THE OLFACTORY MUCOSA OF THE SHEEP

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Summary

The olfactory mucosa of the sheep was studied by light and electron microscopy. The epithelium conforms to the general vertebrate pattern and consists of olfactory receptor cells, supporting, and basal cells. The free edge of the epithelium is made up of long microvilli from the supporting cells and olfactory rods of the receptor cells, each carrying 40–50 cilia. All cell types contain large dark granules which may be the site of olfactory pigment. The basement membrane is not visible in light microscopy and is fine and discontinuous in electron microscopy. Bowman's glands are simple, tubular, mucus-secreting glands in the lamina propria. Their cells contain basal granules resembling those in the epithelial cells. The lamina propria also contains bundles of fine, unmyelinated, olfactory nerve fibres which are the proximal continuations of the receptor cells.

I. Introduction

Schultze, in 1856, was the first to establish that the olfactory epithelium consisted of three types of cells: sensory, supporting, and basal. The sensory cells are unusual in that they retain their primitive position on the surface of the body, but extend their proximal portions as olfactory nerve fibres to the glomerular zone of the olfactory bulb.

The finer details of olfactory membrane structure have come under scrutiny with the electron microscope mainly as a result of renewed interest in the nature of the olfactory process. Moulton and Beidler (1967) give a comprehensive review of recent findings on structure and function. Nevertheless, considerable gaps remain in our knowledge, especially in the detailed structure of the membrane in the domesticated ruminants.

The present study has shown that the olfactory mucosa of the sheep conforms to the general pattern. It is situated on part of the ethmoturbinates and its extent can be seen readily in the fresh state by its yellow colour, which distinguishes it from the highly vascularized epithelium over the nasal septum and the remainder of the turbinates.

II. Materials and Methods

Immediately after decapitation the olfactory area of the sheep was exposed by sectioning the head in the mid-sagittal plane. Cold 4% glutaraldehyde in cacodylate buffer at pH 7·3 was run over the olfactory area and small strips were then excised. These were fixed in fresh glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in alcohol, and embedded in Epon. Sections were stained with uranyl acetate and Reynold's lead citrate.

Sections for light microscopy were fixed in 5% formalin. Paraffin sections were stained with haematoxylin and eosin (H & E), periodic acid–Schiff (PAS), alcian blue, and Sudan black B.

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III. Results

(a) *Light Microscopy*

Light microscopy of the olfactory mucosa of the sheep shows it to be covered by a pseudostratified epithelium ending in an irregular brush border. Several zones are clearly discernible in the epithelium. These are: a free border, a wide cytoplasmic zone free of nuclei, a nuclear zone 6–8 nuclei in depth, and a line of basal cells. The free border is made up predominantly of microvilli from the supporting cells but also includes the rounded projecting rods of the olfactory cells. This region is strongly positive to PAS technique, and is stained a bright blue with alcian blue. The supranuclear cytoplasmic zone is eosinophilic in sections stained with H & E and stands out as a dark band in sections stained with Sudan black B. In the nuclear zone those of the supporting cells occupy a superficial position and are placed closer together than the nuclei of the olfactory cells. The nuclei of basal cells form a single layer slightly below the main nuclear zone but this is frequently interrupted by long gaps in which no nuclei can be seen. PAS technique shows no evidence of a basement membrane, but PAS-positive granules can be seen in the cytoplasm deep in the epithelium, most frequently just above the nuclei of the basal cells. These granules are clearly evident in sections stained with Sudan black B.

Below the epithelium the lamina propria of the olfactory mucosa is very largely occupied by the numerous simple tubular glands of Bowman, whose ducts open on the epithelial surface. The apical portions of their secretory cells are stained by alcian blue and are PAS-positive. Below the nucleus the cytoplasm of these cells contains granules which, like those at the lower level of the epithelium, stain with Sudan black B and are positive to PAS. In sections stained with Sudan black B the basal cytoplasm of secretory cells produces a darker zone around the glandular alveolus resembling the staining in the supranuclear level of the epithelium.

