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Functional anatomy of the lungs of the green lizard, *Lacerta viridis*

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INTRODUCTION

The mammalian pulmonary alveolus has been studied extensively by electron microscopy and numerous descriptions of its morphological elements are now available (for recent reviews, see Weibel, 1973; Kilburn, 1974). Rather surprisingly, the microstructure of the gas-exchange areas of the lower vertebrates has received little attention. For example, only a few brief reports have been published on the ultrastructure of reptilian lungs (Okada *et al.* 1962; Nagaishi *et al.* 1964).

It was therefore decided to study the lungs of a reptile, the green lizard (*Lacerta viridis*) by light microscopy and electron microscopy. The lung structure of this animal is compared with that of mammals and the functional significance of the various features is discussed.

MATERIALS AND METHODS

Adult lizards (*Lacerta viridis*) were obtained from a commercial source and maintained in the laboratory for at least 7 days before use. Batches of animals showing evidence of parasitic infection were discarded. The lizards were decapitated and small blocks of lung tissue were excised and fixed for 3 hours at 4 °C in either 3% glutaraldehyde in 0·1 M phosphate buffer (pH 7·3) or an osmium tetroxide–glutaraldehyde mixture (Hirsch & Fedorko, 1968). The blocks were then washed for 18 hours in cacodylate-HCl buffer (pH 7·3) containing 0·25 M sucrose, post-fixed for 1 hour in 2% osmium tetroxide solution, rapidly dehydrated in absolute ethanol and embedded in Durcupan or Aquon (Gibbons, 1959). Thin sections were cut on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined in an AEI 801 electron microscope.

The remainder of each lung was fixed in 4% formaldehyde solution, dehydrated, cleared and embedded in paraffin wax. Sections were cut at 10 μm and stained with either haematoxylin and eosin or resorcin and fuchsin.

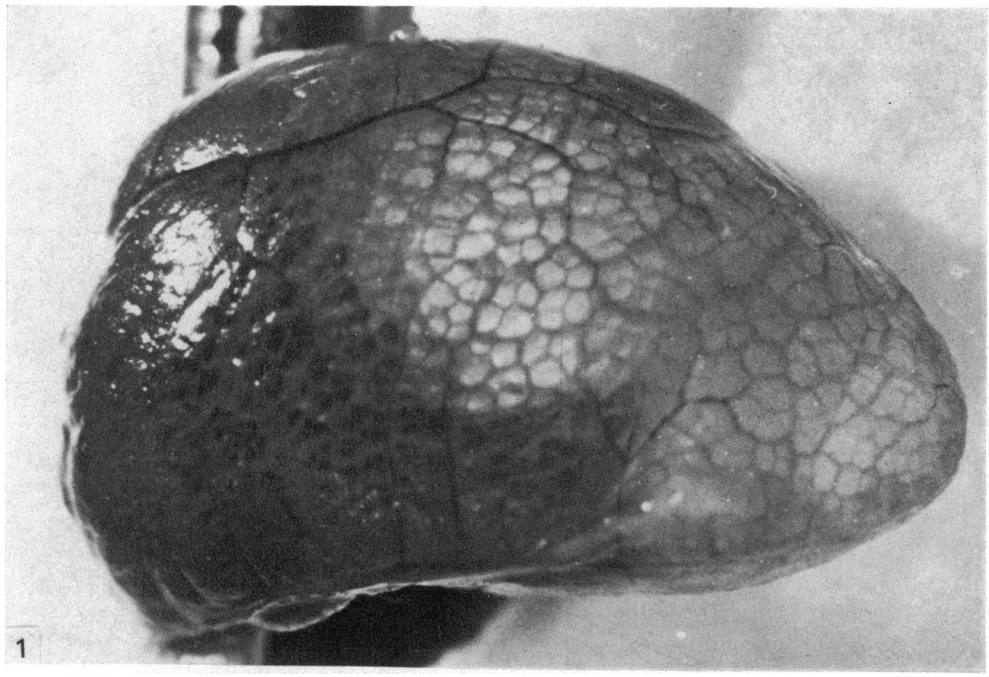
Some lungs were also distended with aldehyde fixative and then photographed intact or after partial dissection.

OBSERVATIONS

General topography

The lungs of *Lacerta viridis* are spindle-shaped sacs, approximately 4 cm long when fully expanded. Each bronchus enters its lung on the medial side about 1 cm from the apex. Once inside the lung, the bronchus opens directly into the axial air channel without further branching.

