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A morphological study of the tracheal epithelium of the snake *Natrix maura*

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INTRODUCTION

The ultrastructure of the epithelium of the trachea of mammals has been widely studied (Jeffery & Reid, 1975; Pack, Al-Ugaily & Morris, 1981; Dalen, 1983; Plopper *et al.* 1983; Wilson, Plopper & Hyde, 1984; Robinson, Venning, Kyle & Widdicombe, 1986).

However, in reptiles, these studies are very scanty and only refer to lizards and turtles (Tesik, 1984; Pastor *et al.* 1987, 1988). These studies showed marked differences between the tracheal epithelium of turtles and those of lizards. While, in turtles, the epithelium is similar to that described in mammals, in lizards the epithelium is different and shows a type of secretory cell that may have a similar function to the serous cells described in mammals. In a previous work with conventional light microscopy the histological structure of the trachea of *Natrix maura* has been described and, by means of conventional histochemical techniques and lectins, the mucoglycoproteins were studied (Castells *et al.* 1989).

An electron microscopy study of the tracheal epithelium of a snake (*Natrix maura*) has now been carried out to elucidate the possible morphological variations of the tracheal epithelium between *Chelonia*, *Squamata* and *Ophidia*.

MATERIAL AND METHODS

Light microscopy

The animals were killed by the injection of an overdose of sodium pentobarbitone into the peritoneal cavity. Portions of the trachea from adult specimens of both sexes, ranging in weight from 150 to 200 g, of six *Natrix maura* were fixed by immersion in 10% buffered formalin. The samples were routinely processed and embedded in paraffin. The sections, 5 μm thick, were stained with haematoxylin and eosin. Cell numbers per unit length of epithelium were determined with the aid of a calibrated eye piece graticule. In three sections from four different levels of each trachea the number of secretory and ciliated cells per 1·8 mm of epithelium was counted.

Electron microscopy

Thin sections were obtained from different levels of the trachea. The samples were fixed in 2·5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon 812. Ultrathin sections were cut using a Reichert–Imy Ultracut ultramicrotome and stained with uranyl acetate and lead citrate. Electron microscopy was performed with a Zeiss EM/10 CR. For scanning electron microscopy (SEM), after washing in buffer and postfixation the specimens were dehydrated in