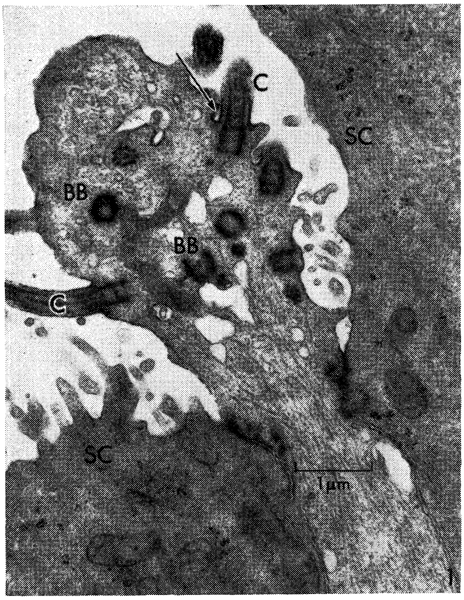
Deep to the glandular layer the lamina propria has a moderately rich blood supply but its distinctive feature is the presence of large bundles of unmyelinated nerve fibres originating from the proximal portions of the olfactory cells.

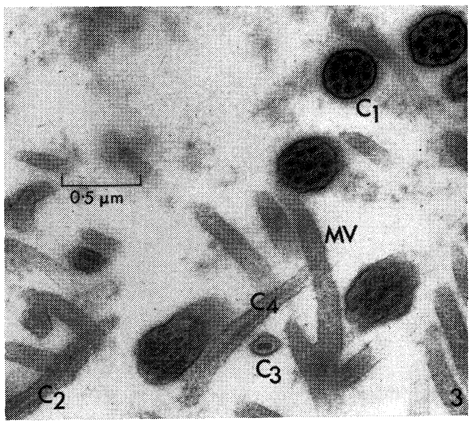
(b) *Fine Structure*

At low magnifications ($\times 4000$) the division into an apical and a nuclear zone in the epithelium stands out sharply. The two main cell types, supporting and olfactory, are easy to recognize in the apical zone, mainly because of the specializations at their apices, but also by differences in electron density and cytoplasmic organelles. However, in the nuclear zone it was impossible to determine with certainty which nucleus belonged to each cell. Basal cells are much fewer in number than the other cell types but can be recognized easily by their position and their electron-dense cytoplasm.

(c) *Olfactory Cells*

The olfactory cells are narrow, elongated, and extend the full height of the epithelium. Narrowest at their proximal portion, they reach greatest width at the nuclear region; the distal cytoplasm constricts to a well-defined neck before increasing in diameter again to form a rounded apical structure which projects above the





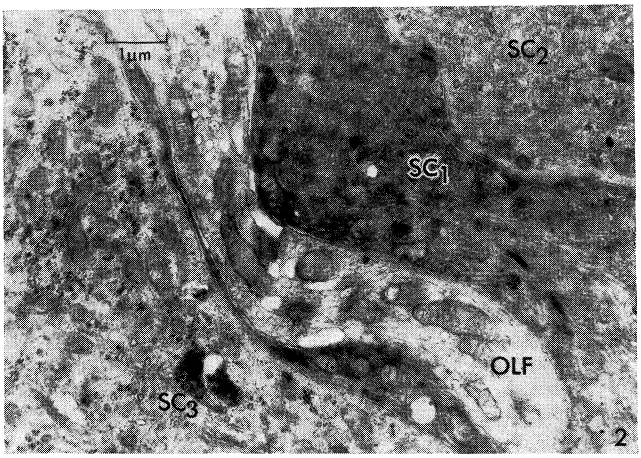


Fig. 1.—Olfactory rod flanked by supporting cells (*SC*). Sections of cilia (*C*) and basal bodies (*BB*) can be seen. Vesicles of varying sizes occur throughout the cytoplasm. An arrow indicates a probable site of origin of a pinocytic vesicle. Longitudinally orientated microtubules are numerous in the cytoplasm just below the rod. Junctional complexes anchor the neck of the olfactory cell to the supporting cells.

Fig. 2.—Apical cytoplasm of olfactory and supporting cells. Two types of supporting cells can be seen: SC_1 is a "dark" cell and SC_2 is a "light" cell. Longitudinally orientated microtubules, mitochondria, and pale cytoplasm distinguish the distal part of the olfactory cell (*OLF*). Extensive smooth endoplasmic reticulum occurs in the apical part of supporting cells of both types. SC_3 shows perinuclear cytoplasm with abundant mitochondria, ribosome clusters, and some rough endoplasmic reticulum.