The outer wall of the lung is thin and the internal septa can be seen through it



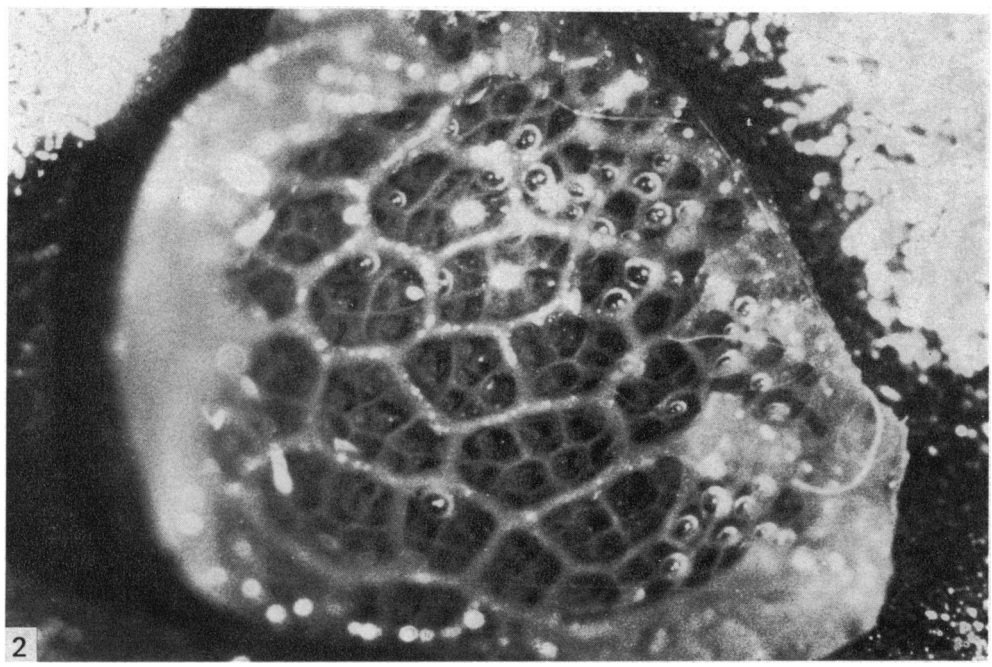


Fig. 1. External surface of a lung from *Lacerta*. The lines of attachment of the septa are visible through the thin lung wall. ×9.

Fig. 2. View of the internal surface of the lung showing the arrangement of the septa. ×11.

(Fig. 1). The major external longitudinal arteries are also conspicuous in fresh specimens.

The interior of the lung contains numerous thin septa. These are attached peripherally to the lung wall; centrally, their free ends are bulbous and serve to delineate the margins of the axial air channel. When a lung is opened longitudinally and the interior examined through a dissecting microscope, it becomes obvious that the septa can be classified into three groups on the basis of their length (Fig. 2). The longest septa divide the internal surface of the lung into polygonal recesses. The intermediate septa subdivide these recesses into smaller areas, each of which is further subdivided by the shortest septa into shallow depressions (about 250–400 μm in diameter). These three orders of air spaces are referred to in this study as 'air sacs'.

The cores of the septa consist of a loose mat of collagenous and elastic tissue together with lesser amounts of smooth muscle fibres. On each face the septa bear a close-meshed plexus of capillaries. The epithelium overlying these capillaries is attenuated and cannot be resolved satisfactorily with the light microscope.

Electron microscopy

The septa in the lung of *Lacerta viridis* are covered by a continuous layer of epithelium which consists of two distinct types of pneumonocytes.

The type I pneumonocyte is typically squamous with a thick central region which contains the nucleus and thinner peripheral sheets of cytoplasm (Figs. 3, 4). The nucleus is ovoid in shape and shows numerous indentations of its membrane. A profile of a nucleolus is usually present (Fig. 4). Most of the organelles are concentrated in the perinuclear region of the cytoplasm. These consist mainly of short tubular mitochondria, a small Golgi complex and elements of smooth-surfaced endoplasmic reticulum. In contrast to the central region of the cell, the cytoplasmic sheets are attenuated and contain very few organelles (Figs. 5, 8). At its extremity, each sheet is united to an adjacent type I or type II pneumonocyte by a tight junction (zonula occludens). The intercellular gaps deep to these junctions often display a Z-shaped profile in micrographs (Fig. 5).

The type II pneumonocyte is roughly cuboidal in form (Fig. 6). Its nucleus is centrally placed and has an irregular outline. Pores are frequently observed in the nuclear envelope. The cytoplasm is closely packed with organelles (Figs. 3, 5). Mitochondria are particularly numerous and take the form of short tubules with transverse cristae. A Golgi complex, consisting of 4–6 stacks of flattened cisternae and associated small vesicles, is usually located near the nucleus. Profiles of agranular endoplasmic reticulum are common but the granular variety is scarce. Multivesicular bodies are numerous, particularly in the vicinity of the Golgi complex.The inclusions are probably the most conspicuous group of organelles. These bodies tend to be concentrated in the apical portion of the cytoplasm but they also occur either singly or in small groups in the infranuclear region (Fig. 6). Each body has a rounded profile and is limited by a membrane about 10 nm in thickness. The inclusion contents are invariably heavily stained with uranyl and lead salts and appear either as amorphous masses or as irregular tangles of membranous material. The latter appearance is particularly common in tissue fixed in a mixture of osmium tetroxide and glutaraldehyde (Fig. 7). In most cells a proportion of the inclusion bodies shows vacuolation of their contents. The apical surface of each type II pneumonocyte bears short blunt microvilli (Fig. 6). These microvilli do not appear to have an organized