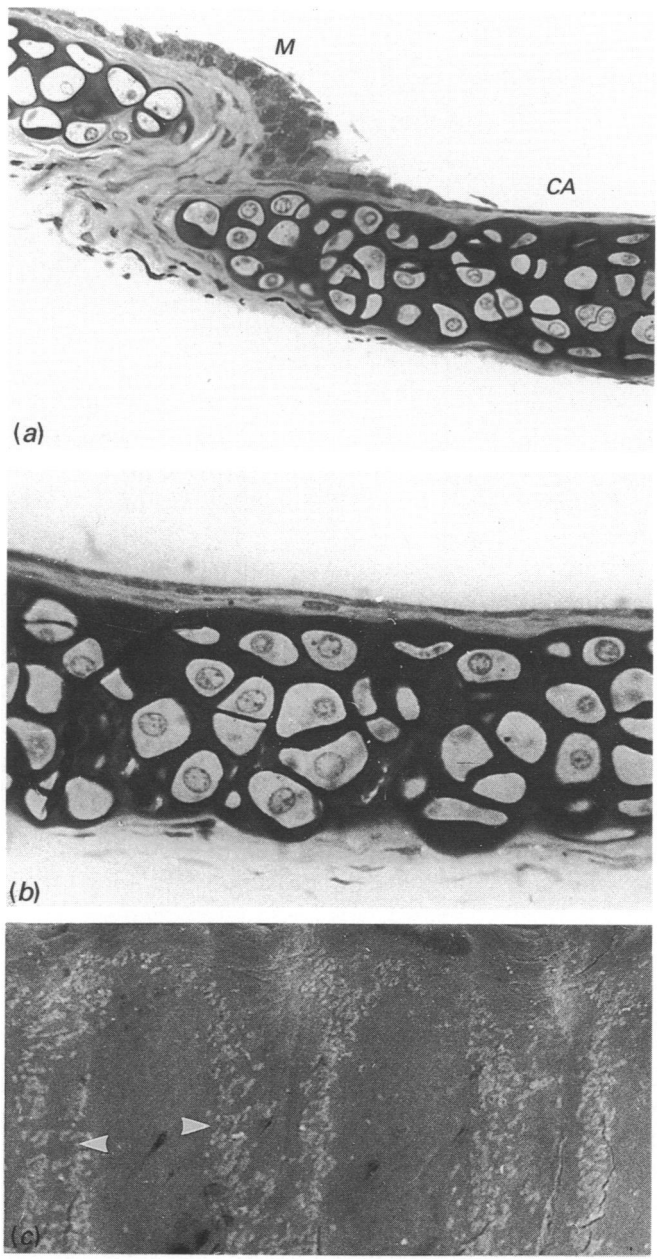
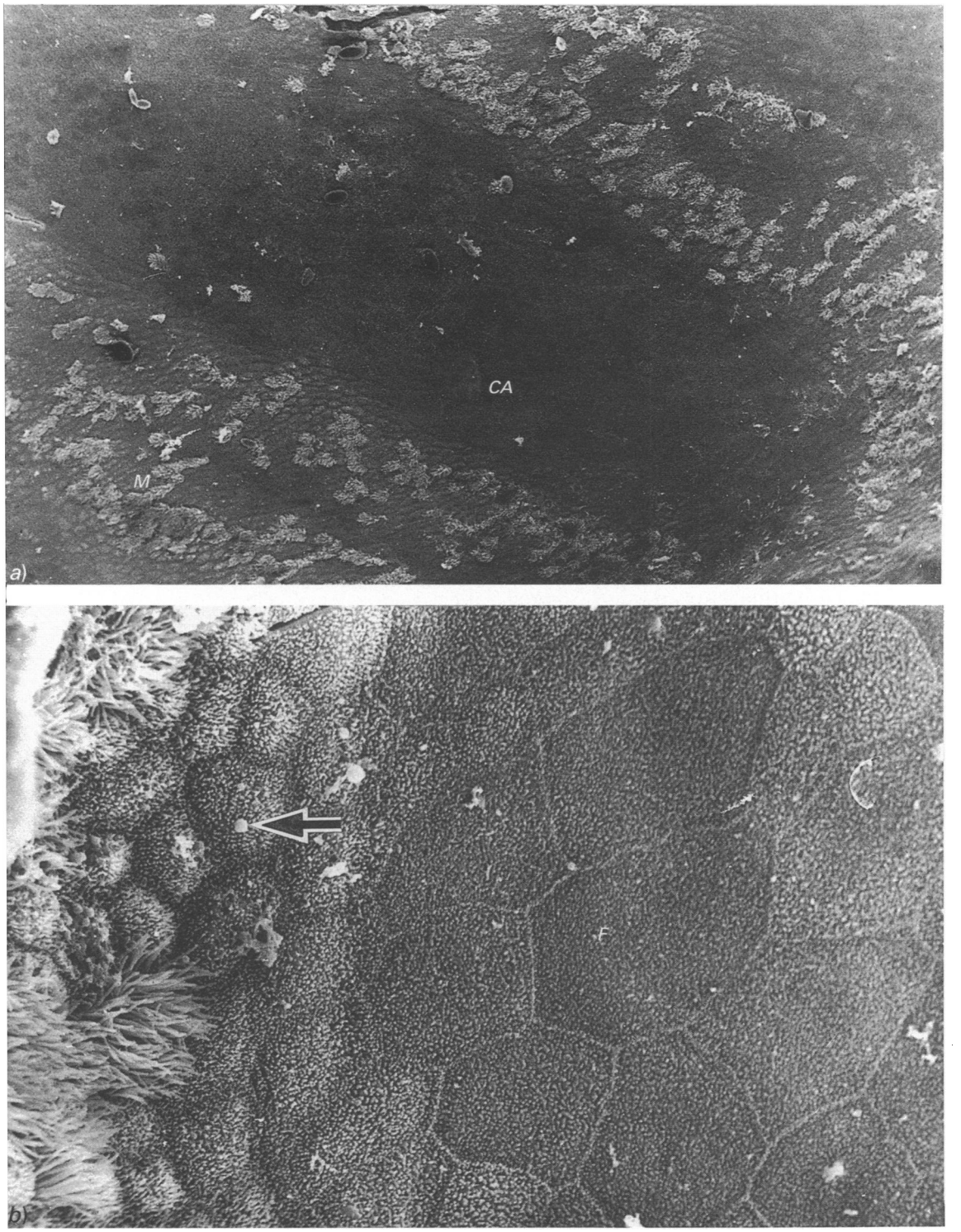


Fig. 1 (*a–c*). (*a*) Epithelium over the membranous (*M*) zone changing into a flat epithelium in the cartilaginous zone (*CA*). Toluidine blue. × 350. (*b*) At a higher magnification, the thin epithelial cells in the cartilaginous portion can be observed. Toluidine blue. × 500. (*c*) Scanning electron microscopy micrograph, at low magnification, of *Natrix maura* trachea. The ciliated cells principally appear in the membranous portion (arrowheads)., × 50.

