

Ultrastructural characterisation of the olfactory mucosa of the armadillo *Dasypus hybridus* (Dasypodidae, Xenarthra)

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ABSTRACT

The ultrastructure of the olfactory mucosa of the armadillo *Dasypus hybridus* was studied. A comparison with the olfactory mucosa of another armadillo (*Chaetophractus villosus*) was made. The olfactory mucosa of *D. hybridus* shows many features which are similar to those of other mammals. Interestingly, it differs from the olfactory mucosa of the armadillo *C. villosus*. A suggestion is made that these differences may be due to differences in the digging habits of these species. In *Dasypus*, the supporting cells (SCs) showed dense vacuoles, multivesicular bodies and lysosome-like bodies probably related with the endocytotic system. The SCs show a dense network of SER presumably associated with xenobiotic mechanisms. The olfactory receptor neurons exhibit lysosome-like bodies and multivesicular bodies in their perikarya. These organelles suggest the presence of an endocytotic system. Duct cells of Bowman's glands exhibit secretory activities. Bowman's glands are compound-branched tubulo-acinar mixed glands with merocrine secretory mechanisms.

Key words: Olfactory epithelium; Bowman's glands.

INTRODUCTION

Olfactory stimuli impinging upon the olfactory mucosa play a critical role in feeding, mating and interspecific or intraspecific communication necessary for survival in most vertebrates. Those stimuli play an important role in the regulation of the electrical activity of the brain (Affanni & Garcia Samartino, 1984) and serve chiefly as activators of complex sensorimotor systems whose patterns of performance are determined primarily by other senses (Herrick, 1933).

The olfactory receptor neurons (ORNs) and the vomeronasal receptor neurons are the only neurons that are in direct contact with the external environment. Moreover, they retain the ability to regenerate throughout life (Graziadei & Monti Graziadei, 1979; Jia & Halpern, 1998), allowing the complete reconstitution of the olfactory epithelium under physiological or experimental conditions. The supporting cells (SCs) are involved in maintaining the ionic composition of the mucus layer and the

xenobiotic metabolism (Lewis & Dahl, 1995). The basal cells (BCs) are considered to be the stem cells of the olfactory neurons (Caggiano et al. 1994). Additionally, knowledge about the fine structure of the associated Bowman's glands (BG) is important on account of the influence of the mucous layer on events associated with olfactory transduction and detoxification (Lewis & Dahl, 1995).

The olfactory mucosa (OM) has been the subject of detailed electron microscopical studies in various species of mammals (Graziadei, 1973; Farbman, 1992; Morrison & Costanzo, 1992). However, less attention has been focused on mammals of the order Xenarthra. In previous investigations we described the ultrastructure of the OM of another armadillo, *Chaetophractus villosus*, which exhibits a variety of novel and interesting features (Ferrari et al. 1998a). The genus *Dasypus* was considered to be a more primitive group than *Chaetophractus* (Jorge et al. 1985; Cetica et al. 1998). Moreover, both genres show different activity patterns and modal feeding specialisation. Armadillos of the genus *Chaetophractus* are generalist feeders.

