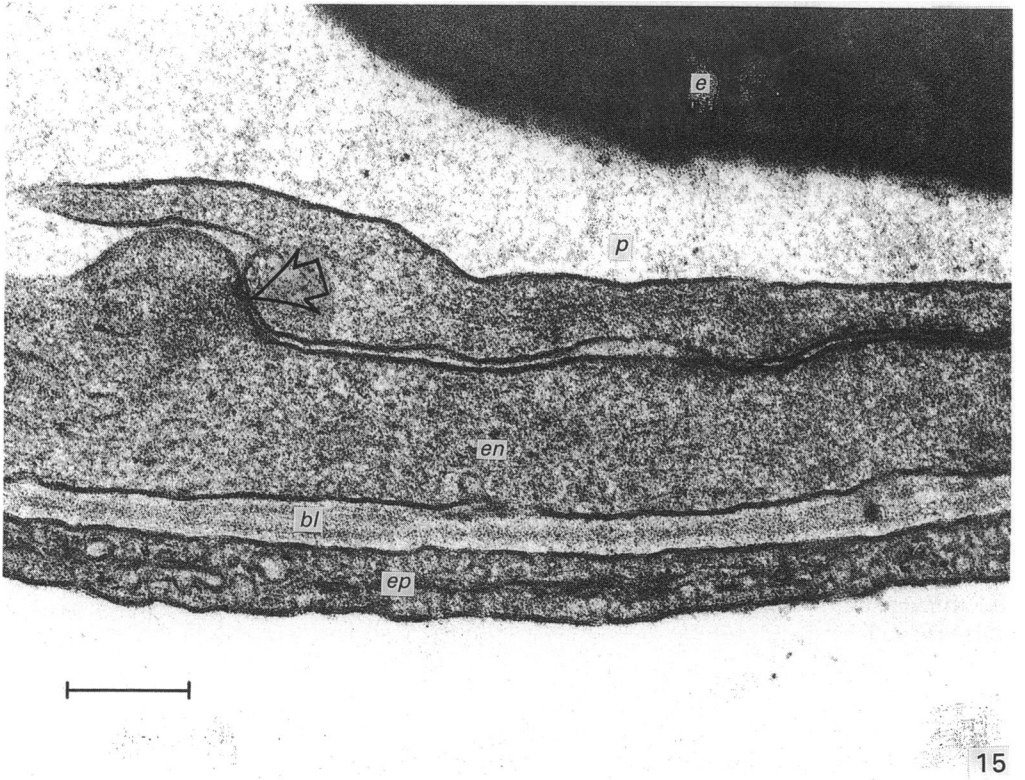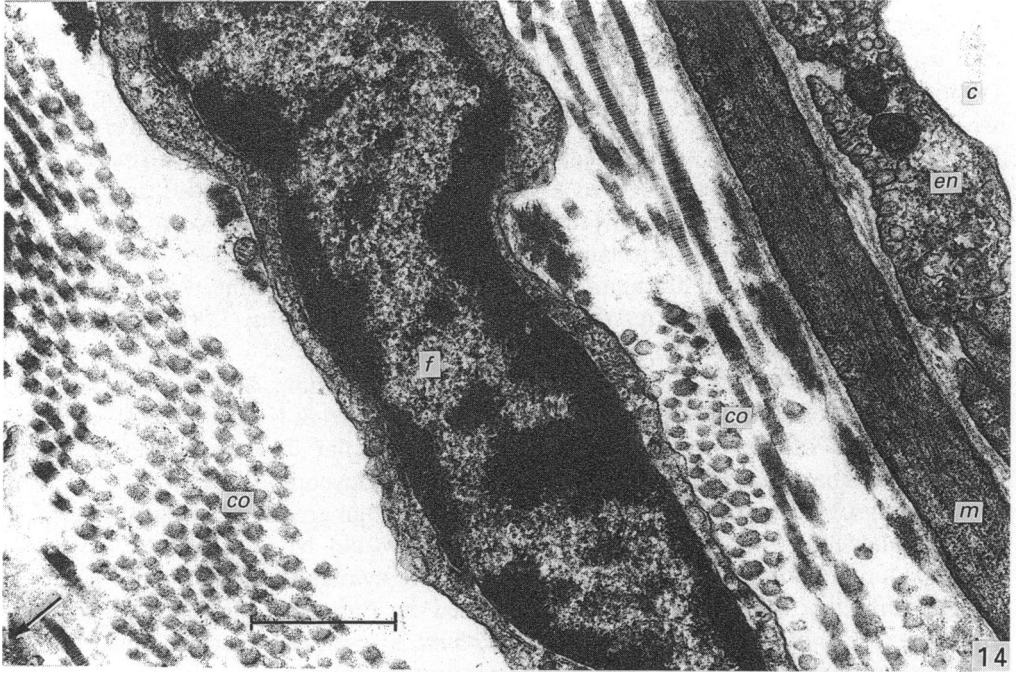
The evident increased compartmentation in the ophidian lung may be a compensation for both the absence of a functional left lung and for the conversion of the caudal part of the extant lung, which may comprise as much as three-quarters of the lung, into a saccular membranous, non-respiratory chamber. Differences in the anatomy of the ophidian lung are to be anticipated in view of the wide range of aerobic capacities exhibited by different snakes (Ruben, 1976) and the diverse non-respiratory functions of these lungs (Graham, Gee & Robinson, 1975). In spite of these pulmonary structural and functional disparities, the basal and active metabolic rates in many snakes are comparable with or surpass those of other reptiles at the same body temperature (Bennet & Dawson, 1976). This would strongly imply that the snake lung is at least equally efficient in gas exchange.