Fig. 2 (*a–b*). (*a*) Scanning electron micrograph of tracheal surface epithelium of *Natrix maura*. Note the differences between the membranous (*M*) and the cartilaginous zone (*CA*). × 150. (*b*) Region of transition between the membranous and the cartilaginous zones. The flattened non-ciliated cells of the cartilaginous zone (*F*) show marked cell margins and numerous microvilli. The non-ciliated cells from the membranous portion show convex apices (arrow). × 2500.


CA
M
a)
b)

acetone, critical-point dried, mounted on aluminium stubs, sputtered with gold and studied with a JEOL T-300 scanning electron microscope.

RESULTS

Light microscopy

The trachea of *Natrix maura* is short and extends as a C-shaped groove along the ventral side of the lung (intrapulmonary trachea, Luchtel & Kardong (1981)). The trachea of *Natrix maura* was lined by a pseudostratified epithelium composed of basal, ciliated and secretory cells. The tracheal epithelium had different thickness and different structure depending on the area. The epithelial cells lining the membranous zones (i.e. the intercartilaginous zone, which does not usually contain smooth muscle) were columnar and composed of basal, secretory and ciliated cells with an average thickness lying between 15 and 20 μm (Fig. 1*a*) whereas the cartilaginous zone showed a flattened epithelium (Fig. 1*b*) composed of flat basal and secretory cells with an average thickness from surface to basal membrane of only 2–3 μm. The numbers of secretory and ciliated cells at the different levels of the trachea were similar. Between 30 and 40 ciliated cells and 160–170 secretory cells were counted per 1·8 mm of sections of the epithelium.

Scanning electron microscopy

Variations in the morphology of the cells were observed according to the area studied. The general aspect was similar to that observed by light microscopy (Figs. 1*c*, 2*a*). The cartilaginous portions showed flattened non-ciliated cells with marked cell margins and microvilli (Fig. 2*b*). In the membranous portion, patches of ciliated cells were observed together with non-ciliated cells that showed microvilli and occasionally rounded apical surfaces (Fig. 3*a–b*).

Transmission electron microscopy

The tracheal epithelium of *Natrix maura* showed ciliated, secretory, endocrine and basal cells (Fig. 4*a*, *b*).

Ciliated cells had an electron-lucent cytoplasm and showed numerous electron-dense mitochondria in the apical portion of the cytoplasm. Microvilli were usually present among the cilia. Abundant lysosomes were observed in the cytoplasm (Fig. 4*a*).

Secretory cells showed a morphology that varied with the area studied. Thus, in the membranous zone, secretory cells were cuboidal or columnar; cytoplasmic protrusions were usually observed in the tracheal lumen (Fig. 4*c*, *d*). Secretory granules were in different states of maturation. These granules were of five types: (*a*) granules of low electron density and of granular content; (*b*) granules of low electron density with a