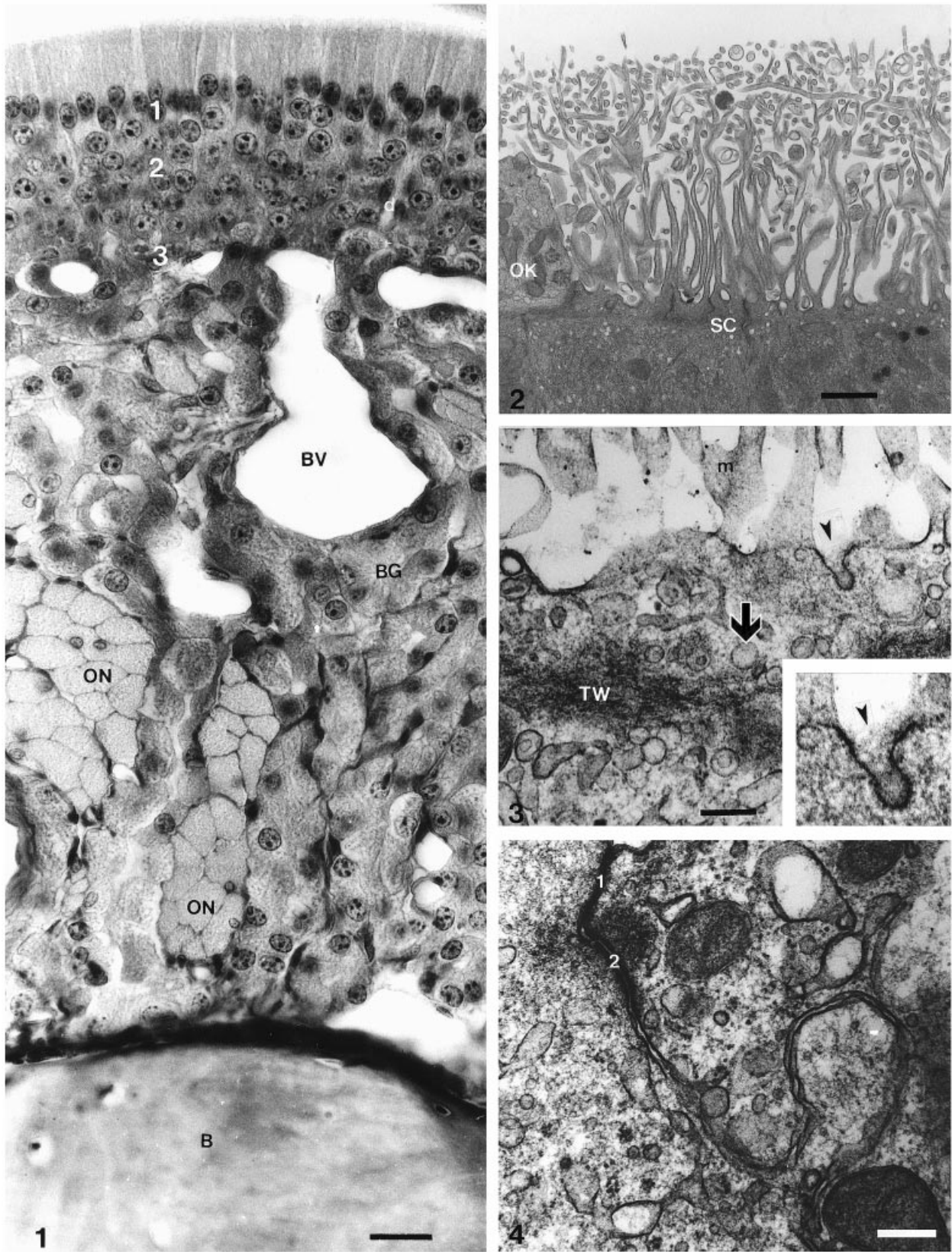


Fig 1-4. For legends see opposite.

They consume virtually any type of animal tissues as well as different types of fruits and tubers. In contrast, the genus *Dasybus* is insectivorous (Redfort, 1985). A comparison of the OM structures in both species might be interesting if the different digging and feeding habits are in some way reflected in the organisation of those structures. Both species of armadillo exhibit a highly developed rhinencephalon with enormous olfactory bulbs, olfactory tubercles and pyriform cortex (Benítez et al. 1994; Ferrari et al. 1998b).

The armadillo *Dasybus hybridus* shows the extraordinary reproductive characteristic of producing regular litters of monozygous twins. This species bears especial interest because it produces identical twins with 7–12 offspring by litter (Fernandez, 1913). It therefore appears an ideal species for physiological, morphological and tissue transplantation studies.

The aims of this paper are (1) to describe the histological and ultrastructural composition of the olfactory mucosa of *Dasybus hybridus*, including the cellular components of the olfactory epithelium (OE) and its associated Bowman's glands, and (2) to compare the findings with those obtained from the OM of *Chaetophractus villosus* and other mammalian species.

MATERIALS AND METHODS

Ten armadillos (*Dasybus hybridus*; 6 females, 4 males) were used. They were maintained and fed as previously reported, with a special diet consisting of eggs, milk, high meat, vitamins, minerals and water ad libitum (Ferrari, 1997). A natural light-darkness cycle was maintained in the breeding rooms. For light microscopic studies, they were anaesthetised with ketamine hydrochloride (40 mg/kg, i.m.) and sodium thiopental (60 mg/kg, i.p.). They were then perfused via the aorta with saline followed by Bouin's fluid or neutral buffered formol saline. After samples of the endoturbinals had been removed the tissues were dehydrated and embedded in paraffin. Serial sagittal and transverse sections (5–8 µm thick) were stained either with haematoxylin and eosin or Masson's trichrome.

For electron microscopy, immersion and perfusion fixation was performed. For perfusion fixation, the animals were perfused transcidentally with 1 l of modified physiological saline (0.8% NaCl, 0.8% sucrose, 0.4% glucose), followed by 2 l of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH: 7.4). The effect of this fixation on the cytological characteristics was studied in 2 animals. The fixative was dropped onto the exposed surface of the nasal cavity at the level of the dorsal endoturbinals. These specimens were then fixed by immersion in the same fixative at room temperature for 2–3 h. The tissues were stored overnight in the same buffer with 30% sucrose. Following 3–5 min washes in buffer, they were postfixed in 1% O_3O_4 in the same buffer, at pH 7.2, for 1 h, washed as previously, dehydrated through an ethanol series, cleared in acetone, and infiltrated with Araldite. Semithin and ultrathin sections were cut with diamond or glass knives with a Sorvall Porter-Blum ultramicrotome. For light microscopy semithin sections were stained with toluidine blue. For electron microscopy, ultrathin sections were contrasted with uranyl acetate (1% in 70% ethanol w/v) and lead citrate (Reynolds). They were studied with a Siemens Elmiskop I or a Zeiss M-109 Turbo electron microscope.