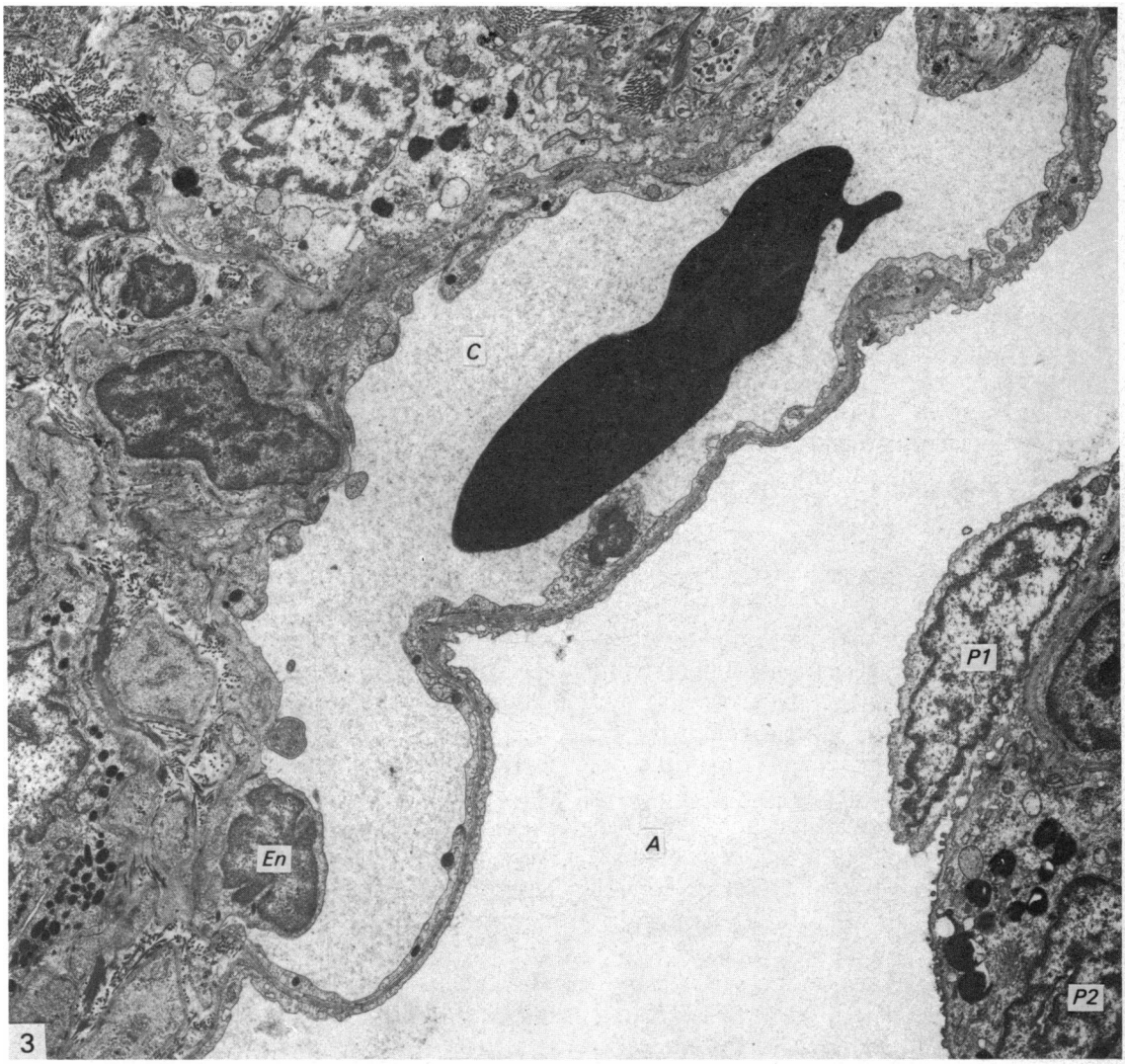


Fig. 3. Low power electron micrograph showing the edges of two septa. *A*, lumen of air sac; *C*, lumen of pulmonary capillary; *En*, nucleus of capillary endothelial cell; *P1*, nucleus of a type I pneumonocyte; *P2*, nucleus of a type II pneumonocyte. × 6500.

internal structure. Externally, however, their plasma membranes are covered by a thin layer of filamentous material.

The pulmonary capillaries lie underneath the epithelial layer and usually cause it to bulge out into the lumina of the air sacs (Figs. 3, 4). The capillaries consist of an endothelial layer which is surrounded in many places by the cytoplasmic processes of pericytes; in turn, both the endothelial cells and the pericytes are invested by a thick basal lamina. The capillary endothelium lacks pores or fenestrations but becomes extremely attenuated in regions where it forms the inner layer of the air–blood barrier (Fig. 8). Numerous caveolae are present on both the luminal and outer membranes of the endothelial cells and micropinocytotic vesicles abound within the intervening cytoplasm. In addition, densely stained bodies are often observed in the