Different terms have been used to describe the gas exchange components of the reptilian lung: they have been inappropriately called 'alveoli' by Okada *et al.* (1962) and 'air sacs' by Meban (1978). Duncker (1978), on the basis of the internal architecture of the amniote lung, designated the ophidian lung 'unicameral' due to the fact that it consists of a single chamber. The less ambiguous term 'faveolus' was proposed to describe the gas exchange components in such lungs (Duncker, 1978).

The anatomical disposition between the blood and the air in the lung of the mamba appears to differ with the relative proximity to the central air duct. A double capillary system appears to occur proximal to the faveoli, the areas delineated by the primary and secondary septa, while a single capillary system largely prevails at the faveolar level. A single capillary system provides a larger surface area for gas exchange as the whole of the capillary surface participates in gas exchange. The notable bulging of the blood capillaries in the ophidian lung further increases the area of the respiratory surface (Hughes, 1978; Stinner, 1982). The double capillary system characterises the lungs of the primitive air breathers like the Dipnoi (Hughes & Weibel, 1976, 1978; Maina & Maloiy, 1985; Maina, 1987*a*) and amphibians (Goniakowska-Witalinska, 1978, 1986; Meban, 1977, 1980; Maina & Maloiy, 1988; Maina, 1989*a*) and is a transient feature during the initial stages of the development of mammalian lung (Burri, 1974; Burri, Dbaly & Weibel, 1974; Pinkerton *et al.* 1982). It would appear, in this respect, reasonable to suggest that the ophidian lung and, in general, the reptilian lung somehow manifest an intermediate developmental stage in the evolutionary hierarchy of the lung from the primitive to the more advanced air-breathing vertebrates. This conforms with the generally accepted status of the reptiles in the evolution of the extant vertebrates.