Fig. 3.—Olfactory cilia (*C*) and microvilli (*MV*) sectioned above the epithelium. C_1 has the full complement of 9+2 filaments. C_2 and C_3 have two filaments, and C_4 a single central filament. A branching microvillus can be seen close to C_4.

surface of the epithelium. This structure carries the olfactory cilia and is shaped rather like a mace (Fig. 1). It is here called the olfactory rod, following de Lorenzo (1957) but is also referred to as the olfactory vesicle (van der Stricht 1909), or olfactory

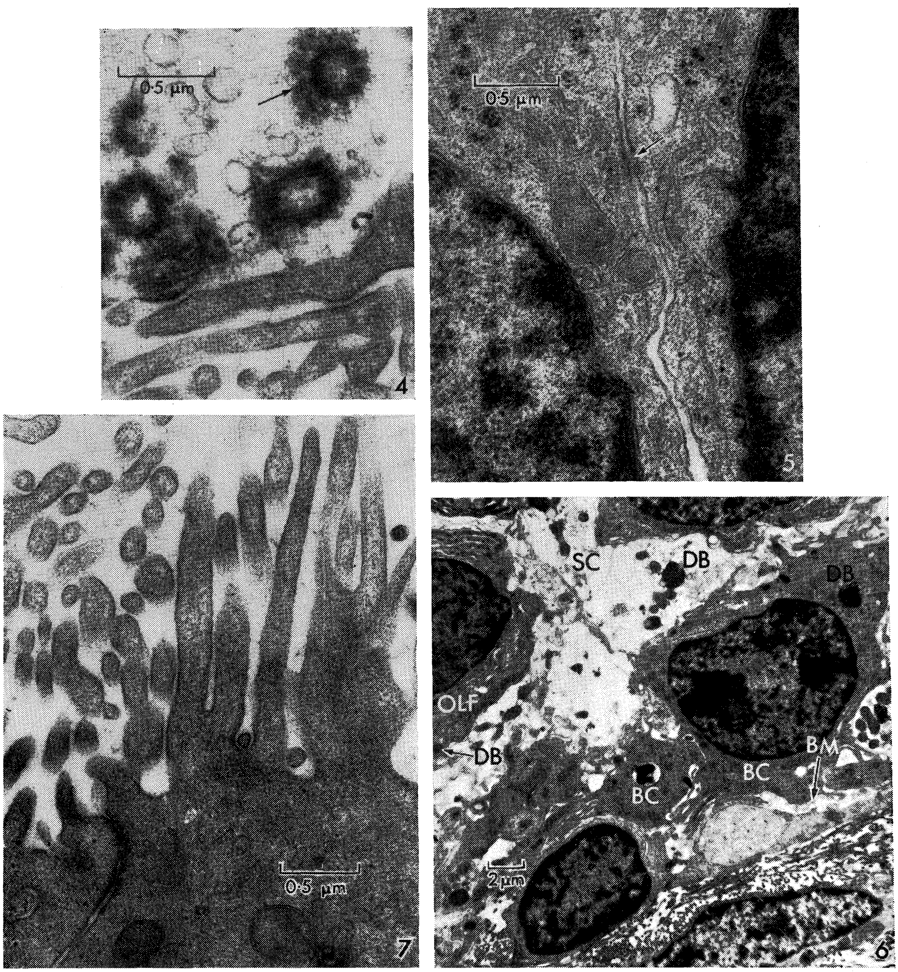


Fig. 4.—Radially arranged dense filaments around the basal body of an olfactory cilium.

Fig. 5.—Olfactory nuclei and perinuclear cytoplasm. Arrow indicates a thickening in adjacent olfactory cell membranes.

Fig. 6.—Base of olfactory epithelium. Basal cells (*BC*) have irregular thin processes extending between olfactory (*OLF*) and supporting (*SC*) cells. Dense bodies (*DB*) occur in all cell types. The basement membrane (*BM*) is poorly defined.

Fig. 7.—Supporting cell microvilli. Note pale core, denser peripheral cytoplasm, and occasional branching.

knob (Trujillo-Cénoz 1961). The cytoplasm of the cell has a fairly low electron density when compared with that of the supporting cells. A well-developed system of microtubules is evident in the supranuclear part of the cell, continuing into the rods. Mitochondria are most numerous in the narrow apical portion of the cell, but a few may be seen in the centre of the rods. Most of the mitochondria have their long axes parallel to the length of the cell, cristae are narrow, and the matrix has a greater electron density than the surrounding cytoplasm (Fig. 2). In the dendritic part of the cell and in the rods there are a number of vesicles of various sizes. Narrow indentations of the cell membrane around the base of the cilia (Fig. 1) indicate active pinocytosis.