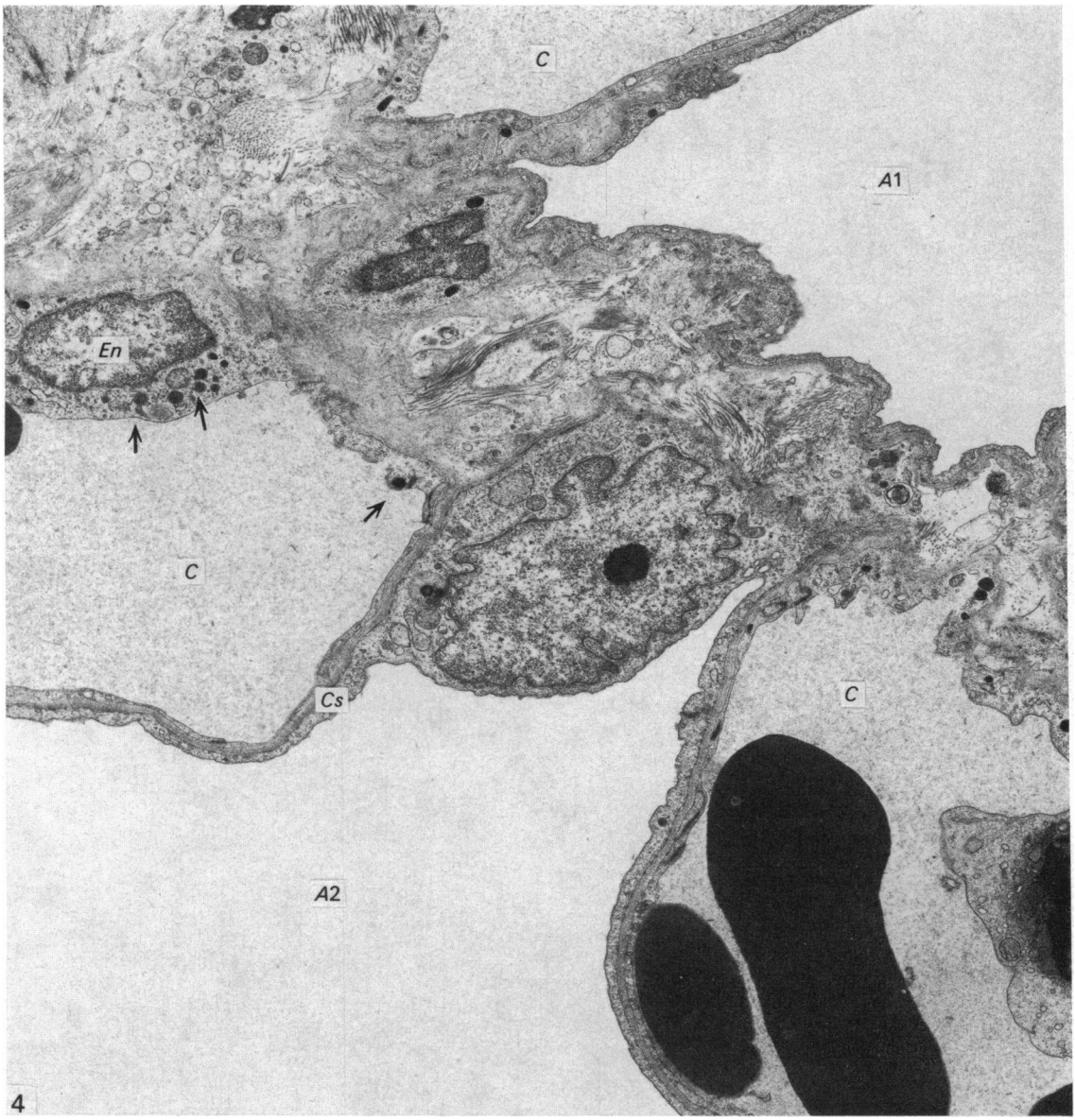
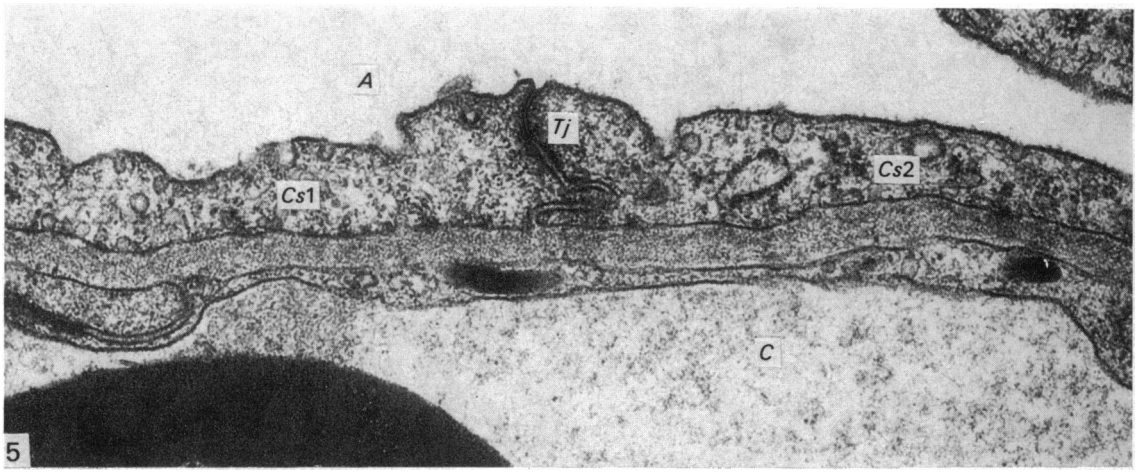


Fig. 4. Low power view of a section through a thin portion of a septum. *A1*, lumen of an air sac; *A2*, lumen of an adjacent air sac; *C*, lumen of a pulmonary capillary; *Cs*, cytoplasmic sheet of a type I pneumonocyte; *En*, nucleus of a capillary endothelial cell; arrows, small densely stained granules in endothelium. × 8500.

capillary endothelium. These have circular or oval profiles and rarely exceed 0·3 μm in their maximum diameter.