The pneumocytes in the lung of the black mamba are clearly morphologically well differentiated into the Type I and Type II cells. These cells are ultrastructurally similar to those described in the other snake lungs (Okada *et al.* 1962; Nagaishi *et al.* 1964; Brooks, 1970; Luchtel & Kardong, 1981; Pohunkova & Hughes, 1985) and, in general, other reptilian lungs (Meban, 1977, 1978; Klika, Tesik & Nedved, 1976; Klemm, Gatz, Westfall & Fedde, 1979; Perry, 1983). In the lungs of the lower vertebrates, like the lungfishes and amphibians, the pneumocytes are not well differentiated (Meban, 1973, 1977; Goniakowska-Witalinska, 1978, 1985, 1986; Maina & Maloiy, 1985; Maina 1987*a*). On the surface of the lung of the pneumonate

Fig. 14. Interstitial septum, the space between the blood capillary (*c*) and the epithelium (arrow): *en*, endothelial cell; *m*, smooth muscle; *co*, collagen fibres; *f*, fibrocyte. These structural components may be important for support and mechanical ventilation of the lung. Scale bar: 1 μm.

Fig. 15. Blood–gas barrier which, in the thinnest parts, is made up of the epithelial cell (*ep*), a common basal lamina (*bl*) and endothelium (*en*). The arrow shows an intercellular junction. *p*, plasma layer; *e*, erythrocyte. Scale bar: 0·3 μm.

gastropod *Trichotoxon copleyi*, only a single population of microvilli bearing squamous cells has been observed (Maina, 1989b). In this respect the reptilian lung shows cytoarchitectural features of the pneumocytes comparable with those of other amniotes. A complete differentiation of the alveolar pneumocytes may decrease the cellular density in the gas exchange region of the lung concomitantly reducing, even though to an unknown extent, the oxygen consumption by the pulmonary tissue itself (Maina, 1987b).

The concentration of the respiratory tissue in the anterior part of the ophidian lung may facilitate gas exchange during both inspiration and expiration as, in a bellows-like action, the posterior saccular part ventilates the faveoli. However, the overall efficiency of these lungs in gas exchange is limited by the notable inequalities of ventilation and perfusion that prevail in these extremely elongated lungs (Stinner, 1982). The morphology of the respiratory tissue in the snake lung is somewhat similar to that of the lizards (Perry & Duncker, 1978; Klemm *et al.* 1979; Perry, 1983; Hlastala, Standaert, Pierson & Luchtel, 1985). This may be accounted for by the fact that snakes presumably evolved from burrowing or aquatic lizard-like ancestors, the varanid or lanthanotid lizards being the most probable candidates (Estes, 1983). The ophidian lungs, indeed those of the reptiles in general, would be expected to be more elaborate than the lungs of the extant amphibians, as the reptiles rely wholly on the lungs for gas exchange, the surface of the body being covered by scales. These functional demands appear to have been structurally satisfied in the lungs of the black mamba examined here as the lung is profusely vascularised and hence has an extensive respiratory surface area and the blood–gas barrier in some areas was extremely thin, factors that enhance the diffusing capacity of a lung.

SUMMARY

The lung of a snake, the black mamba (*Dendroaspis polylepis*), has been investigated by scanning and transmission electron microscopy. This species has only one lung, the right, which is long and occupies most of the pleuro-peritoneal cavity. Grossly, the lung could be divided into two discrete anatomical regions: an anterior respiratory area made up of a honeycomb network of capillary-bearing partitions, and a posterior membranous saccular region. The exchange region consisted of a central air duct, the bronchus, which was delineated both dorsally and laterally by morphologically and spatially distinct hierarchically arranged septa. The primary septa gave rise to the secondary septa from which the much deeper peripherally situated tertiary septa that formed the immediate openings to the faveoli arose. The faveoli were rather parallel elongated pockets separated by partitions, the interfaveolar septa, and terminated peripherally on the pleura. A double capillary disposition of the blood capillaries was observed on the relatively thick primary and secondary septa. These septa were lined by a heterogenous epithelium made up of ciliated cells, secretory cells, and smooth squamous cells. This epithelium was continued from the trachea and the bronchus. At the faveolar level the blood capillaries exhibited a single system where they formed a matrix on both sides of the partitions. The surface of the faveoli was covered by two types of cells: Type I cells were squamous and their remarkably attenuated cytoplasmic arborisations were notably extensive while the Type II cells were rather cuboidal, bore stubby microvilli and contained the characteristic osmiophilic lamellated bodies. On the basis of the clearly evident complete differentiation of the pneumocytes and the presence of both the double and single capillary systems, it was observed that this lung, and apparently the reptilian lung in general, manifests a transitional developmental

