The olfactory apparatus of the bandicoot (*Isoodon macrourus*): fine structure and presence of a septal olfactory organ

JEAN E. KRATZING

Department of Veterinary Anatomy, University of Queensland, St Lucia, Brisbane

INTRODUCTION

The fine structure of the olfactory epithelium of vertebrates has been widely investigated: it exhibits a high degree of uniformity, particularly in land-dwelling vertebrates (Graziadei, 1971). Among mammals, olfactory receptor cells differ little between such diverse animals as the mole (Graziadei, 1966), the dog (Okano, Weber & Frommes, 1967), and the sheep (Kratzing, 1970). The supporting cells, however, are less uniform, variations being seemingly related to the secretory activity of these cells in some species. Reports of rarer cells include a 'fourth cell type' described by Okano *et al.* (1967) in the dog, and a 'fifth cell type' described by Andres (1969) in the cat.

Variations in the anatomical distribution of the sensory epithelium have received little attention. A patch of olfactory epithelium on the nasal septum isolated by respiratory mucosa from the main sheet of sensory epithelium was described by Rodolfo-Masera (1943) and designated a *septal olfactory organ*. Rodolfo-Masera described the organ in a number of fetal and adult rodents and stated that it was found on the posterior and ventral region of the septum caudal to the vomeronasal organ. He investigated several species other than rodents, and described the presence of the organ in two *Didelphys* fetuses, though poor preservation prevented an adequate histological description. There does not appear to have been any other investigation of the occurrence or significance of this isolated patch of sensory epithelium.

The present study forms part of an investigation of the olfactory and vomeronasal organs of a number of marsupial families. The olfactory apparatus of marsupials has not previously been studied in detail, though the group includes many species in which olfaction plays an important role in survival. The bandicoot is a ground dwelling, nocturnal, omnivorous animal with well developed olfactory and vomeronasal organs and a distinct separate septal organ.

MATERIAL AND METHODS

Specimens for electron microscopy were taken from four male and two female bandicoots trapped in the wild. Fixation was by perfusion of 1·5 % glutaraldehyde in 0·1 % cacodylate buffer, pH 7·2, containing 4 % polyvinylpyrrolidone (Bohman & Maunsbach, 1970). The perfusate was introduced via the left ventricle with the animal deeply anaesthetized with pentobarbitone sodium. After fixation the nasal region was sectioned transversely into 2–3 mm slices. These were decalcified in 4·13 % EDTA for approximately 14 days at 4 °C (Warshawsky & Moore, 1967).

Portions of the conchae and dorsal and ventral areas of the nasal septum were removed for electron microscopy. Tissues were post-fixed in osmium tetroxide, dehydrated in alcohol and embedded in Araldite. Sections were stained with uranyl acetate and Reynolds' lead citrate, and viewed in a Siemens Elmiskop 1A electron microscope. Thick sections (1 μm) stained with toluidine blue were used for orientation.

Specimens for light microscopy were taken from two young adult males and fixed by perfusing with 5 % formalin containing 4 % polyvinylpyrrolidone. The snout region was decalcified in a solution of picric acid, formalin and formic acid. Serial sections were cut at 5–6 μm and every tenth section stained with haematoxylin and eosin. Representative sections were stained with alcian blue and periodic acid-Schiff.

RESULTS

Light microscopy

Distribution of olfactory epithelium

The long nasal region of the bandicoot is divided completely into left and right cavities only in its rostral third. Caudal to this, the left and right cavities are in continuity across the ventral border of the septum (Fig. 1A–C). A complex system of nasal conchae provides a very large surface covered by mucous membranes. The ventral concha (maxilloturbinate) extends well forward to the vestibular region and is covered by non-olfactory ciliated epithelium. The middle concha (nasoturbinate) is a complex scroll in the main part of the nasal cavity and continues rostrally as a simpler ridge on the nasal bone towards the vestibule. The mucosa over the middle concha is mixed, with the proportions covered by olfactory epithelium increasing caudally.

The ethmoturbinate complex in the caudal part of the nasal cavity is also covered by a mixed epithelium which is predominantly olfactory, but changes to a low epithelium containing goblet cells over the part of the scrolls which face ventrally. The dorsal concha lies partly within the space of the maxillary sinus, and a limited amount of olfactory epithelium occurs dorsally in the more rostral part of the sinus. Caudally, the sinus is lined with ciliated epithelium overlying a glandular lamina propria.

The vomeronasal organ consists of a tube on each side of the base of the nasal septum in the rostral third of the nasal cavity. In this region the septum completely separates the nose into a right and a left cavity. Caudal to the vomeronasal tubes, the septum loses its attachment to the floor of the nasal cavity and ends ventrally with two rounded lateral extensions (Fig. 1 C). The bony septum underlying this structure has roughly the shape of an inverted letter 'T' and supports a glandular lamina propria well supplied with blood vessels and nerves.