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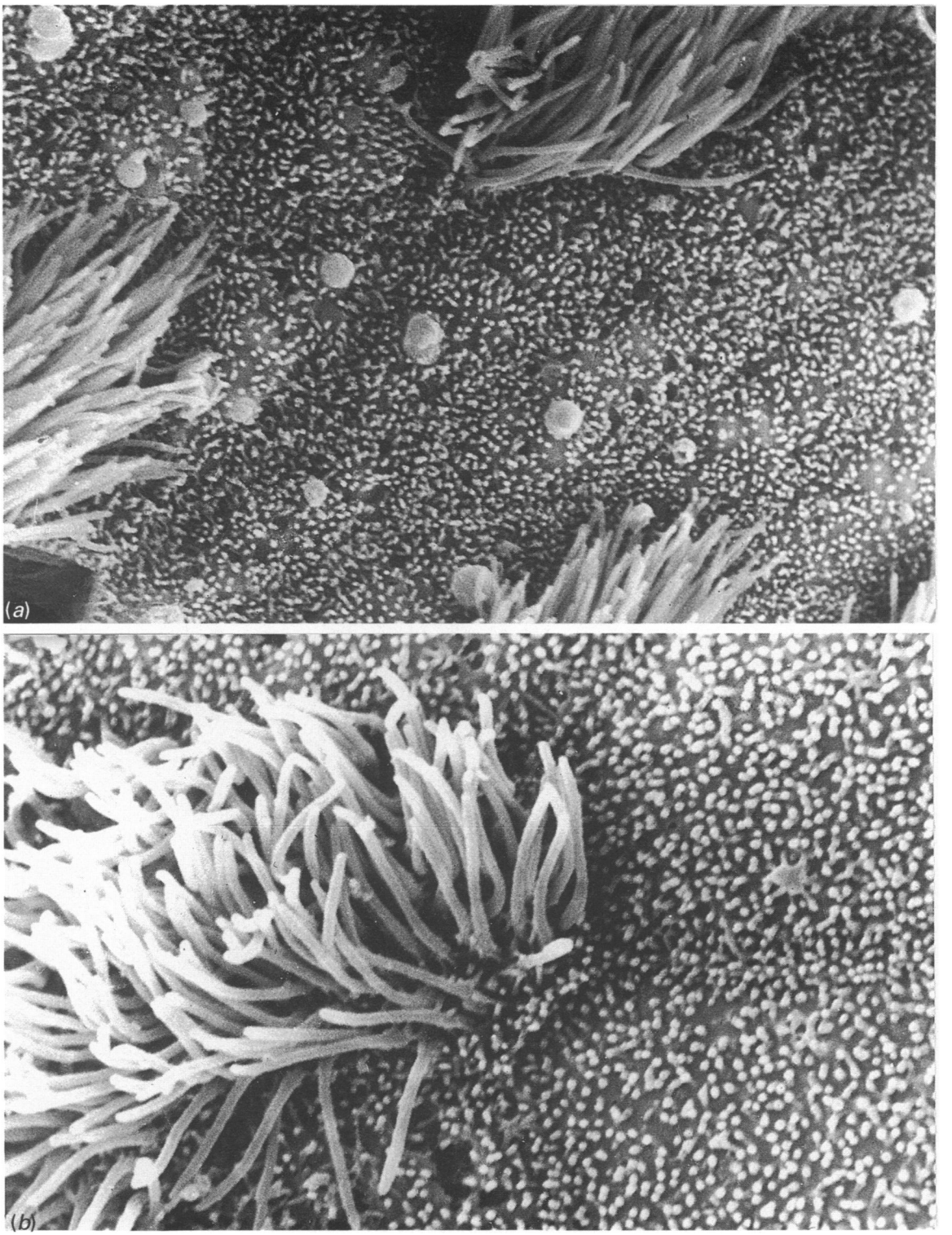
Fig. 3 (*a–b*). (*a*) Ciliated cells and non-ciliated cells from the membranous portion. Some non-ciliated cells have rounded apical surfaces, probably produced by secretory material. × 8000. (*b*) Microvilli are abundant in non-ciliated cells and also among the cilia. × 10000.

Fig. 4 (*a–d*). (*a*) Tracheal epithelium of *Natrix maura*. Basal (*B*), ciliated (*C*) and secretory (*S*) cells are present. Note the dense accumulation of mitochondria (*M*) in the apical portion of the ciliated cells. × 2500. (*b*) Cluster of endocrine cells (*E*) in tracheal epithelium showing an electron-lucent cytoplasm. × 3500. (*c*) Secretory columnar cells of tracheal epithelium of *Natrix maura*. Cytoplasmic protrusions can be seen in some cells (arrows). × 1400. (*d*) Cuboidal secretory cells, some showing distinct types of secretion granules. × 2000.


(a)
(b)