Apart from the pulmonary blood vessels, the bulk of each septum consists of longitudinally orientated bundles of smooth muscle and a loose network of collagen and elastic fibres. Fibrocytes and macrophages are scarce. Many septa have relatively thin areas which correspond to sites where both capillaries and smooth muscle bundles are absent (Fig. 4). Septal perforations or apertures do not appear to exist.



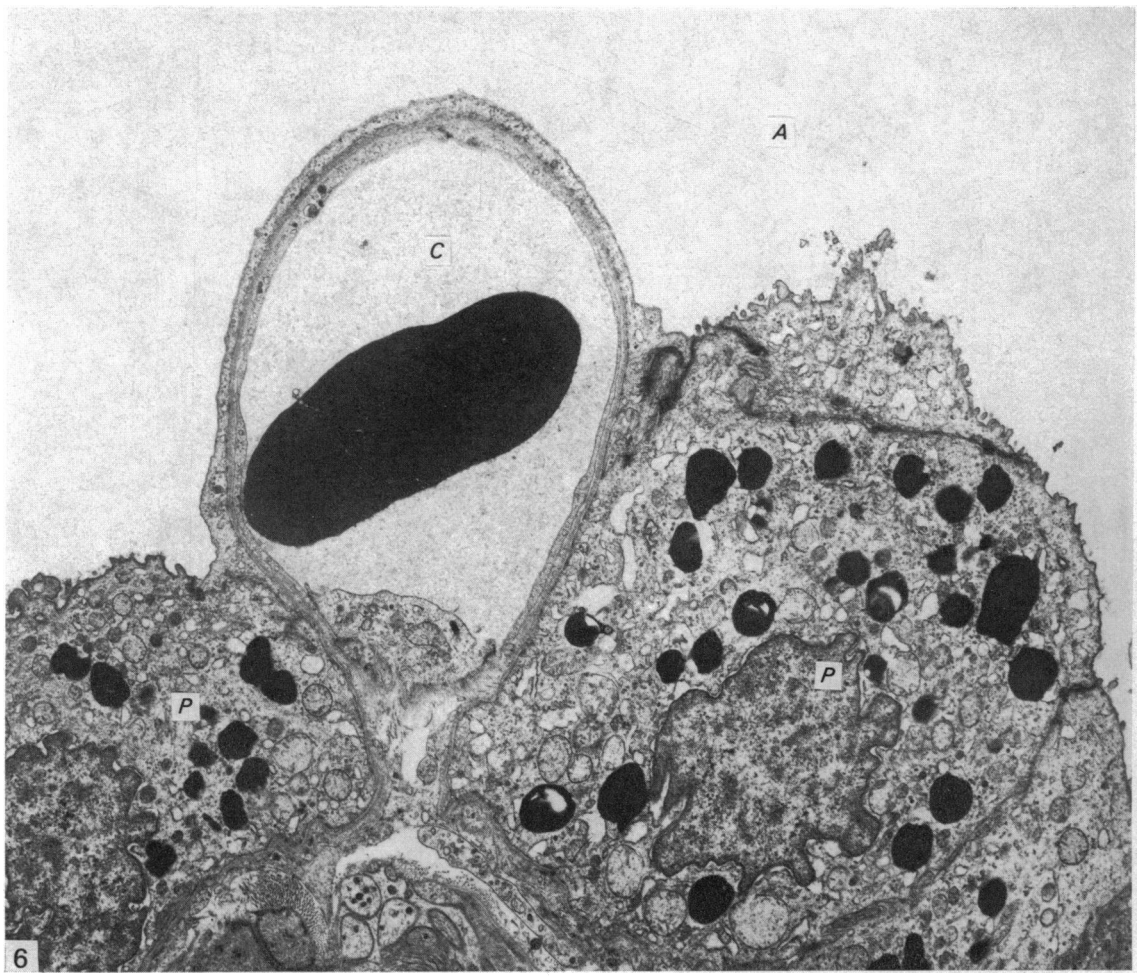


Fig. 5. Junction between the cytoplasmic sheets (*Cs*1, *Cs*2) of two type I pneumonocytes. Note the Z-shaped profile of the intercellular gap. *A*, air sac lumen; *C*, lumen of pulmonary capillary; *Tj*, tight junction. ×32000.

Fig. 6. Section through a pulmonary capillary (*C*) and two type II pneumonocytes (*P*). The pneumonocytes contain osmiophilic inclusion bodies. Note also how the capillary bulges into the air sac lumen (*A*). ×8000.