Olfactory mucosa extends down from the roof of the nasal cavity over most of the septum, but is replaced ventrally by non-sensory ciliated, pseudostratified epithelium containing numerous goblet cells. The septal olfactory organ consists of two strips of sensory epithelium surrounded by this ciliated mucosa, and extends along the lateral and ventral aspects of the ventral flanges of the septum. It begins well forward in the nasal cavity, caudal to the termination of the vomeronasal organ, becomes more ventral in position and terminates before the pharyngeal end of the septum. A careful investigation of two sets of serial sections did not show any continuity between the olfactory epithelium of the septal organ and that of the general sheet of olfactory mucosa over the dorsal part of the septum.
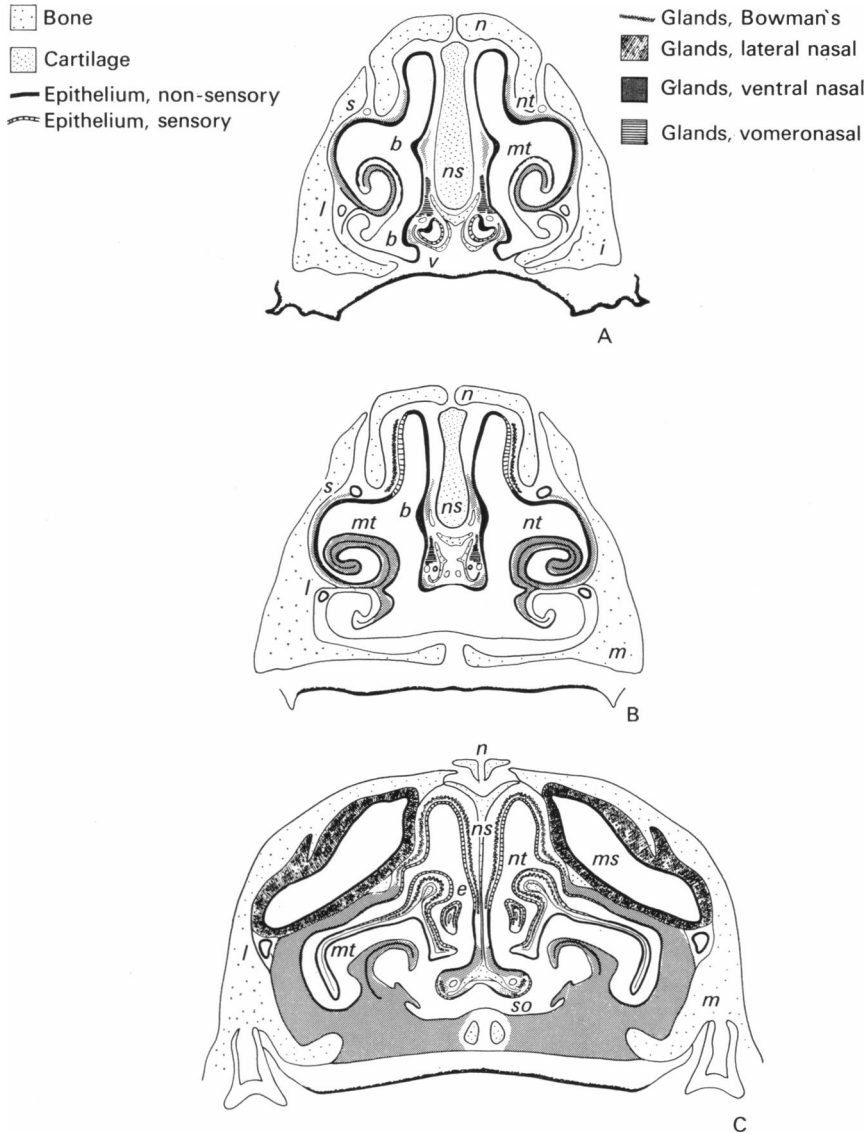
Fig. 1 (A–C). Diagrammatic cross section of the bandicoot snout showing distribution of types of epithelium and glands: (A) at the level of the third incisor; (B) at the level of the canine tooth; (C) at the level of the first molar. *b*, swell body; *e*, ethmoturbinate; *i*, incisive bone; *l*, lacrimal duct; *m*, maxilla; *ms*, maxillary sinus; *mt*, maxilloturbinate; *n*, nasal bone; *ns*, nasal septum; *nt*, nasoturbinate; *s*, duct of Steno; *so*, septal olfactory organ; *v*, vomeronasal organ.

The epithelium of the septal olfactory organ is distinguished by a characteristic olfactory epithelial structure with nuclear stratification into a superficial row of supporting cell nuclei, a wider zone of sensory cell nuclei, and a single line of basal cell nuclei. Immediately under the epithelium, and opening on to its surface, are typical Bowman's glands. Underneath these, and extending beyond the sensory epithelium are the glands of the nasal septum. These have distinctive staining reactions and structural features and their ducts open on to the respiratory mucosa.

They resemble glands in the ventrolateral part of the nasal cavity. Goblet cells are absent from the sensory mucosa but are frequent in the surrounding epithelium.

The sensory epithelium of the septal organ is slightly lower than its counterpart on the rest of the septum, and the nuclei are tightly packed. In other respects, the structure of the two regions is similar.

In the rostral third of the nasal cavity, there are a pair of 'swell bodies' on the nasal septum, a region raised by underlying glandular and vascular tissue and a similar pair in the region of the vomeronasal tubes. These swell bodies do not occur on the septum further caudally (Fig. 1A, B). The epithelium over the swell bodies is pseudostratified and non-ciliated, without olfactory cells.

Fine structure

The cell types are similar in all the olfactory regions. Sensory cells (Fig. 2A), supporting cells, basal cells and the duct cells of Bowman's glands in general have the structure described for their counterparts in other mammalian olfactory mucosa. Two other cell types occur which have unusual features. One, the brush cell (Fig. 2B), resembles closely the 'fifth cell type' described by Andres (1969), while the other, a pale cell (Fig. 8A–C), does not appear to have been described previously in olfactory epithelium.

Supporting cells. The nuclei of supporting cells form a distal row in the nuclear zone. Rough endoplasmic reticulum lies just above the nucleus, smooth reticulum and mitochondria occur throughout the cytoplasm, but there is no evidence of secretion droplets. Microvilli bordering the cells are about 2–2·5 μm long, usually unbranched, and do not have a fibrillar core (Figs. 2, 8C).