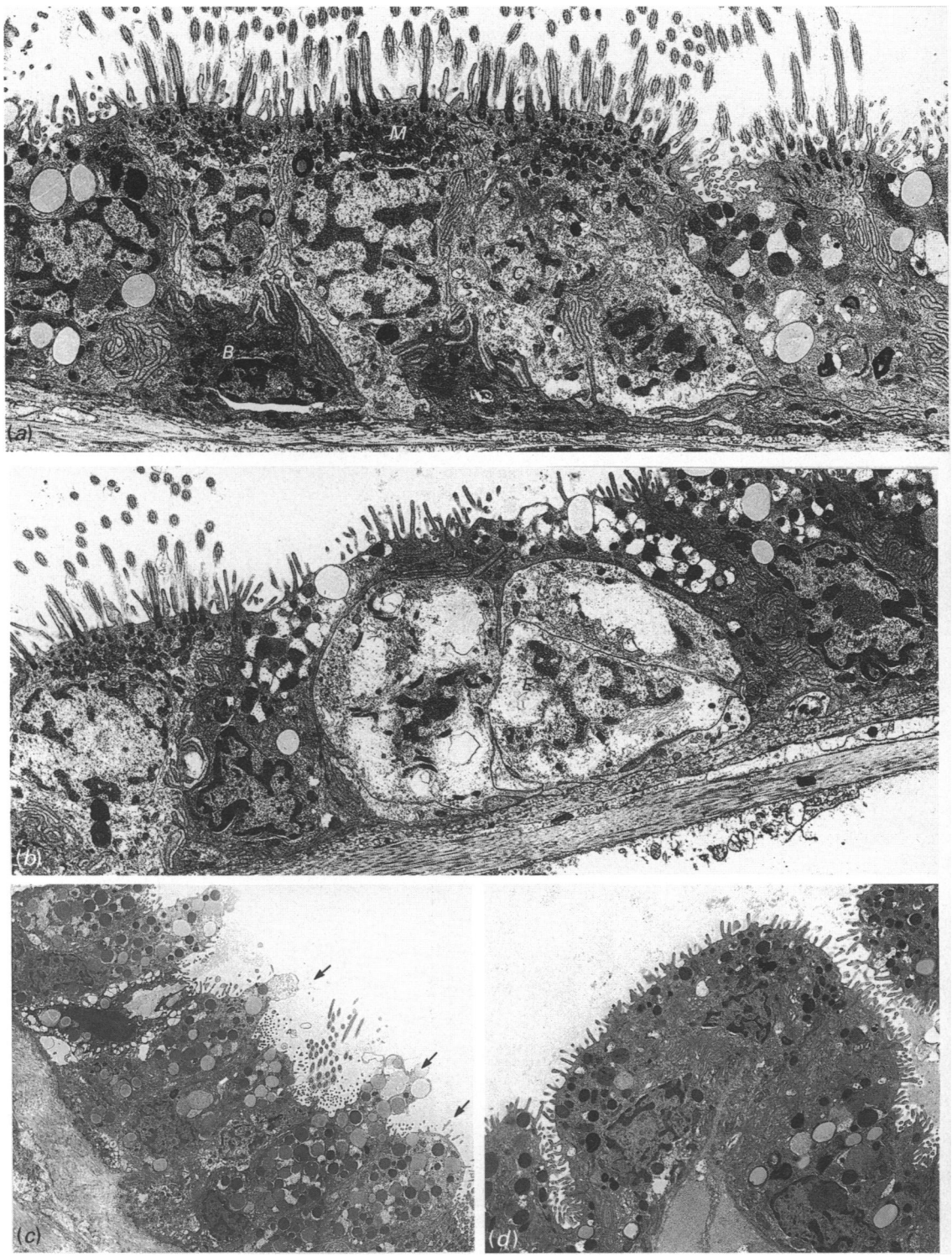


Fig. 4(*a–d*). For legend see p. 50