RESULTS

Structural differences were not observed between the male and the female OM. The OM includes the OE and the subjacent lamina propria in which the olfactory nerves and the Bowman's glands (BG) occur. The OE is a pseudostratified epithelium containing 3 morphologically identified cell layers: supporting cells (SCs), olfactory receptor neurons (ORNs) and basal cells (BCs). Ducts of BG and the apical neck region of acini were observed within the epithelium (Fig. 1).

Supporting cells

The SCs form the superficial layer of the epithelium (Fig. 1). They have a columnar profile extending between the epithelial surface and the basement

Fig. 1. Histological section of *Dasybus hybridus* olfactory mucosa showing row of nuclei of supporting cells (1), rows of olfactory receptor neurons (2), and row of basal cells (3) and a Bowman's gland duct traversing the epithelium (d). The lamina propria shows Bowman's gland (BG), olfactory nerves (ON) and blood vessels (BV). *b*, bone. Bar, 40 µm.

Fig. 2. Apical region of the olfactory epithelium. Microvilli of supporting cells (SC) are seen between olfactory knobs (OK). Bar, 2.4 µm.

Fig. 3. Detail of the apical region of a supporting cell with a terminal web (TW) consisting of bundles of microfilaments running parallel to the apical cellular surface. Small vesicles (arrow) and coated endocytotic vesicles (arrowhead) are observed. Inset. Higher magnification of a coated endocytotic vesicle (arrowhead) in the apical surface. *m*, microvilli; bar, 0.3 µm.

Fig. 4. Detail of apical region of supporting cells showing relationship between neighbouring cells. 1, zonula occludens; 2, zonula adherens. Bar, 0.3 µm.