Olfactory cells. The nuclei of olfactory cells lie in a wide band towards the base of the epithelium. Their perinuclear cytoplasm contains a prominent Golgi apparatus and extensive rough endoplasmic reticulum (Fig. 3A, B). A proximal axonic process leaves the epithelium to join the fila olfactoria in the lamina propria. The distal dendritic process extends to the epithelial surface and ends in an olfactory rod carrying a number of cilia (Fig. 2A). Cilia are of the $9+2$ type for an initial segment of about 0·5 μm, after which they narrow abruptly to a long terminal section with microtubules reducing to a single central pair.

Structural variation in olfactory cells is evident in all specimens and in all areas of the sensory epithelium, particularly in the septal organ, and this is most obvious in the perikaryon and distal process. The cytoplasmic density of the perikaryon varies from light to very dark. Light cells have a well-developed Golgi apparatus, limited amounts of rough endoplasmic reticulum and a few mitochondria. Their cytoplasm is studded with free ribosomes (Fig. 3A). Dark cells have well organized rows of rough reticulum, more numerous mitochondria and dark bodies which are probably lysosomes (Fig. 3B).

Perikarya showing all gradations from light to very dark can be found, and pycnotic nuclei are seen in some of the very dark cells. Disintegrative fragments also occur, but cannot be referred to any particular cell type. Both dark and light cells appear throughout the nuclear zone in close proximity to one another, but more light cells are found towards the epithelial base. Cells in this position sometimes show a cluster of centrioles close to the nucleus (Fig. 5). Light cells near the epithelial base resemble one type of basal cell, but may be distinguished by tracing their proximal processes through the basal lamina (Fig. 4), or by the presence of a basal cell process separating most of the cell body from the basal lamina.
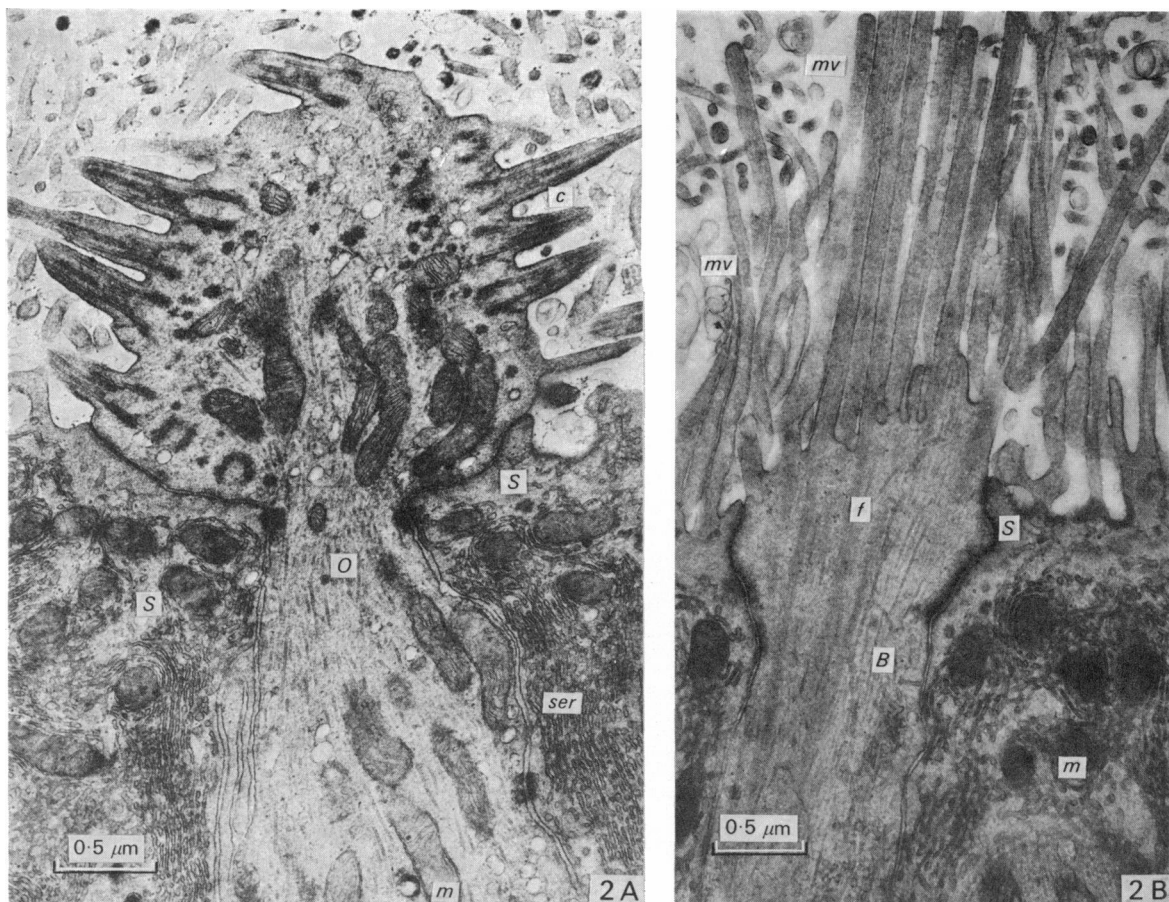
Fig. 2 (A–B). Surface of the olfactory epithelium. Olfactory cells (*O*), supporting cells (*S*) and brush cells (*B*) all project into the border of cilia (*c*) and microvilli (*mv*). *f*, microfilaments; *m*, mitochondria; *ser*, smooth endoplasmic reticulum.

While variations in cytoplasmic density also occur in the dendritic process, variation in the number of cilia and basal bodies is more noticeable. A section of a typical rod carries eight to twelve cilia, but some dendritic processes have simple rounded ends with few or no cilia and a cluster of basal bodies well below the surface.