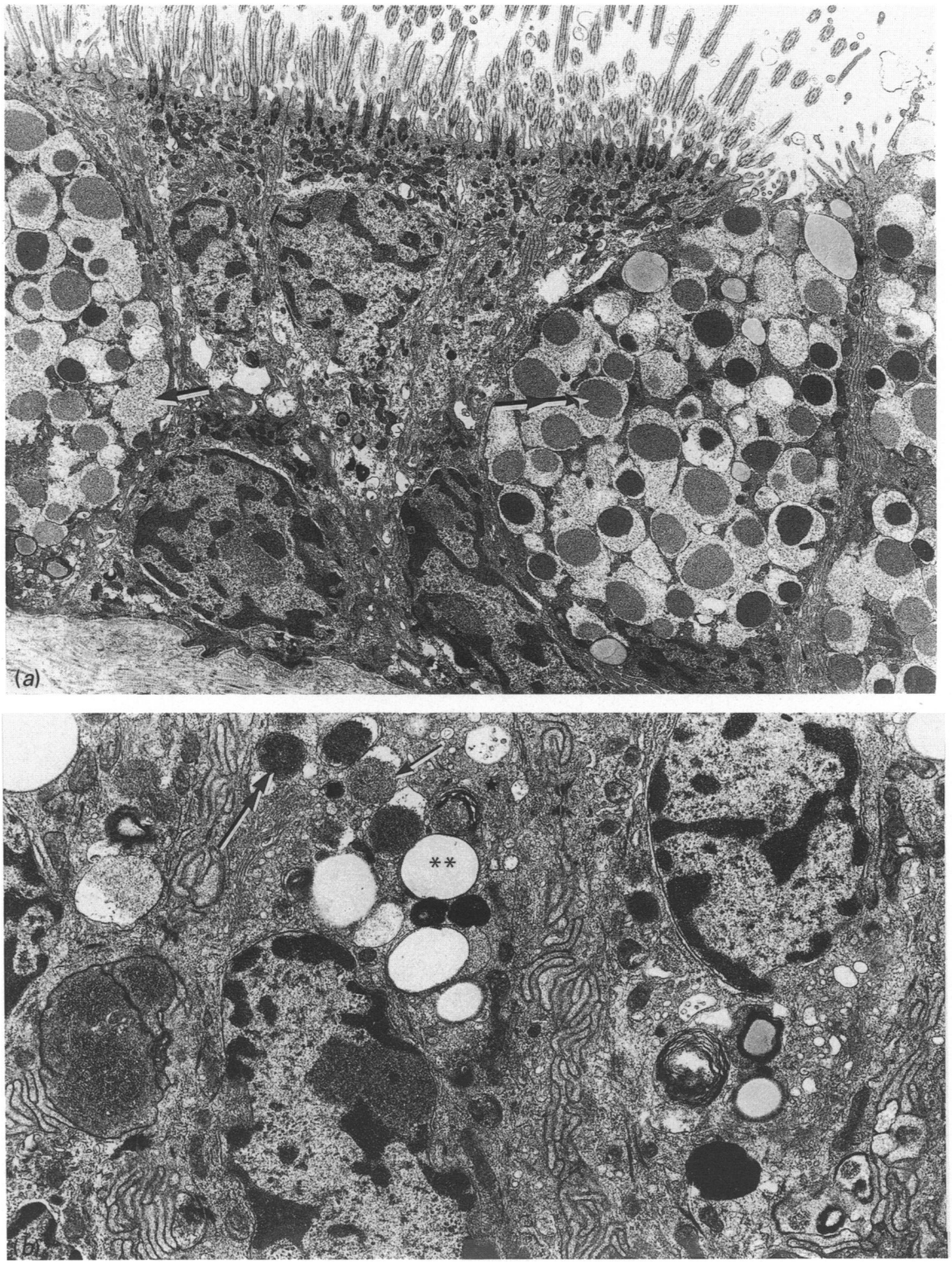
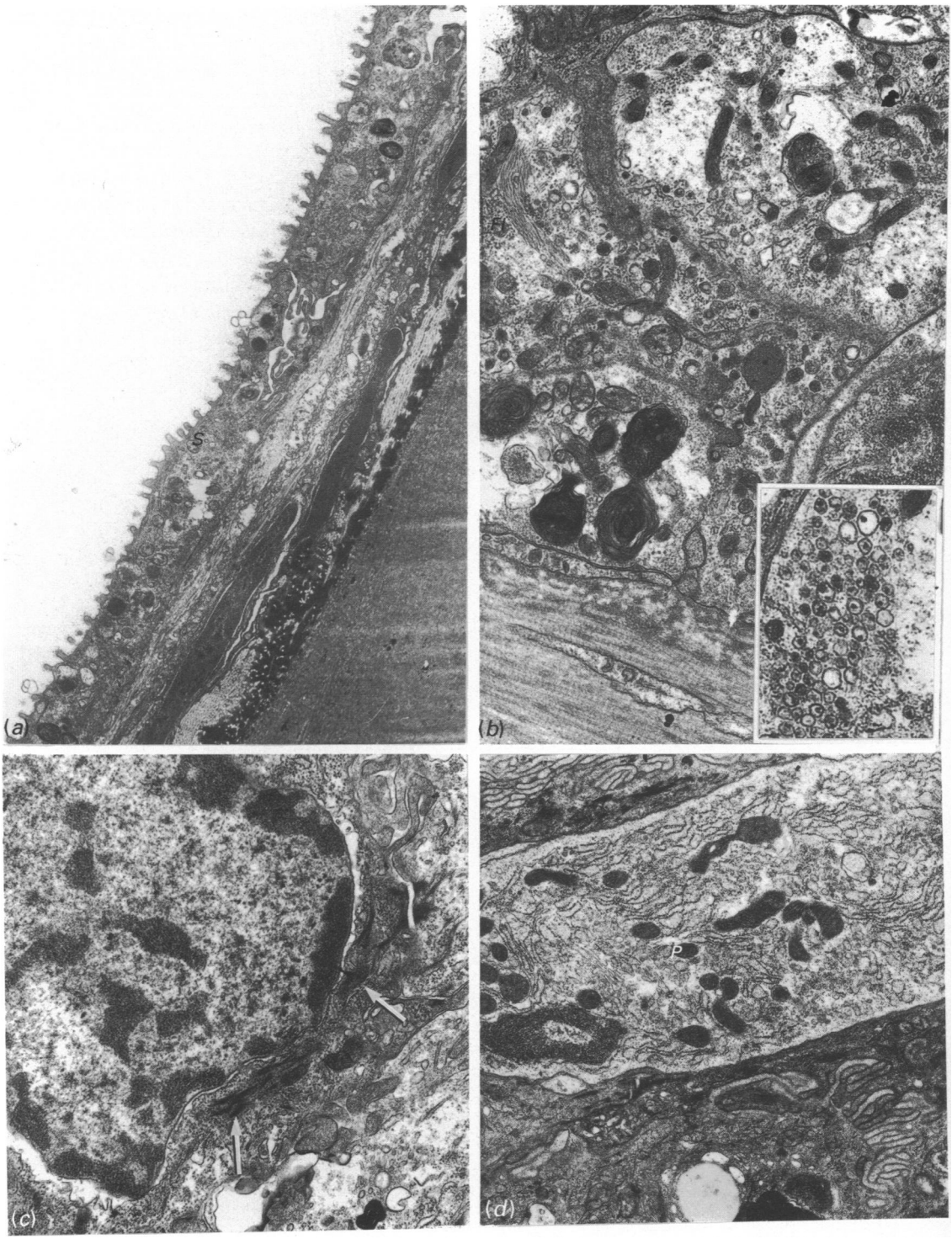