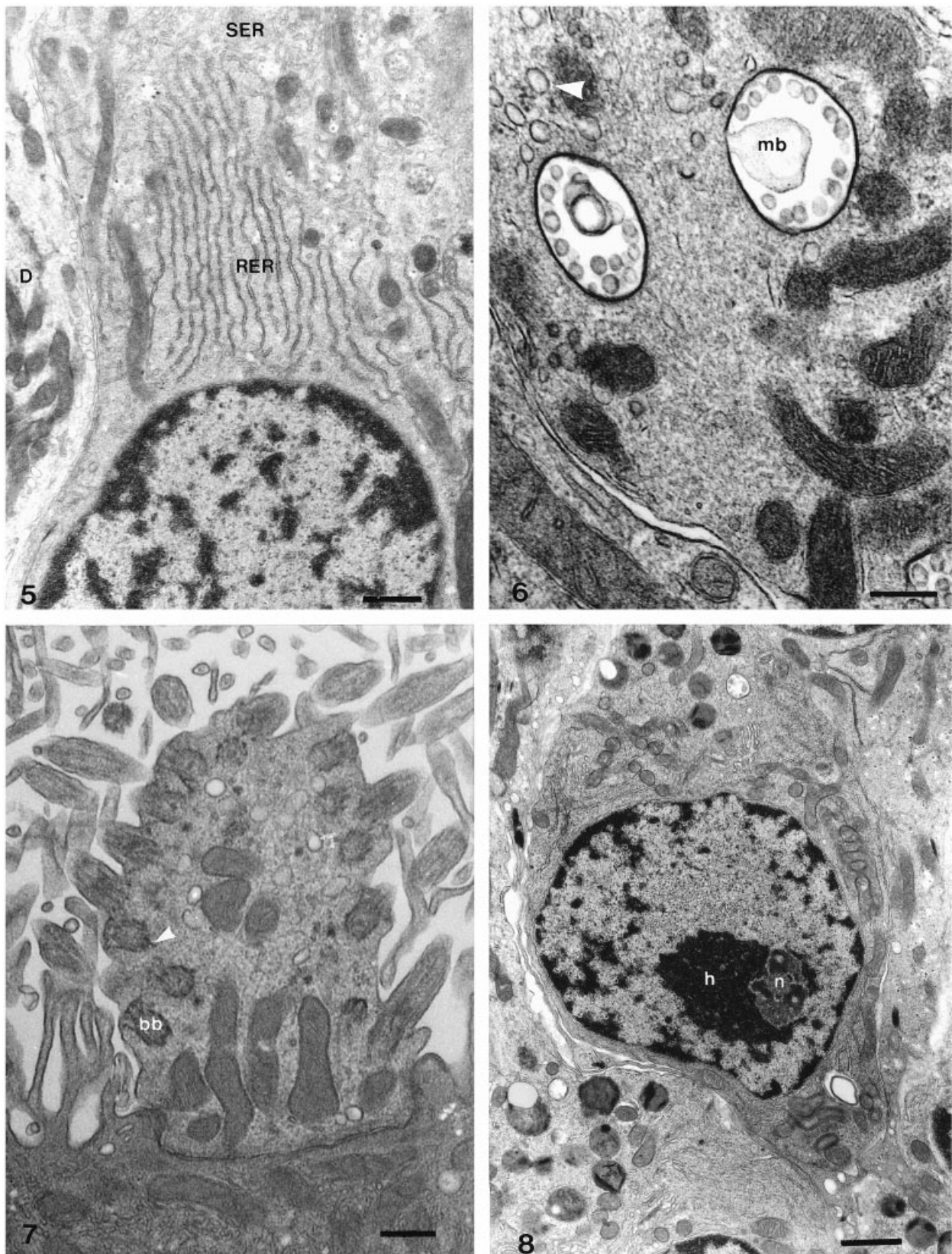


Fig. 5-8. For legends see opposite.

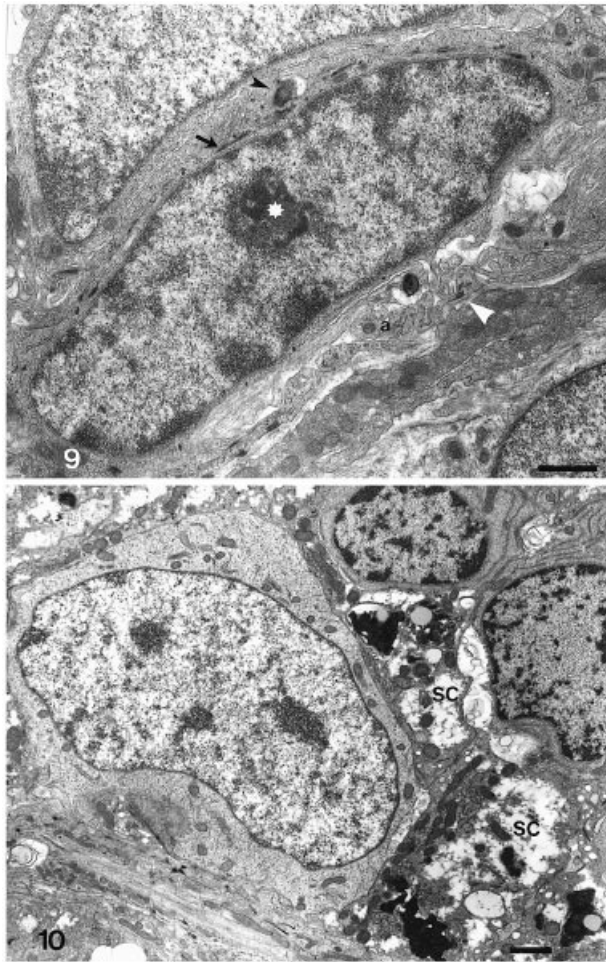


Fig. 9. Flat basal cell contacting the basal lamina (white arrowhead). The nucleus exhibits scattered clumps of chromatin and a nucleolus (*). The cytoplasm shows bundles of tonofilaments (arrow) and centrioles (black arrowhead). Cytoplasmic processes ensheath bundles of olfactory axons (a). Bar, 0.8 μm .

Fig. 10. Globose basal cell showing pale cytoplasm and a nucleus. The cytoplasm is filled with free ribosomes clumped in rosettes and mitochondria. Basilar processes of supporting cells contain dense vacuoles (SC). Bar, 1.25 μm .

membrane. The surface is covered with long microvilli (Fig. 2). Coated endocytotic vesicles are frequently observed between the microvilli (Fig. 3). The presence of zonulae occludentiae and zonulae adherentiae indicated a close relationship between ORNs and SCs as well as between neighbouring SCs (Fig. 4). Desmosomes were not observed in the junctional

complex. Bundles of microfilaments running parallel to the apical cellular surface constitute the terminal web, which forms a zone free from organelles where only a few small vesicles are observed (Fig. 3). The nucleus, located in the upper region of the epithelium, is oval and shows peripheral clumps of heterochromatin (Figs 1, 5). Most of the cytoplasm of the SCs occupies a supra and perinuclear position. The apical cytoplasm has abundant SER, tubular mitochondria and free ribosomes. In the supranuclear region of the SCs, the RER is organised in flattened cisternae tending to be arranged in parallel stacks (Fig. 5). Multivesicular bodies, RER, SER, small vesicles, tubular mitochondria and intermediate filaments are frequently observed in the perinuclear cytoplasm (Fig. 6). The infranuclear region narrows into a thin process expanded in the basal zone and forming basilar expansions. Dense vacuoles are frequently observed in this region. They are pleomorphic with contents varying from homogeneous highly electron dense to fibrillar or lamellar material. Droplets of clear and homogeneous material are frequently found within these vacuoles (Fig. 10). Mitochondria with a dense matrix are also observed in these processes.