Basal cells. Two very different types of cell lie at the base of the epithelium (Fig. 6). One is more numerous and might be described as a typical basal cell. It is irregular in outline with long, thin cytoplasmic processes which make contact with other cells, and curve around bundles of olfactory axonic processes. The long axis of the nucleus is usually parallel with the basal lamina. The cytoplasm of these cells is relatively electron-dense and contains prominent dark fibrillar bundles. A centriole is often seen near the nucleus, and occasionally a cilium may extend into the intercellular space. Basal cell processes can often be seen surrounding sections of dark cytoplasm filled with abundant mitochondria.

The second type of basal cell resembles a light olfactory cell; it has pale cytoplasm, lacks fibrillar bundles, and is filled with abundant free ribosomes. Like the more

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3 B

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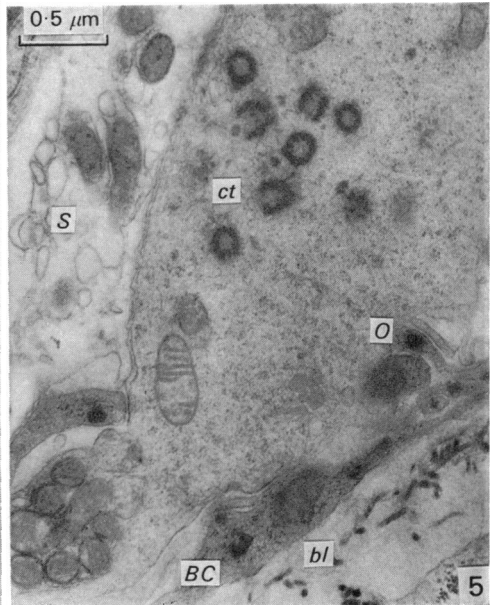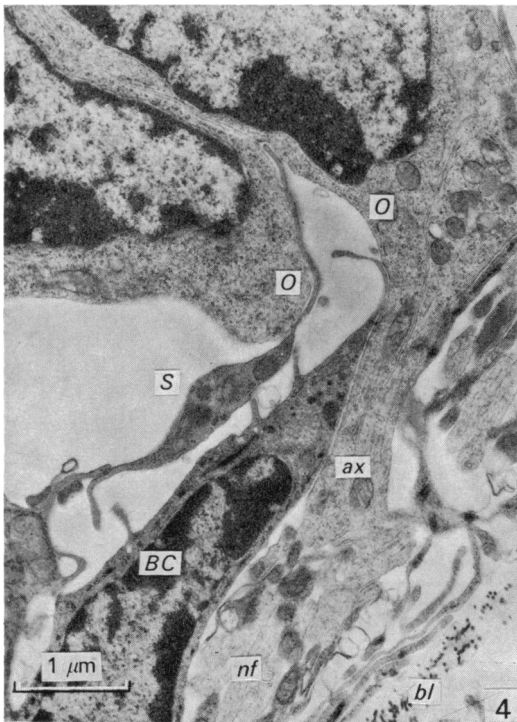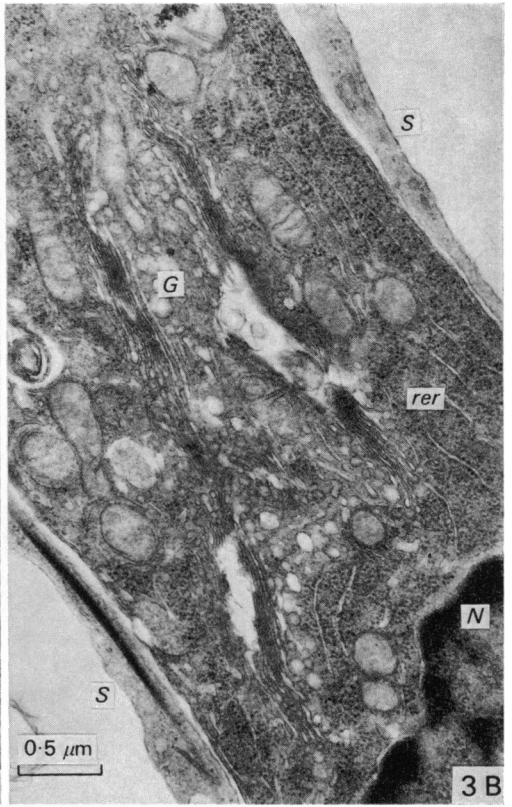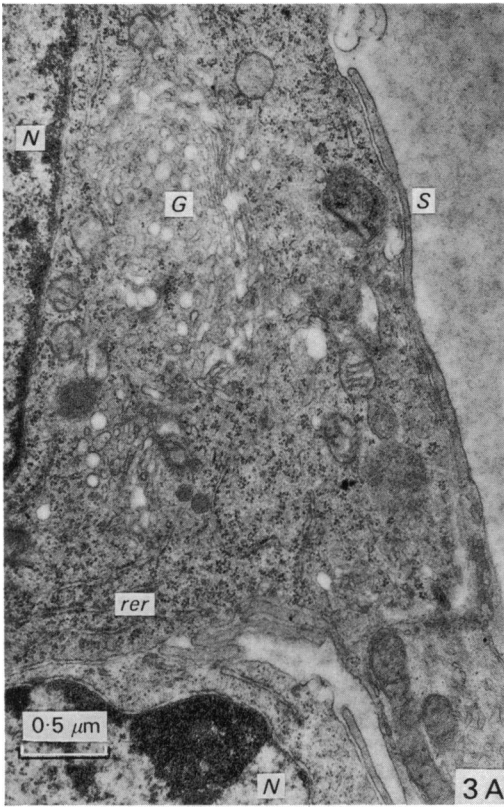
frequent type, it also encloses bundles of axons. Mitotic cells can be seen infrequently at basal cell level (Fig. 7). Macrophages occur up to the level of sensory cell nuclei, but are more frequent at basal cell level. They are more numerous in the septal organ than in the other olfactory sites.