Fig. 5(*a–b*). For legend see p. 55


(a)
S
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core of moderate strong electron density; (*c*) granules of moderate electron density and with a granular content; (*d*) round very electron-dense granules of variable diameter; (*e*) granules of low electron density with partially myelin-like appearance. Occasionally, the content of this type of granule seemed to have been extracted and the granule seemed to be empty (Fig. 5*a–b*).

In the cartilaginous zone secretory cells were usually flat; they contained few secretory granules and had abundant apical microvilli (Fig. 6*a*).

Endocrine cells (solitary or in clusters) had an electron-lucent cytoplasm. The main ultrastructural characteristic was the presence of secretory granules with a thin halo surrounding an electron-dense core. The diameter of the granules varied between 90 and 120 nm (Fig. 6*b*). No nerve supply to these bodies was seen.

Basal cells were characterised by intermediate filaments and abundant ribosomes in the cytoplasm (Fig. 6*c*). They showed an electron-dense cytoplasm and numerous interdigitations with adjacent cells. Intra-epithelial plasma cells were also identified (Fig. 6*d*).

DISCUSSION

Light microscopic examination of the epithelium of the trachea of *Natrix maura* showed marked differences from those reported in turtles but were similar to those observed in lizards (Tesik, 1984; Pastor *et al.* 1988). The epithelium of the trachea of lizards, like that described in the present study, presented two distinct areas: cartilaginous and membranous; there was a distribution of epithelial cells similar to that in the trachea of *Natrix maura*. The morphology was quite different from that observed in turtles which is very similar to that of mammals.

The findings obtained by SEM study of the trachea of *Natrix maura* were similar to those reported in *Lacerta lepida* (Pastor *et al.* 1988). The presence of an irregular distribution of ciliated cells, and their relative paucity compared with turtles and mammals, may suggest that the mucociliary transport in the trachea of genus *Squamata* and *Ophidia* is low.

The tracheal epithelium of *Natrix maura* is composed of four types of cells: basal, ciliated, endocrine and secretory cells. Cells that have been observed in mammalian tracheobronchial epithelium (Clara cell, brush cell, etc.) were not found in the tracheal epithelium of *Natrix maura* (Jeffery, 1983).