and structural stage in the evolution of the lungs of the air-breathing vertebrates from lower through to higher vertebrates. The gross and ultrastructural heterogeneity of the organisation of the ophidian lung is illustrated and the dearth of pulmonary morphological data in this taxon is pointed out.

REFERENCES

BEDDARD, F. E. (1903). On the trachea, lungs, and other points in the anatomy of the Hamadryad snake (*Ophiophagus bungarus*). *Proceedings of the Zoological Society of London*, 319–328.

BENNET, A. F. & DAWSON, W. R. (1976). Metabolism. In *Biology of Reptiles* (ed. C. Gans & W. R. Dawson), pp. 127–223. New York: Academic Press.

BRATTSTROM, B. H. (1959). The function of the air sac in snakes. *Herpetologica* 15, 103–104.

BRONGERSMA, L. D. (1949). On the main branches of the pulmonary artery in some Viperidae. *Bijdragen tot de dierkunde* 28, 57–64.

BRONGERSMA, L. D. (1951). Some remarks on the pulmonary artery in snakes with two lungs. *Zoologische verhandelingen* 14, 13–36.

BROOKS, R. E. (1970). Lung alveolar cytosomes: a consideration of their significance. *Zeitschrift für Zellforschung und mikroskopische Anatomie* 106, 484–497.

BURRI, P. H. (1974). The post-natal growth of the rat lung. III. Morphology. *Anatomical Record* 180, 77–98.

BURRI, P. H., DBALY, J. & WEIBEL, E. R. (1974). The post-natal growth of the rat lung. I. Morphometry. *Anatomical Record* 178, 711–730.

BUTLER, G. W. (1895). On complete or partial suppression of the right lung in Amphisbaenidae and of the left lung in snakes and snake-like lizards and amphibians. *Proceedings of the Zoological Society of London*, 691–712.

CANTOR, T. (1841). Observations upon pelagic serpents. *Transactions of the Zoological Society of London* 2, 303–313.

COPE, E. D. (1894). On the lungs of the Ophidia. *American Philosophical Society* 33, 217–224.

COPE, E. D. (1900). Crocodilians, lizards and snakes of North America. *Annual Report of the Smithsonian Institute* (1898), pp. 153–1270. Washington, DC: U.S. National Museum.

DUNCKER, H. R. (1978). General morphological principles of amniotic lungs. In *Respiratory Function in Birds, Adult and Embryonic* (ed. J. Piiper), pp. 2–15. Berlin: Springer-Verlag.

ESTES, R. (1983). The fossil record and early distribution of lizards. In *Advances in Herpetology and Evolutionary Biology* (ed. A. Rhodin & K. Miyata), pp. 365–398. Cambridge, MA: Harvard University Press.

GEORGE, J. C. & SHAH, R. V. (1956). Comparative morphology of the lung in snakes with remarks on the evolution of the lung in reptiles. *Journal of Animal Morphology and Physiology* 3, 1–7.

GOIN, C. J. & GOIN, O. B. (1962). *Introduction to Herpetology*. San Francisco: W. H. Freeman.

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

GONIAKOWSKA-WITALINSKA, L. (1985). The structure of the lung of the tree frog (*Hyla arborea* L.). In *Vertebrate Morphology* (ed. H. R. Duncker & G. Fleischer), pp. 375–377. Stuttgart, New York, Giessen: Gustav Fischer Verlag.

GONIAKOWSKA-WITALINSKA, L. (1986). Lung of the tree frog *Hyla arborea* L. A scanning and transmission electron microscope study. *Anatomy and Embryology* 174, 379–389.