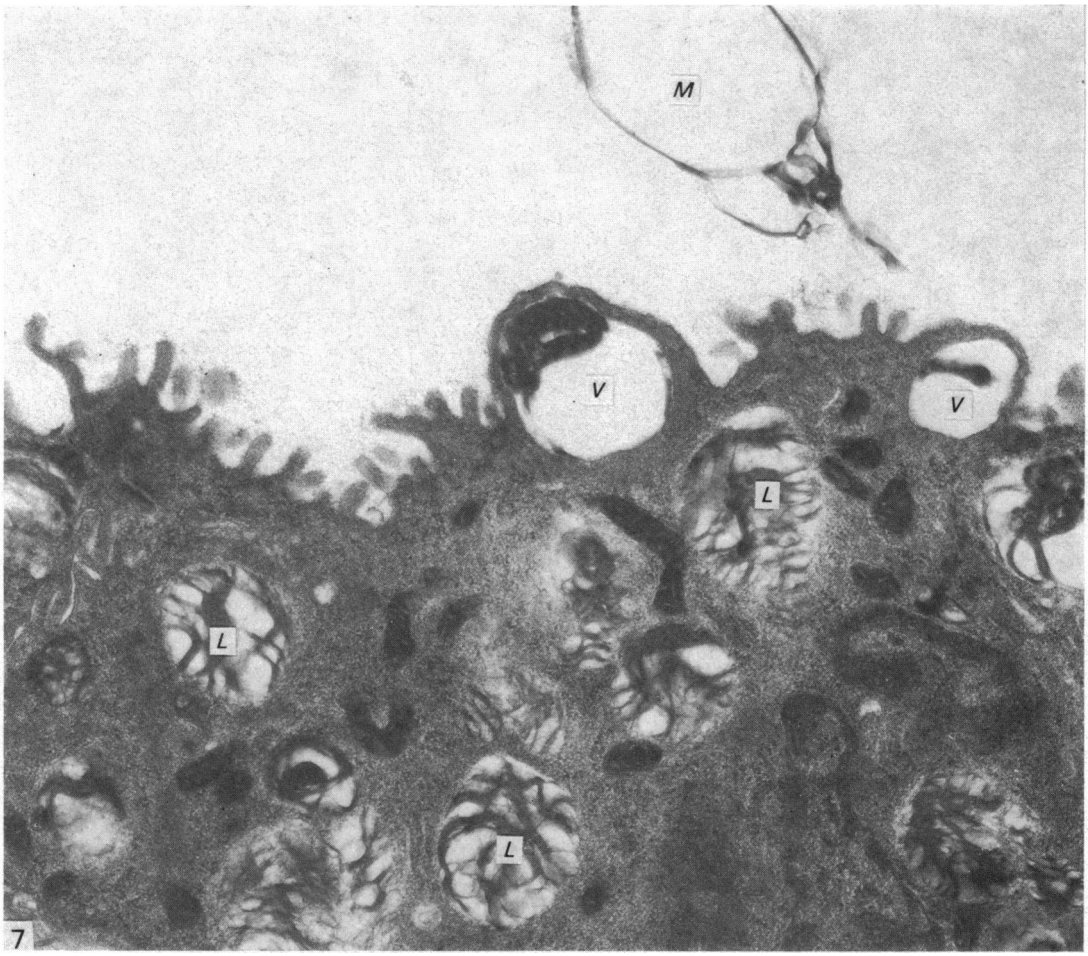
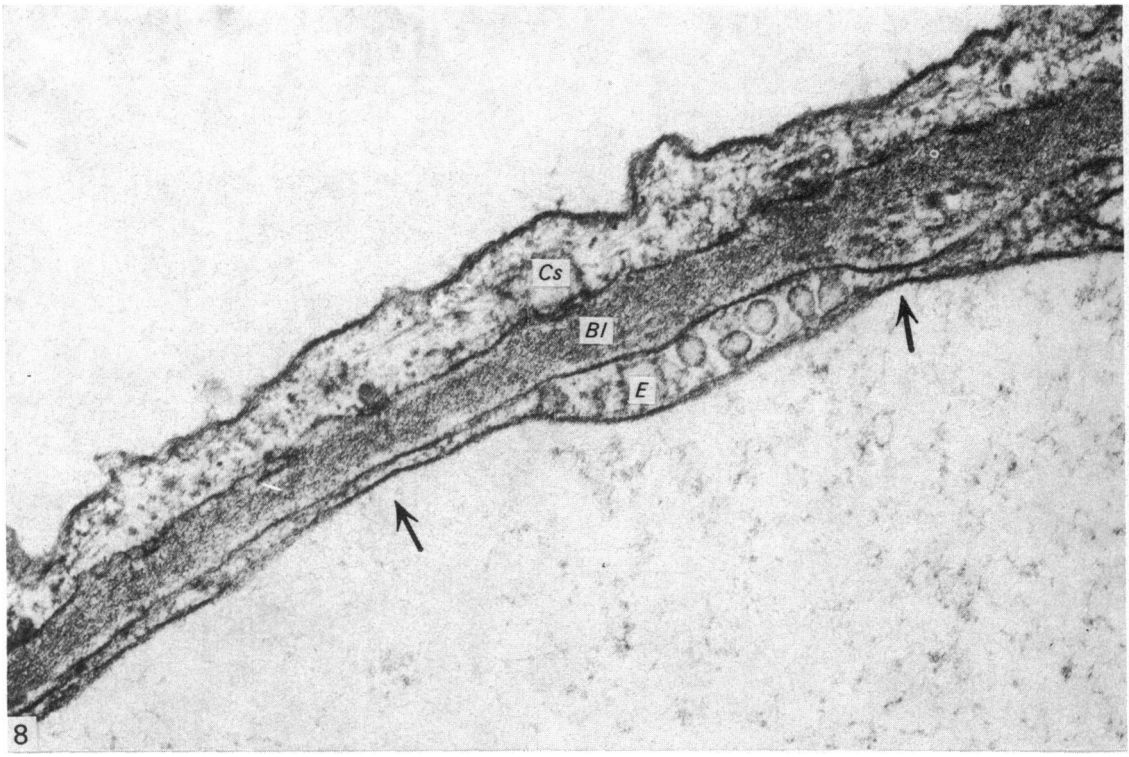