Olfactory receptor neurons

The ORNs have a typical flask-like shape. The perikaryon occupies the midportion of the epithelium (Fig. 1). Peripheral dendritic processes are seen extended from the cell body towards the apical surface whereas a proximal axonal process traverses the basal lamina. The dendritic terminal portion protrudes from the free surface of the epithelium forming the olfactory vesicle or olfactory knob (Fig. 7). Numerous cilia, projecting from the olfactory vesicle, arise from corresponding basal bodies within the olfactory knob (Fig. 7). The internal structure of the cilia appears similar to that of ordinary motile cilia, due to the usual 9+2 pattern of microtubules and the structure of the basal corpuscle. Basal feet are seen extending radially from the basal bodies (Fig. 7). Dendrites are clearly distinguished from the surrounding SCs by

Fig. 5. Supranuclear region of a supporting cell with abundant RER organised as parallel stacks of flattened cisternae (RER) and dense SER network (SER). A dendrite (D) is distinguishable from the surrounding supporting cells by its electron lucent cytoplasm. Bar, 0.5 μm .

Fig. 6. Detail of perinuclear cytoplasm of a supporting cell with multivesicular bodies (mb), small vesicles (arrowhead) and tubular mitochondria. Bar, 0.3 μm .

Fig. 7. Longitudinal section of an olfactory knob. Numerous cilia projecting from the olfactory vesicle arise from corresponding basal bodies (bb). Basal feet (arrowhead) extend radially from basal bodies. Numerous mitochondria and small vesicles are also constituents of the cytoplasm of the distal process. Bar, 0.25 μm .

Fig. 8. Longitudinal section including the soma of an olfactory receptor neuron. The nucleus with scanty peripheral chromatin and block of central heterochromatin (h) is associated with the nucleolus (n). The perinuclear cytoplasm contains mitochondria, RER, Golgi complex, multivesicular bodies, lysosome-like bodies and lipofuscin granules. Bar, 1 μm .

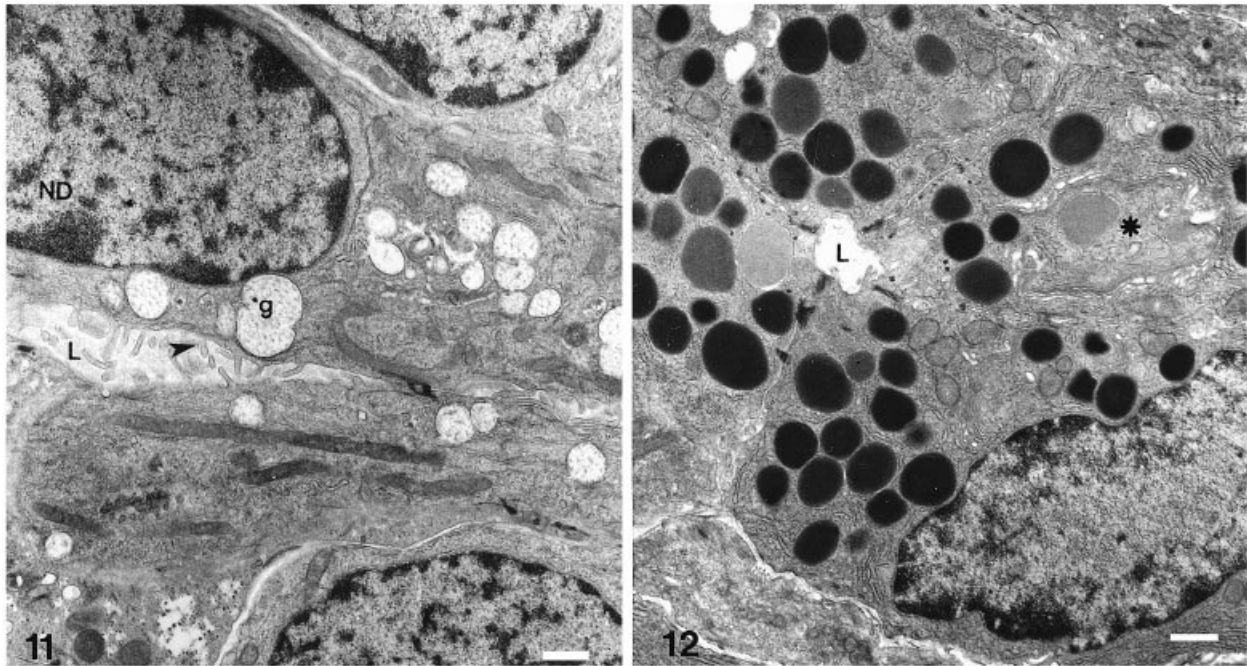


Fig. 11. Bowman's gland duct showing duct cells with microvilli (arrowhead) and secretory granules (g). ND, nucleus of duct cell; L, lumen. Bar, 0.6 μm .