Pale cells. The cytoplasm of these large cells is electron-lucent throughout, making them stand out in sharp contrast to the rest of the epithelium. Mitochondria are numerous except at the cell surface, and have a dense matrix and irregular cristae. Smooth endoplasmic reticulum is more frequent in the supranuclear cytoplasm, but occurs throughout the cell. Vacuoles of irregular profile, multivesicular bodies, and a few darker bodies, probably lysosomes, also occur (Fig. 8 A–C).

The surface of a pale cell protrudes in a manner similar to an olfactory cell. It lacks cilia or basal bodies, but some cells have a pair of centrioles towards the surface. The microvilli of these cells are less regular in shape than those of the supporting cells, and do not have a fibrillar core. Pinocytic vesicles can be seen pinching off from the cell membrane at the base of the microvilli (Fig. 8 A).

Because of their large size, pale cells are difficult to follow through the full height of the epithelium. Those that have been traced have not shown evidence of neuronal contact with any other type of cell. They appear to rest broadly on the basal lamina, and do not have an axonal process continuing through into the lamina propria in the manner of the receptor cells (Fig. 8 C). They have junctional complexes with supporting cells at the epithelial surface, and, deeper in the epithelium, lateral processes interdigitate with neighbouring cells.

Brush cells. These are the least frequently encountered cells in the epithelium. Counts show one brush cell for about every 200 olfactory cells at the epithelial surface. Brush cells have a number of straight unbranched microvilli about 0·1 μm in diameter, each with a fibrillar core which continues well down into the superficial cytoplasm (Figs. 2B, 9). Like the olfactory cells, brush cells have an enlarged perikaryon, and a proximal and distal process. The nucleus is oval with large and small clumps of chromatin, not readily distinguished from nuclei of supporting cells. The main distinguishing feature of the cytoplasm in all parts of the cell is the presence of fibrillar bundles (Fig. 9). In so far as it is possible to generalize about a cell seen so infrequently, the perinuclear cytoplasm contains sparse rough endoplasmic reticulum, small vesicular profiles of smooth reticulum, free ribosomes and scattered mitochondria. The Golgi complex is well developed. Centrioles have been observed in most of these cells near the nucleus, and in one example a stack of annulate lamellae was observed close to the nucleus (Fig. 9, inset).

No evidence has been found for any neuronal contact between brush cells and any other cell type. Junctional complexes occur between them and supporting cells at the epithelial surface, and folding processes fit into other cells laterally.

Gland cells. Towards the surface, cells lining the ducts of Bowman's glands have

Fig. 3 (A–B). Perinuclear cytoplasm of (A) light and (B) dark olfactory cells. Note increased cytoplasmic density, increased organization of smooth and rough (*rer*) endoplasmic reticulum in (B). *G*, Golgi apparatus; *N*, nucleus; *S*, supporting cell process.

Fig. 4. Base of olfactory epithelium. Axonic process (*ax*) of a young olfactory cell (*O*) joining a bundle of olfactory nerve fibres (*nf*) at the base of the epithelium. *BC*, basal cell; *bl*, basal lamina; *S*, supporting cells.

Fig. 5. Young olfactory cell as base of olfactory epithelium. Early ciliogenesis is indicated by the number of centrioles (*ct*). Note that this cell is separated from the basal lamina (*bl*) by processes of basal cells (*BC*). *S*, supporting cell.
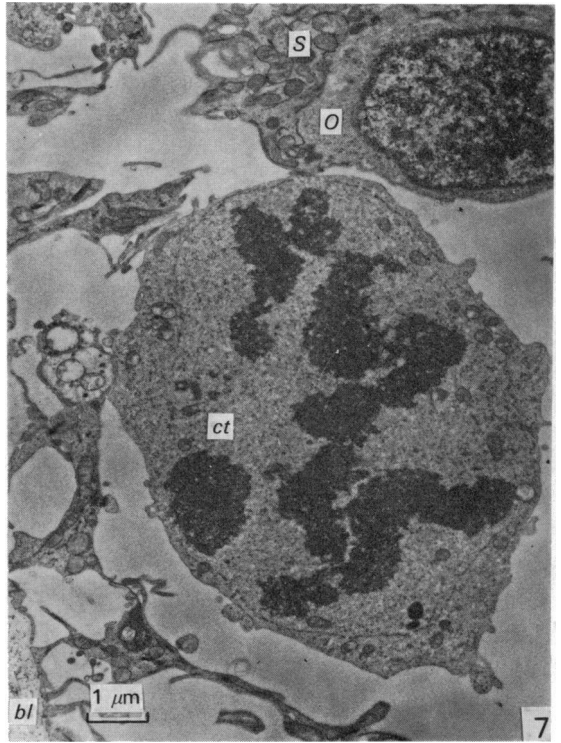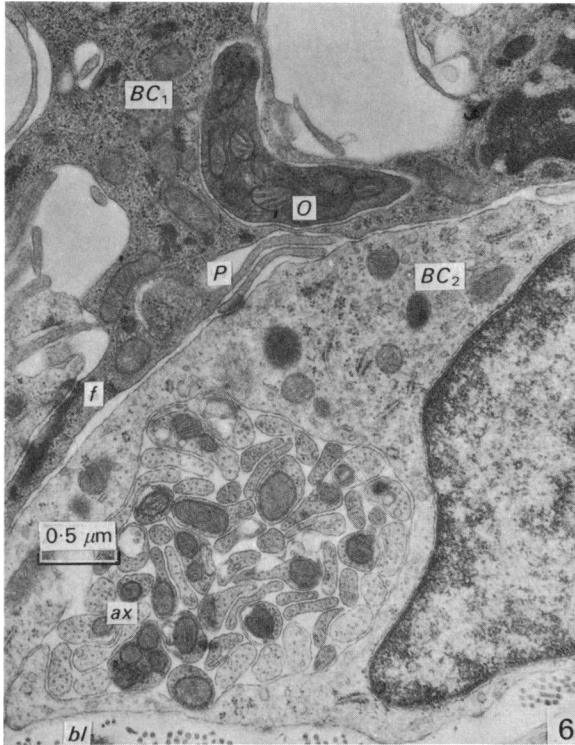
Fig. 6. Two types of basal cell. BC_1 has dense cytoplasm, dark bundles of microfilaments (*f*), slender branching processes (*P*). BC_2 has pale cytoplasm, smooth contours, lacks fibrillar bundles. Both may enclose bundles of olfactory axons (*ax*). The dense process (*O*) is probably from a dark olfactory cell. *bl*, basal lamina.