Fig. 7. Superficial portion of a type II pneumonocyte fixed in an osmium tetroxide–glutaraldehyde mixture. The cell contains numerous inclusion bodies. Some are vacuolated (*V*); others have lamellated contents (*L*). Membranous material (*M*) is present in the air sac. × 20000.

Nerve fibres are often present in the depths of the septa and also, less frequently, in the vicinity of pulmonary capillaries and pneumonocytes (Fig. 9). Nerve bundles consisting of as many as 12 unmyelinated axons have been observed near type II pneumonocytes. The axons usually contain neurotubules and some mitochondria, and are partially surrounded by the cytoplasm of Schwann cells. In no instance have the axons been found to breach the basal lamina of the capillary endothelium or the pneumonocytes. Furthermore, enlarged axonal endings have not been observed.

Deposits of membranous material are often present within the air sacs. The membranes are intensely stained with uranyl and lead salts and are disposed in the form of loose tangles or, less commonly, as orderly three-dimensional lattices. Irrespective of their overall arrangement, the individual membranes measure about 7 nm in thickness.



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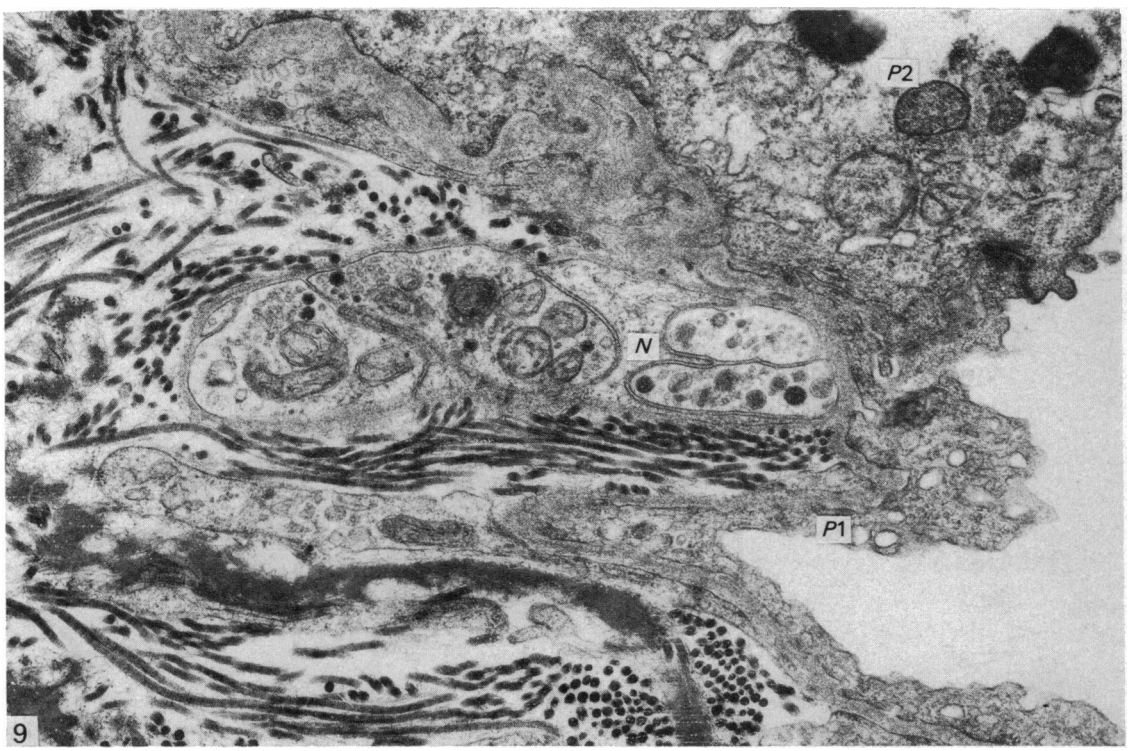

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DISCUSSION

This study has shown that the air spaces bounded by the septa in the interior of the lung of *Lacerta viridis* are relatively large (much larger than the alveoli of mammals or the air capillaries of birds) and that they communicate directly with the axial air passageway. These air spaces are functionally analogous to the alveoli of mammalian lungs in that they are the sites of gas-exchange. However, they differ from the latter in both their dimensions and in their relationship to the conducting airways. It seems best, therefore, to refer to the air spaces of *Lacerta* and other reptiles as 'air sacs' and to use the term 'alveoli' only to describe the terminal air chambers of mature mammalian lungs.