Fig. 12. Survey electron micrograph of Bowman's gland serous acini. The cytoplasm shows numerous secretory granules some of which vary in electron density. L, lumen; asterisk, immature serous secretory granules associated with the Golgi complex. Bar, 1 μm .

their electron lucent cytoplasm with tubular mitochondria, longitudinally oriented neurotubules and small vesicles (Fig. 5). The nuclei are round or irregularly shaped with scattered chromatin clumps (Fig. 8). However, some ORNs show a very large clump of heterochromatin associated with the nucleolus (Fig. 8). The supranuclear region contains enormous Golgi complexes. Mitochondria, free ribosomes, lysosome-like bodies, multivesicular bodies, RER and lipofuscin granules are also seen (Fig. 8). The Golgi complexes are generally associated with small vesicles. The infranuclear cytoplasm exhibits mitochondria, RER, lysosome-like bodies and free ribosomes. The cytoplasm narrows into an axonal process, which crosses the basal lamina before being collected as olfactory nerves in the lamina propria.

Basal cells

Flat basal cells. These are situated above the basal lamina. The nucleus is elongated with the long axis parallel to the basal membrane. It shows scattered clumps of heterochromatin. Nucleoli are frequently observed (Fig. 9). The cytoplasm contains mitochondria, RER, Golgi complex and multivesicular bodies. Conspicuous bundles of tonofilaments and

centrioles are also frequently observed (Fig. 9). Cytoplasmic processes ensheath bundles of olfactory axons before being collected into the olfactory nerves. Hemidesmosomes relate the flat basal cells to the basal membrane (Fig. 9).

Globose basal cells. These are large and pale cells situated among the FBC. They exhibit around profile, with big nuclei and scarce cytoplasm. Both nucleus and cytoplasm are electron lucent (Fig. 10). The cytoplasm is filled with free ribosomes clumped in rosettes, which appear to be the most prominent organelle (Fig. 10).

Ducts of Bowman's glands

Duct cells are elongated with the long axis perpendicular to the basal membrane. They are characterised by oval nuclei and thin cytoplasm. The luminal surface shows short microvilli. Junctional complexes without maculae adherentiae join the ductal cells (Fig. 11). The cytoplasm contains tubular mitochondria, SER, RER, Golgi complex and multivesicular bodies. Secretory granules with flocculent material immersed in a finely granular matrix are seen. These granules are similar to those found in the mucous acinar cells of the BG (Fig. 11).

Bowman's glands

The BG are compound-branched tubulo-acinar mixed glands. The endpieces consist of mucous and serous secretory units. Both types of cell are pyramidal with basal round or irregularly shaped nuclei.

Serous cells are the most numerous. Their round or oval nuclei are located basally (Fig. 12). Their luminal surface is lined with short microvilli. Neighbouring cells within the acini are joined by a typical junctional complex with zonulae occludentiae, zonulae adherentiae and maculae adherentiae (Fig. 12). Electron dense granules are located in the apical cytoplasm (Fig. 12). These membrane-bounded secretory granules are discrete with a structureless matrix. They show varying degrees of electron density from cell to cell and sometimes within the same cell. The cytoplasm contains RER, SER and mitochondria. An extensive supranuclear Golgi apparatus with small vesicles and condensed vesicles can be seen (Fig. 12). The infranuclear region shows a tightly packed set of whorled RER cisterns. The lateral borders are straight with folds showing microvilli. Basal lamina and collagen fibres surround the acini (Fig. 12). No images suggesting secretory activity were observed.

The mucous cells are pyramidal shaped with round nuclei located basally. Their most prominent feature is the large number of mucous secretory granules filling the supra and perinuclear cytoplasm (Fig. 13). The luminal surface is lined with scarce and short microvilli. The apical membranes are in close apposition to junctional complexes (Fig. 13). The apical cytoplasm contains a dense SER network, tubular mitochondria and well developed Golgi complexes generally related to small vesicles and secretory granules (Fig. 14). The secretory granules are membrane-bounded and coalescent (Figs 13, 15). They are filled with a lucent matrix in which a fibrillogranular material is suspended. These granules have flocculent aggregates of varied appearance, from faint to rather dense material (Fig. 15a). In some granules, punctate densities or dense spherules may be observed (Fig. 15b). Scarce mucous cells contain mucous granules with dense punctate material and show no propensity for fusion (Fig. 13). The mode of discharge of the secretory granules may be described as a merocrine process. The infranuclear cytoplasm has lipofuscin granules, mitochondria, numerous cisterns of SER and scanty RER. Collagen fibres surround the basal membrane of the acini (Fig. 13).

None of the grids analysed showed mitotic figures, intercellular canaliculi or myoepithelial cells in the BG.

Olfactory nerves

The unmyelinated olfactory nerves are ensheathed by Schwann cells. The axons are separated into bundles by branched processes of Schwann cells (Fig. 16). Thus each bundle has a group of closely packed axons. Axons show mitochondria, microtubules, neurofilaments and small vesicles. The olfactory Schwann cell shows a round pale nucleus. The cytoplasm is scarce and contains free ribosomes, mitochondria and centrioles (Fig. 16). Collagen fibres appear longitudinally oriented forming a thin endoneurium, enclosing each fascicle of axons (Fig. 16).