GRAHAM, J. B., GEE, J. H. & ROBINSON, F. S. (1975). Hydrostatic and gas exchange functions of the lung in the sea snake *Pelamis platurus*. *Comparative Biochemistry and Physiology* 50A, 477–482.

HLASTALA, M. D., STANDAERT, T. A., PIERSON, D. J. & LUCHTEL, D. L. (1985). The matching of ventilation and perfusion in the lung of the tegu lizard, *Tupinambis nigropunctus*. *Respiration Physiology* 60, 277–294.

HUGHES, G. M. (1978). A morphological and ultrastructural comparison of some vertebrate lungs. In *Congressus Morphologicus Symposia* (ed. E. Klika), pp. 393–405. Prague: Charles University Press.

HUGHES, G. M. & WEIBEL, E. R. (1976). Morphometry of fish lungs. In *Respiration of Amphibious Vertebrates* (ed. G. M. Hughes), pp. 213–232. London: Academic Press.

HUGHES, G. M. & WEIBEL, E. R. (1978). Visualization of layers lining the lung of the South American lungfish (*Lepidosiren paradoxa*), a comparison with the frog and rat. *Tissue and Cell* 10, 343–353.

KLAUBER, L. M. (1956). *Rattlesnakes*. Berkeley: University of California Press.

KLEMM, R. D., GATZ, R. N., WESTFALL, J. A. & FEDDE, N. R. (1979). Microanatomy of the lung parenchyma of a tegu lizard, *Tupinambis nigropunctus*. *Journal of Morphology* 161, 257–280.

KLIKA, E., TESIK, I. & NEDVED, J. (1976). Ultrastructure of the air–blood barrier in the great house gecko or tokay (*Gekko gecko*). *Folia morphologica* 24, 29–34.

LUCHTEL, D. L. & KARDONG, K. (1981). Ultrastructure of the lung of the rattlesnake, *Crotalus viridis oreganus*. *Journal of Morphology* **169**, 29–47.

McDONALD, H. S. (1959). Respiratory functions of the ophidian air sac. *Herpetologia* **15**, 193–216.

MAINA, J. N. (1987*a*). The morphology of the lung of the African lungfish, *Protopterus aethiopicus*. *Cell and Tissue Research* **250**, 191–196.

MAINA, J. N. (1987*b*). The morphology and morphometry of the adult normal baboon lung (*Papio anubis*). *Journal of Anatomy* **150**, 229–245.

MAINA, J. N. (1989*a*). The morphology of the lung of the East African tree frog *Chiromantis petersi* with observations on the skin and the buccal cavity as secondary gas exchange organs. A TEM and SEM study. *Journal of Anatomy* **165**, 29–43.

MAINA, J. N. (1989*b*). The morphology of the lung of a tropical terrestrial slug *Trichotoxon copleyi* (Mollusca: Gastropoda: Pulmonata): a scanning and transmission electron microscopic study. *Journal of Zoology* **217** (3).

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

MAINA, J. N. & MALOIY, G. M. O. (1988). A scanning and transmission electron microscopic study of the lung of a caecilian *Boulengerula taitanus*. *Journal of Zoology* **215**, 739–751.

MARCUS, H. (1937). Lungen. In *Handbuch der vergleichenden Anatomie der Wirbeltiere*. III (ed. L. Bolk, E. Goppert, E. Kallius & W. Lubosch), pp. 909–988. Berlin–Wien: Urban and Schwarzenberg.

MEBAN, C. (1973). The pneumocytes in the lung of *Xenopus laevis*. *Journal of Anatomy* **114**, 234–244.

MEBAN, C. (1977). Ultrastructure of the respiratory epithelium in the lungs of the newt *Triturus cristatus*. *Acta zoologica* **58**, 151–156.

MEBAN, C. (1978). Functional anatomy of the lungs of the green lizard *Lacerta viridis*. *Journal of Anatomy* **125**, 421–431.