The ultrastructure of basal and ciliated cells is similar to that found in mammals and other reptiles (Breeze & Wheeldon, 1977; Pastor *et al.* 1987, 1988). The endocrine cells have been recognised in the trachea and lungs of mammals, birds, reptiles and amphibians and apparently contained serotonin (Diagustine & Sonstegard, 1984). The clusters of endocrine cells observed in the trachea of *Natrix maura* are similar to the

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Fig. 5 (*a–b*). (*a*) Secretory cells with granules. Granules of Type a (arrow) and b (double arrow). × 6000. (*b*) Cytoplasm of secretory cells with Type c granules (arrow), d (double arrow) and e (asterisk). Note the presence of some granules with the contents extracted (double asterisk). × 30 000.

Fig. 6 (*a–d*). (*a*) Tracheal epithelium over the cartilaginous zone of *Natrix maura*. Flattened secretory cells (*S*) with different types of secretory granules are observed. × 8000. (*b*) Endocrine cell of tracheal epithelium. In the cytoplasm several lysosomes, cytoplasmic filaments (*FI*) and secretory granules are observed. × 13 000. Inset: Detail of the endocrine granules. × 35 000. (*c*) Basal cell showing electron-dense cytoplasm and filaments (arrows). × 25 000. (*d*) Intraepithelial plasma cell (*P*) in the trachea of *Natrix maura*. Note the presence of numerous cisternae of granular endoplasmic reticulum.

neuroepithelial bodies in the lungs of vertebrates. No such clusters have been described in the extrapulmonary airways of the adult vertebrate. The result of our study is unexpected and it contradicts the idea that the neuroepithelial bodies are placed only in intrapulmonary airways (Diagustine & Sonstegard, 1984). The presence of endocrine cells in the trachea of Ophidians suggests a wide distribution of these cells in the extrapulmonary airways of vertebrates.

The secretory cells of *Natrix maura* are ultrastructurally similar to the granular or secretory cells found in *Lacertidae* but are different from the mucous cells of *Chelonia* (Tesik, 1984; Pastor *et al.* 1987, 1988; Perry *et al.* 1989). The characteristics of the secretory cells of *Natrix maura* are similar to those reported in the mammalian, serous cells of the tracheal epithelium and in the glands of the respiratory system (Jeffery & Reid, 1975; Spicer, Shulte & Thomopoulus, 1983). From a histochemical point of view, the secretory cells of *Natrix maura* can be labelled with Con-A and WGA lectins (Castells *et al.* 1989). This pattern is similar to that observed in other serous cells in the respiratory system of mammals (Spicer *et al.* 1983). In mammals the serous cells usually appear, in pathogen-free animals, as the only secretory cell of the airway tract. Infections cause a change of serous cells into mucous cells (Huang, Haskell & McDonald, 1989). This fact suggests that the epithelium of lizards and snakes could be free of infective agents. The myelin-like degeneration observed in some granules of the secretory cells of the tracheal epithelium of *Lacertidae* were also observed in *Natrix maura*. This finding suggests that it is probable that such degeneration in the secretory granule is related to different functional states of the epithelium. All these findings suggest that the secretions in lizards and snakes are different from those found in turtles. These secretions are probably of the serous type with abundant water and proteins (mainly immunological proteins) while in turtles they are of mucous type with abundant carbohydrate and high viscosity (Spicer *et al.* 1983).

Plasma cells were identified in tracheal epithelium of *Natrix maura*. Similar cells have been reported in the tracheal epithelium of reptiles and of mammals (Breeze & Wheeldon, 1977; Tesik, 1984; Pastor *et al.* 1988). It is very likely that these cells play an important role in the formation of an epithelial immunobarrier, as occurs in mammals (Brandtzaeg, 1988).

In conclusion, the tracheal epithelium of *Natrix maura* has a similar structure to that of lizards and differs from that described in turtles, birds and mammals.

SUMMARY

The epithelium of the trachea of the *Natrix maura* snake was studied by conventional light microscopy and transmission and scanning electron microscopy. The epithelium is formed of basal, ciliated, endocrine and secretory cells. It shows different thickness and distribution of the cells, depending on the area (covering the cartilaginous or the membranous zone). Secretory cells show a morphology similar to that found in lizards but it is different from the mucous cells reported in the extrapulmonary airways of turtles, birds and mammals. The ultrastructure of the secretory cells is similar to that reported for serous cells in the airways of mammals. Intra-epithelial plasma cells are also found within the epithelium.

The present results show that there are marked morphological differences between the tracheal epithelium of lizards and snakes and that of turtles, birds and mammals.

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