DISCUSSION

The OM of *Dasypus hybridus* appears organised in a similar way as most of mammals. Interestingly, when *Dasypus hybridus* OM is compared with *ChaetophRACTUS villosus*, some morphological differences are evident. The SCs of *Dasypus hybridus* appear lack secretory granules, as is true of most of mammals in which these cells appear to be nonsecretory (Getchell & Getchell, 1992). This characteristic differs from those found in mice (Frisch, 1967) and *ChaetophRACTUS villosus* (Ferrari et al. 1998a). Apocrine-like protrusions, apoptotic bodies and the peculiar SER arrangement, characteristic of *C. villosus*, are not found in *D. hybridus*. However, we found a dense network of SER in *D. hybridus*. An abundance of SER in other cellular systems has been associated with several functions (Ponzio, 1996) including lipid and steroid synthesis, glucose metabolism and detoxification. The SER is much more strongly developed in *C. villosus* than in *D. hybridus*. This might be related to the extraordinarily powerful digging activity displayed by *C. villosus* in comparison with that of *Dasypus*. This behaviour permanently exposes the nasal cavity to a variety of soil materials in spite of the protective nasal structures situated at the entrance of the nostrils (Affanni et al. 1987). Presumably that exposure determines an intense mandatory xenobiotic activity. The xenobiotic function of SCs was described by Lewis & Dahl (1995). Of course, the less developed SER of *Dasypus* does not exclude xenobiotic activity.

The functional role of SCs has not yet been analysed extensively. Some authors suggest a role similar to glial cells (Rafols & Getchell, 1983). They are also thought to participate in the regulation of the ionic composition of the mucous layer (Getchell et al. 1984), mucus secretion, molecular transport and guiding