Fig. 7. Mitotic cell at base of olfactory epithelium. The small cluster of centrioles (*ct*) suggests formation of a young olfactory cell of the type seen in Fig. 5. *O*, olfactory cell; *S*, supporting cell.

a cytoplasm containing fibrillar bundles which is similar to that of basal cells. Deeper in the epithelium, the lining cells change to the secretory type, but most of the secretory part of these simple tubular glands lies below the basal lamina.

DISCUSSION

The structure of typical olfactory cells in the bandicoot does not appear to differ significantly from that described for other mammals. In particular, surface details closely resemble those described in the cat by Andres (1969). Minor differences in ciliary basal feet may be noted. Transitional fibres are present, but basal feet and basal filaments, which are reported for other mammals, are not present in the bandicoot. The structural variation in olfactory cells suggests a population of cells at different stages of development and deterioration. Electron-lucent cytoplasm studded with free ribosomes, and a paucity of rough endoplasmic reticulum is characteristic of olfactory cells in fetal and neonatal rats, as are clusters of centrioles in the perikaryon or well below the surface of the dendrite (Kratzing, 1971). Similar clusters have been noted in the olfactory cells of pouch young of the bandicoot and possum (Kratzing, unpublished observations). All these features are
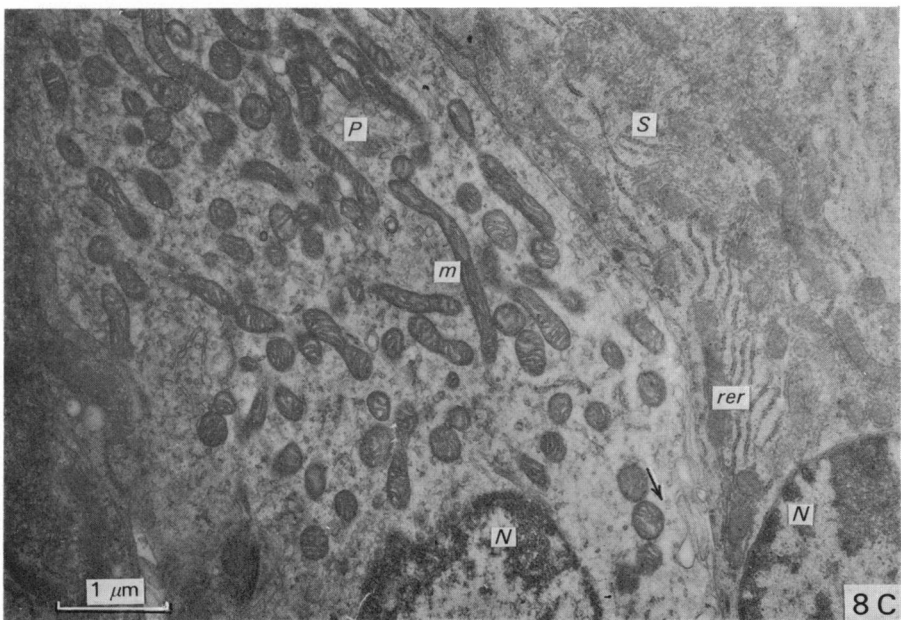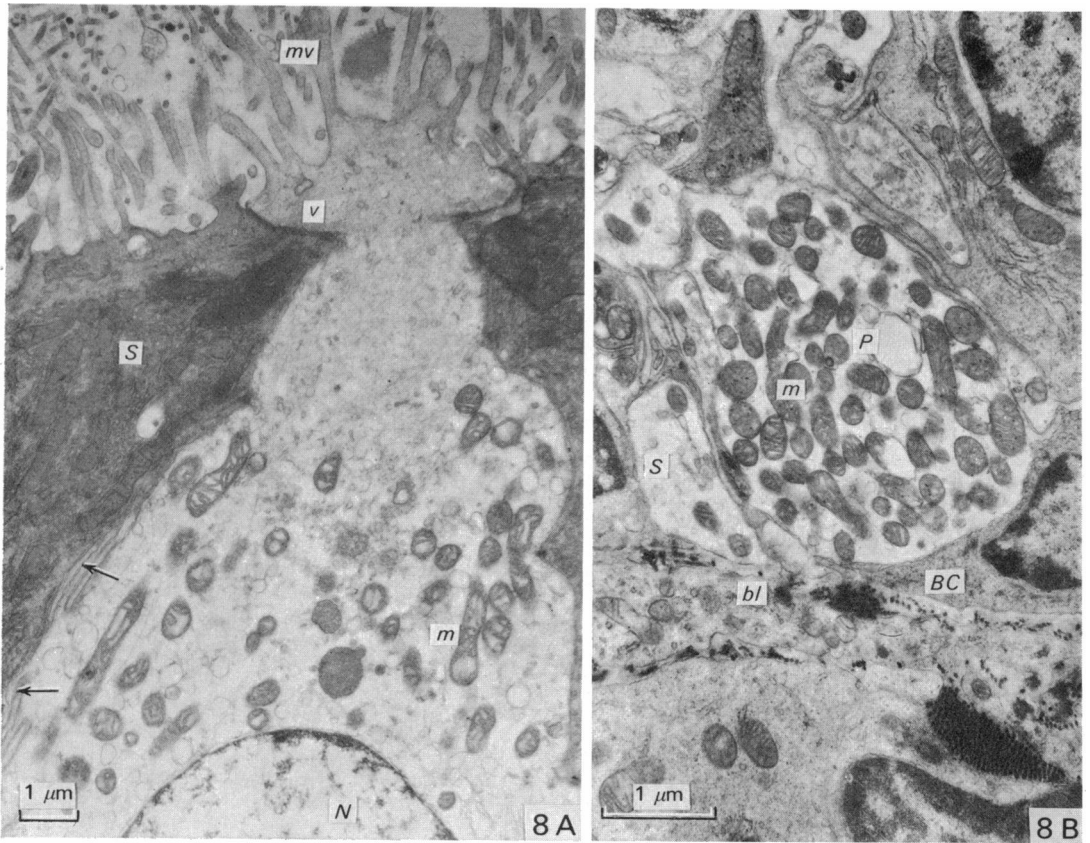
Fig. 8 (A–C). Pale cell (*P*): surface (A), basal (B) and supranuclear (C) regions, surrounded by supporting (*S*) and basal (*BC*) cells. Mitochondria (*m*) with dense matrix are frequent throughout the cytoplasm. Microvilli (*mv*) are more irregular than those of supporting cells. *bl*, basal lamina; *N*, nucleus; *rer*, rough endoplasmic reticulum; *v*, vesicles. Arrows indicate lateral interdigitations with other cells.
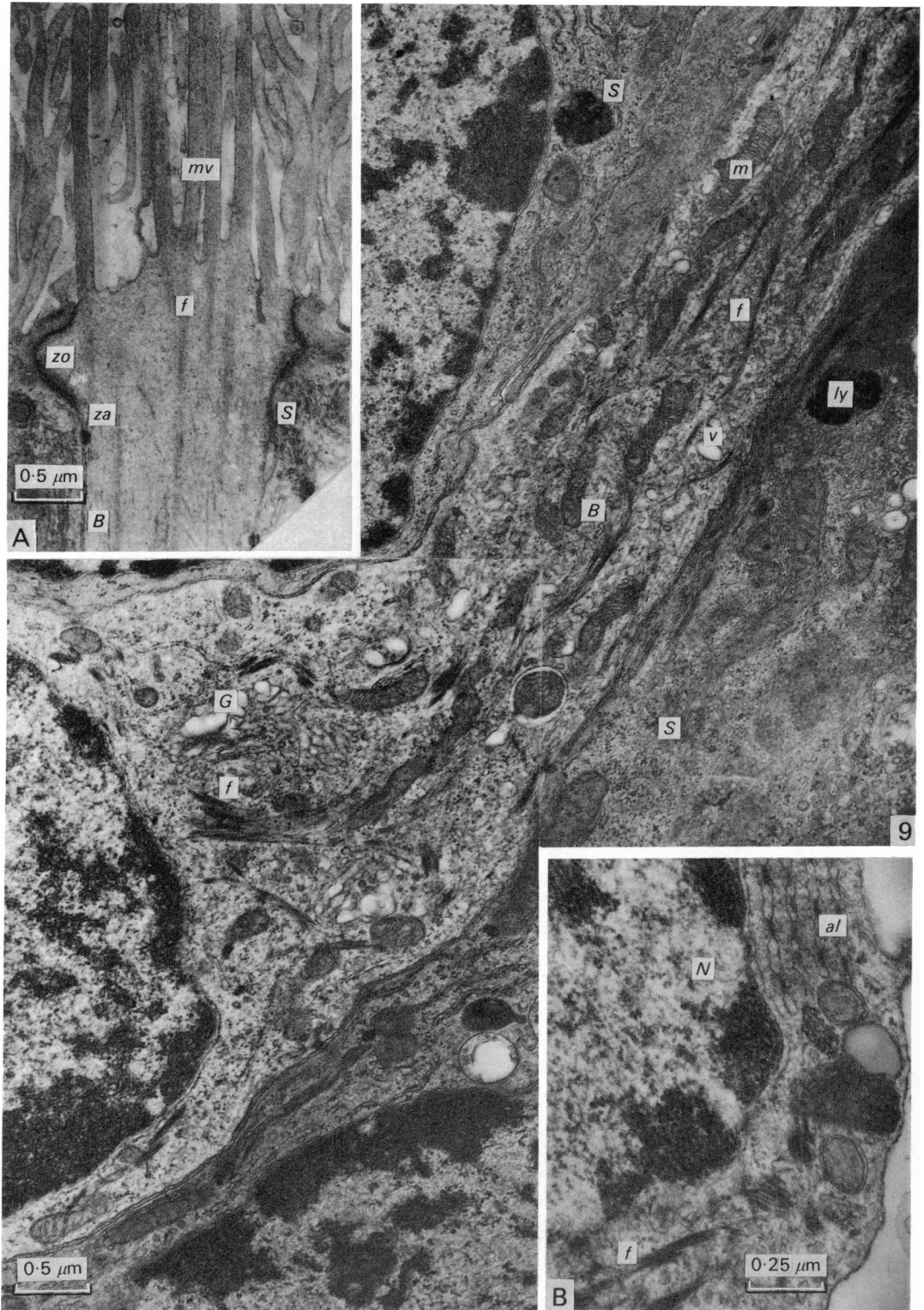
Fig. 9. Brush cell (B) in olfactory epithelium contains mitochondria (m), numerous vesicles (v) and bundles of microfilaments (f). The cell surface (inset A) has straight microvilli with a central core of filaments (f). Junctions with supporting cells (S) show zona occludens (zo) and zona adherens (za). Inset B shows a stack of annulate lamellae (al) close to the nucleus (N). G, Golgi apparatus; ly, lysosome.

found in the light olfactory cells in the present study. By contrast, dark cytoplasm, pycnotic nucleus, and an increase in vesicles suggest a degenerative or ageing cell.