MEBAN, C. (1980). Thickness of the air–blood barriers in vertebrate lungs. *Journal of Anatomy* **131**, 299–307.

NAGAISHI, C., OKADA, Y., ISHIKA, S. & DAIDO, S. (1964). Electron microscopic observations of the pulmonary alveoli. *Experimental Medicine and Surgery* **22**, 81–118.

OKADA, Y., ISHIKA, S., DAIDO, S., KIM, J. & IKEDA, S. (1962). Comparative morphology of the lung with special reference to the alveolar epithelial cells. II. Lung of the Reptilia. *Acta tuberculosea japonica* **12**, 1–10.

PARKER, H. W. (1977). *Snakes: A Natural History*, 2nd ed. Ithaca: Cornell University Press.

PATTLE, R. E., SCHOCK, C., CREASEY, J. M. & HUGHES, G. M. (1977). Surpellic films, lung surfactant, and their cellular origin in newt, caecilian, and frog. *Journal of Zoology* **182**, 125–136.

PERRY, S. F. (1978). Quantitative anatomy of the lungs of the red-eared turtle, *Pseudemys scripta elegans*. *Respiration Physiology* **35**, 245–262.

PERRY, S. F. (1983). Reptilian lungs: functional anatomy and evolution. *Advances in Anatomy, Embryology and Cell Biology* **79**, 1–81.

PERRY, S. F. & DUNCKER, H. R. (1978). Lung architecture, volume and static mechanics in five species of lizards. *Respiration Physiology* **34**, 61–81.

PINKERTON, K. E., BARRY, B. E., O'NEIL, J. J., RAUB, J. A., PRATT, P. C. & CRAPO, J. D. (1982). Morphologic changes in the lung during the lifespan of Fisher 344 rats. *American Journal of Anatomy* **166**, 155–174.

POHUNKOVA, H. & HUGHES, G. M. (1985). Ultrastructure of the lungs of the garter snake. *Folia morphologica* **XXXIII**, 254–258.

PORTER, K. R. (1972). *Herpetology*. London, Philadelphia: W. B. Saunders.

RUBEN, J. A. (1976). Aerobic and anaerobic metabolism during activity in snakes. *Journal of Comparative Physiology* **109**, 147–157.

SKINNER, H. A. (1973). *Snakes and Us: An Introduction to East African Herpetology*. Nairobi, Dar-es-Salaam: East African Literature Bureau.

STINNER, J. N. (1982). Functional anatomy of the lung of the snake *Pituophis melanoleucus*. *American Journal of Physiology* **243**, R251–R257.

THOMPSON, J. C. (1913). Contributions to the anatomy of the Ophidia. *Proceedings of the Zoological Society of London*, 414–425.

THOMPSON, J. C. (1914). Further contributions to the anatomy of the Ophidia. *Proceedings of the Zoological Society of London*, 379–402.

UNDERWOOD, G. (1967). *A Contribution to the Classification of Snakes*. Publication number 653. London: Trustees of the British Museum of Natural History.

UNDERWOOD, G. (1976). A systematic analysis of boid snakes. In *Morphology and Biology of Reptiles* (ed. A. d'A. Bellairs & C. B. Cox), pp. 151–175. London: Academic Press.

VARDE, M. R. (1951). Morphology and histology of the lung in snakes. *Journal of the University of Bombay* **19**, 79–89.

WEBB, J. E., WALLWORK, J. A. & ELGOOD, J. M. (1978). *Guide to Living Reptiles*, 2nd ed. London: The Macmillan Press.

WOOD, G. L. (1980). *Guinness Book of Animal Facts and Feats*, 2nd ed. London: Guinness Superlatives Ltd.

WOLF. S. (1933). Zur Kenntnis von Bau und Funktion der Reptilienlungen. *Zoologische Jahrbücher (Anatomie)* **57**, 139–190.