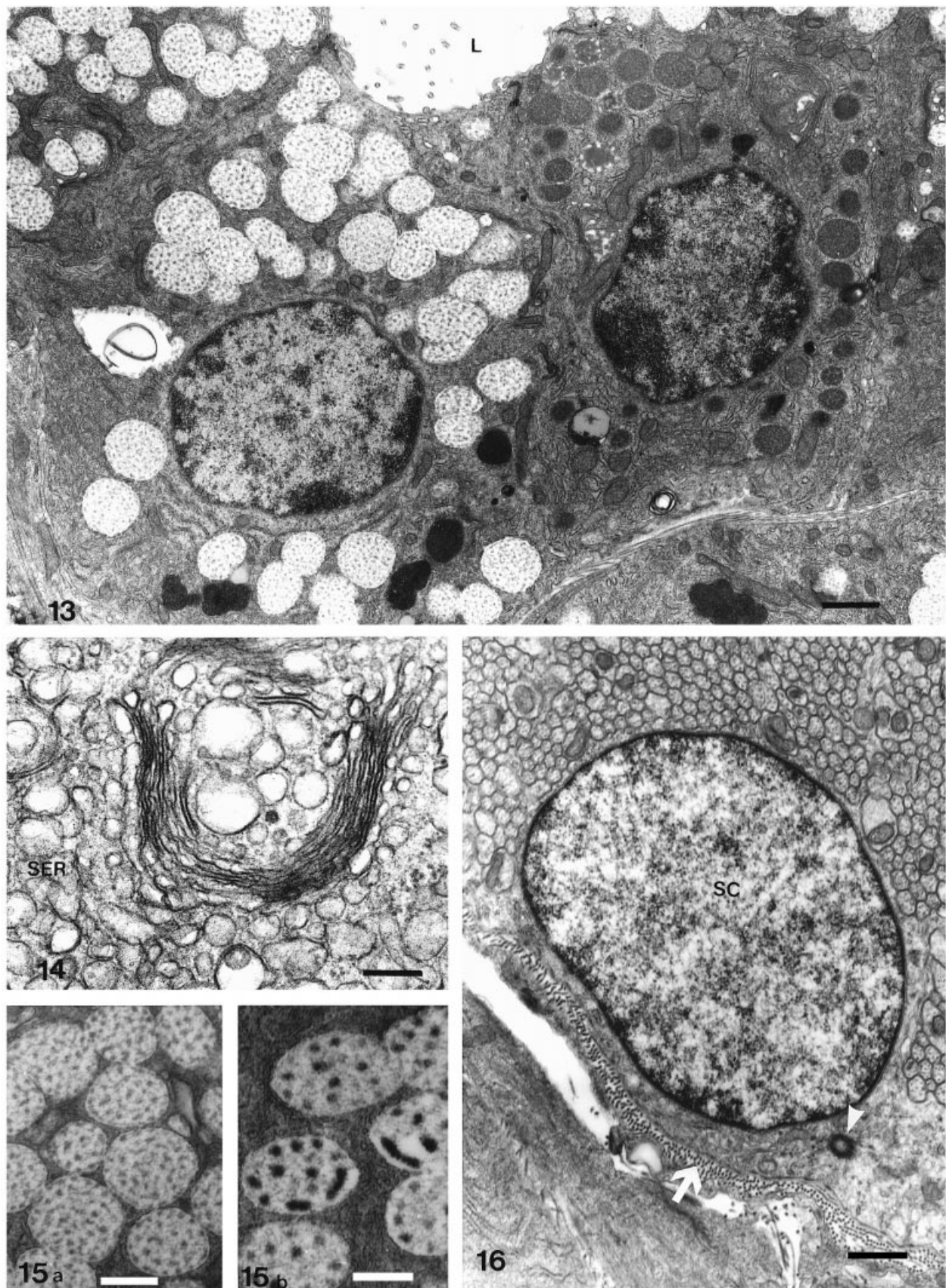


Fig. 13–16. For legends see opposite.

task for developing neurons and their processes (Morrison & Constanzo, 1992).

An interesting feature of *Dasypus*, similar to the one described in *C. villosus* (Ferrari et al. 1998b) is represented by the dense vacuoles in the basilar processes of SCs. These basally located vacuoles presumably represent an endocytotic system comparable to that described by Bannister & Dodson (1992). This system involves endocytotic coated-vesicles, small vesicles, multivesicular bodies and dense basal bodies (Bannister & Dodson, 1992). Furthermore, these organelles are seen in the SCs cytoplasm of *D. hybridus*. Endocytosis in supporting cells may help to establish an odourant-clean zone on the epithelial surface (Pevsner et al. 1985).

The ORNs of *Dasypus hybridus* possessed lysosome-like and multivesicular bodies in their perikarya. These neuronal organelles would suggest the presence of an endocytotic system capable of taking exogenous material from the mucus into the cell (Bannister & Dodson, 1992). The endocytotic mechanism could help to reduce background odourant levels at the olfactory transduction sites, by taking up and metabolising odorants (Bannister & Dodson, 1992).

The duct cells of BG exhibit secretory activity similar to those of mice, dogs, rabbits (Getchell & Getchell, 1992) and *C. villosus* (Ferrari et al. 1998a). The general histological structure of the BG of *Dasypus* coincides with reports from other mammals in which there are serous and mucous cells (Getchell & Getchell, 1992). The proportion of serous endpieces is greater than that of the mucous ones. This differs from that found in *C. villosus* in which this relation is inverted (Ferrari et al. 1998a). This feature might be related to the secretion of different products in each species, as demonstrated by classic histochemistry and lectin binding studies (Ferrari, 1997; Ferrari et al. 1999, 2000). The mucous secretion in *Dasypus hybridus* seems to be released by a merocrine mechanism, as was previously observed in *ChaetophRACTUS villosus* (Ferrari et al. 1998a). Contrasting with *C. villosus*, in *Dasypus hybridus* we did not observe an apocrine secretory mechanism. Studies regarding BG have been less intense than those for the OM. It is known,

however, that BG play an important functional role in immunological processes (Kaliner, 1991), xenobiotic activity (Lewis & Dahl, 1995), and the production of odour-binding proteins and growth factors (Farbman & Buchholz, 1996), as well as regeneration of the OM (Schowb et al. 1995).

Myoepithelial cells were not seen in the BG of *Dasypus*. This absence has also been noted in other mammals (Getchell & Getchell, 1992) including *ChaetophRACTUS villosus* (Ferrari et al. 1998a), a characteristic also shared with vomeronasal glands (Carmanchahi et al. 1999).

According to Jorge et al. (1985) and Cetica (1998), *Dasypus hybridus* is more primitive than *ChaetophRACTUS villosus*. However, the OM of *Dasypus hybridus* shows characteristics similar to those of more recent mammals. The complex ultrastructural features of the OM of *ChaetophRACTUS villosus* might have evolved from simpler structures comparable to those of *Dasypus hybridus*, as an adaptation to its intense digging habits.

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Fig. 13. Survey electron micrograph of a region of a Bowman's gland acinus showing mucous cells, with a typical mucous cell containing coalescent granules with flocculent aggregates suspended in a light matrix (left) and an atypical mucous cell with discrete granules with dense punctate material (right). L, lumen. Bar, 1 μ m.

Fig. 14. Golgi complex of mucous cells. Note the proximity of the Golgi complex to apparently newly generated secretory granules. SER, network of smooth endoplasmic reticulum. Bar, 0.025 μ m.

Fig. 15. Gallery of mucous secretory granules. (a) These granules have flocculent aggregates immersed in a lucent matrix, from faint to rather dense material. (b) Granules with electron lucent matrix in which denser inclusions are suspended. Bars, 0.5 μ m.

Fig. 16. Transverse section of a fascicle of an olfactory nerve. Each nerve is composed of unmyelinated axons enclosed by Schwann cells (SC) and collagen fibres (arrow) forming the endoneurium. Arrowhead, centriole. Bar, 0.6 μ m.

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