It has been shown that receptor and supporting cells may be replaced in the olfactory mucosa of adult animals (Graziadei & Metcalf, 1971*a, b*). Andres (1969) postulated a process of replacement of the olfactory rod, the deeper part of the cell remaining to serve as a replacement centre. In the bandicoot the process may be more complete, the whole cell being replaced from some undifferentiated cell deep in the epithelium. Andres (1966) suggested that the source of newly differentiating olfactory cells was a 'blastema cell' located above the basal cells, with cytoplasm closely resembling the light olfactory cell. Graziadei & Metcalf (1971*a*) produced evidence that new receptors develop from 'stem cells' among the ordinary basal cells of the epithelium. In the bandicoot the more numerous type of basal cell is already well differentiated, and is unlikely to be the precursor of new neurons. However, the rarer, less differentiated basal cell (Fig. 6) may well act as a stem cell, serving to provide either an olfactory blastema cell or a more differentiated basal cell, as required. The light olfactory, or blastema, cell closely resembles the 'stem' type of basal cell, but the latter is in extensive contact with the basal lamina, while the blastema cell is separated from it by processes of basal cells. The blastema cell is often found in small groups of 2–4 cells whose membranes are in contact with one another without intervening processes of supporting cells. Mitotic cells were very rarely observed, and then only at the base of the epithelium (Fig. 7). The presence of a cluster of centrioles in this cell suggests an early stage of ciliogenesis, and thus increases the probability that the daughter cells will be olfactory.

If there is thus considerable evidence of development of new olfactory cells, a question arises as to what happens to old cells. Supporting cells do not appear to have a role in removing cell debris. Basal cells often surround dark processes which appear to derive from olfactory cells (Fig. 6), but these are always separated from the basal cell cytoplasm by intact cell membranes. Since macrophages are not uncommon at lower levels of the epithelium it is tempting to postulate that they remove the fragments of degenerating neurons.

The presence of a special patch of sensory epithelium low on the nasal septum may provide extra olfactory perception in two ways: it is the most ventrally situated olfactory area and could act to monitor the air flow in quiet respiration, when only limited amounts of air pass into the more dorsal reaches of the nasal cavity; more-over, its bilateral distribution on the T-shaped end of the septum may help in the appreciation of the direction from which the odour comes. The septal sense organ is not an extension of the vomeronasal organ, whose sensory cells end caudally before the septum becomes incomplete ventrally. The epithelium of the septal organ, which contains typical olfactory cells, is rather more closely packed than the olfactory epithelium over the turbinates, where there is considerable extracellular space, especially towards the basal lamina. The transition from olfactory to non-sensory epithelium around the septal organ is quite abrupt, and there are no transitional cell forms.

The function of the two rarer types of cells seen in the epithelium remains obscure. The brush cell seems to correspond with the 'fifth cell type' described by Andres (1969), though in the bandicoot the cell has a much greater content of cytoplasmic organelles. The shape of the cell, the presence of microvilli, and of microtubules and fibrils in the superficial cytoplasm all suggest a sensory receptor role, but no morphological evidence of synaptic junctions with nerve cell processes has been seen.

Brush cells have been reported in the trachea (Rhodin & Dalham, 1956), and in the lung alveoli of the rat (Meyrick & Reid, 1968). Their function remains uncertain, though a chemoreceptor function has been described for some (Luciano, Reale & Ruska, 1968). In the bandicoot, the brush cell of the olfactory epithelium lacks the glycogen granules present in brush cells in other sites. They occur in the ciliated epithelium bordering the septal olfactory organ, but less frequently than in the sensory epithelium, and have not been detected in the non-sensory epithelium over the ventral turbinate, or the non-sensory areas of ethmoturbinate epithelium. A further study is needed to determine the occurrence of these cells deeper in the respiratory tract.

The role of the pale cells also remains obscure. These cells are seen throughout the olfactory mucosa, but not in the neighbouring respiratory epithelium, or in the sensory and non-sensory epithelia of the vomeronasal organ. The abundance of mitochondria and smooth reticulum suggests the possibility of ionic exchange. Secretion does not appear to be the role of these cells, nor is there any evidence of a sensory function.

SUMMARY

The structure and extent of olfactory epithelium in the bandicoot (*Isoodon macrourus*) were examined by light and electron microscopy. Sensory epithelium covers most of the dorsal conchae, though non-sensory epithelium lines ventrally facing scrolls. The middle conchae are partly covered by olfactory epithelium, the proportion of olfactory to ciliated respiratory epithelium increasing caudally. Ventral conchae are lined by non-sensory ciliated epithelium. The nasal septum ends short of the floor of the nasal cavity in its caudal two thirds. It is covered dorsally by olfactory epithelium. The ventral margin has rounded lateral extensions which carry the isolated strips of olfactory epithelium which form the septal olfactory organ.

The fine structure of the olfactory epithelium is the same in all areas. Cell types include olfactory receptors, supporting cells, two types of basal cell and rarer pale and brush cells. There is considerable morphological variation in olfactory cells, and evidence suggestive of continuing turnover in the receptor cell population.

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