The epithelium covering the septa in *Lacerta* lung is complete and consists of two different cell types. The first, here termed the 'type I pneumonocyte' because of its general similarity to the cell in this position in mammalian lungs, is squamous and spreads out over several pulmonary capillaries. It would appear to be directly involved with the process of gas-exchange. The other type, referred to here as 'the type II pneumonocyte', is cuboidal in shape and contains a greater variety of organelles. Its large osmiophilic inclusions are particularly conspicuous. There can be little doubt that this cell has a secretory function, most likely producing surface-active agents of the type seen in the lungs of other vertebrates (Leeson & Leeson, 1966; Petrik & Riedel, 1968 *a*, *b*; Gil & Weibel, 1970; Meban, 1972, 1973).

Meyrick & Reid (1968) have observed brush cells in the alveolar epithelium of adult rats. These cells have rounded profiles and are characterized by the presence of squat microvilli on their free surfaces and by bundles of thin filaments and particles of β-glycogen scattered throughout their cytoplasm. Cells of this type have not been encountered in the respiratory epithelium of *Lacerta*.

In the past controversy has existed as to whether or not the alveolar structures of mammalian lung have a sensory and motor nerve supply (Larsell & Dow, 1933; Elftman, 1943; Honjin, 1956; Spencer & Leof, 1964). However, two recent electron microscope studies have provided irrefutable evidence of the existence of small nerve bundles in the interalveolar septa of rats (Meyrick & Reid, 1971) and mice (Hung *et al.* 1972). Furthermore, Hung *et al.* have observed two types of specialized nerve endings in mouse lung. One, which they believe to be sensory in function, is located in the septal interstitium or closely related to type I pneumonocytes. The other, supposed to be secretomotor in function, is generally found near type II pneumonocytes. In the present study, bundles of unmyelinated axons have frequently been detected in the depths of the intrapulmonary septa of *Lacerta* and, in a few instances, they have also been observed more superficially in the vicinity of pneumonocytes. However, axons have not been found to penetrate the basal lamina of the pneumonocytes nor have they been found to form specialized endings. Further work is therefore required to established whether the respiratory epithelium of reptiles has a nerve supply.

The present study also demonstrated that the septa in *Lacerta* lung contained

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Fig. 8. Detail of the air–blood barrier. *Bl*, fused basal laminae of pneumonocyte and capillary endothelium; *Cs*, cytoplasmic sheet of a type I pneumonocyte; *E*, cytoplasm of an endothelial cell. Note the attentuation of the endothelium (arrows). × 50000.

Fig. 9. A nerve bundle (*N*) with unmyelinated axons in the edge of a septum. *P*1, cytoplasmic sheet of a type I pneumonocyte; *P*2, a type II pneumonocyte. × 20000.

substantial quantities of smooth muscle. Some of the bundles of muscle fibres run in the free edges of the septa and their contraction evidently decreases the size of the air sac entrance. In this respect they resemble the circular bands of smooth muscle (the 'entrance rings', Weibel, 1973) surrounding the mouths of mammalian alveoli. In contrast, the muscle bundles in the central regions of the septa are arranged longitudinally; their contraction is probably responsible for shortening the septa and hence reducing the capacity of the air sacs. To date, the behaviour of the smooth muscle in the lungs of reptiles has been virtually ignored.

The pulmonary vasculature of *Lacerta* has several structural features which may serve to increase the overall efficiency of the lung. For example, the capillaries which cover each surface of the internal septa are arranged in a network with very close meshes; indeed, the network seems to approach the form which would permit an effective 'sheet-bloodflow' of the type envisaged by Sobin, Tremer & Fung (1970). Again, the individual elements of the capillary network are interesting in that they are evaginated into the air sacs rather than being countersunk into the substance of the pulmonary septa. Such an arrangement obviously allows a greater area of the capillary wall to be exposed to the air and hence facilitates gas exchange. In addition, the capillary endothelium in *Lacerta* is greatly attenuated in the region of the air–blood barrier (for example, see Fig. 8). This attenuation would also help to reduce the resistance to gaseous diffusion.

SUMMARY

The gas-exchange area in the lung of *Lacerta viridis* has been studied by light microscopy and electron microscopy. The interior of the lung in this species is partitioned into air sacs by radially disposed septa. The surfaces of each septum are covered by a continuous epithelium, the cells of which are termed 'pneumonocytes'. Deep to the epithelium there is a close-meshed plexus of capillaries. The middle layer of the septum contains smooth muscle and fibrous tissue. Two varieties of pneumonocytes can be identified. The type I cells are squamous and give off attenuated sheets of cytoplasm which spread widely over the septal surface; these sheets contain few organelles. The type II cells are more compact and possess many organelles; their osmiophilic inclusion bodies are especially conspicuous. The pulmonary capillaries of *Lacerta* are evaginated into the air sacs and often display marked attentuation of their endothelium. The possible functional significance of these features is discussed.

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