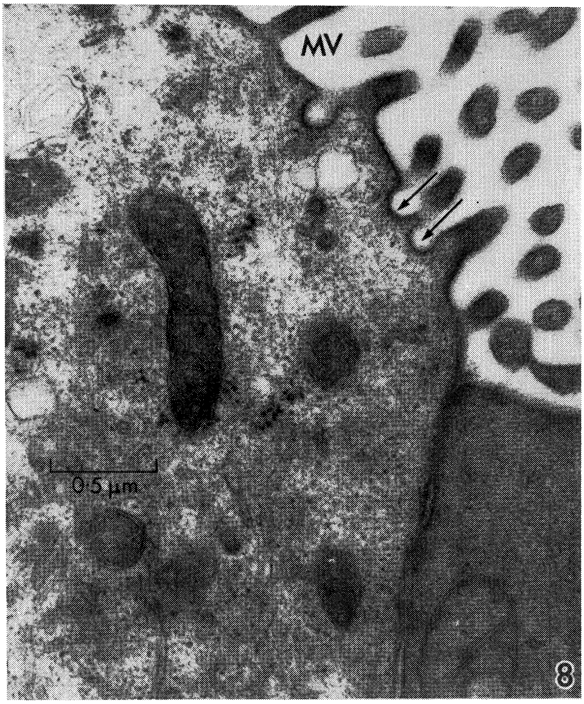
The olfactory rods carry a number of cilia which project into the overlying layer where they extend in various directions among the microvilli of the supporting cells. The length of the cilia was difficult to determine since none remained in focus for all of their length. These cilia have the typical 9+2 arrangement of filaments initially but the number is reduced progressively till terminal sections have one or two filaments (Fig. 3). In the basal bodies of the cilia the two central filaments disappear and the peripheral double filaments become triple. There are no striated rootlet fibres arranged unidirectionally as in the motile cilia of the respiratory mucosa, but some cross-sections of basal bodies are surrounded by a number of dense filaments which appear to be radially arranged (Fig. 4). Longitudinal and oblique sections of rods show that the cilia are given off at several levels and in various directions through the layer of microvilli. While accurate estimates of number cannot be made without serial sections, the number on each rod is probaly about 40–50. Below the rod the cell is tightly attached to the supporting cells by junctional complexes at its constricted neck region, but intercellular spaces are numerous below this level.

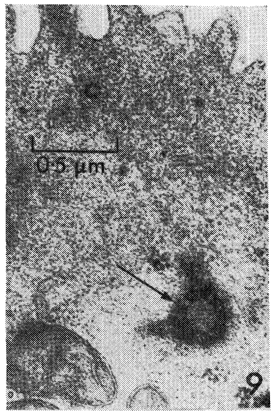
In the perinuclear area of the cytoplasm (Fig. 5) there is a limited amount of rough endoplasmic reticulum and clusters of ribosomes. Smooth endoplasmic reticulum also occurs in this region, but typical Golgi were not prominent. Occasionally in the nuclear area and in the cytoplasm below the nucleus, there were dark bodies of varying electron density, usually without a limiting membrane and apparently identical with those in supporting cell cytoplasm (Fig. 6). The proximal portion of olfactory cells has a cytoplasm of low electron density, and microtubules are the only organelles to be seen in this area. The axonal part of the cell passes through the basal level of the epithelium surrounded somewhat incompletely by processes of the basal cells to join the fila olfactoria.

The nuclei of olfactory cells are oval, with a dark stippling of chromatin material which forms a slightly more prominent band just inside the nuclear membrane. There is usually a pronounced nucleolus, but two nucleoli occur in many cells.

(*d*) *Supporting Cells*

The supporting cells have wide rounded apices which produce a fringe of long, irregular, and sometimes branching microvilli. It is these which give the epithelium its ciliated appearance in light microscopy, and they form an enveloping mesh around the olfactory cilia. Figure 7 shows the irregular branching of the microvilli. While variable in length, most of them are about 2 μm long and about $0 \cdot 15$ μm in diameter. The cytoplasm of the microvilli has a slightly condensed layer close to the cell membrane but separated from it by a pale zone, and there is a less electron-dense central core to the structure. No other cytoplasmic organization is evident in the microvilli or in the layer of cell cytoplasm just below their bases. However, the rest





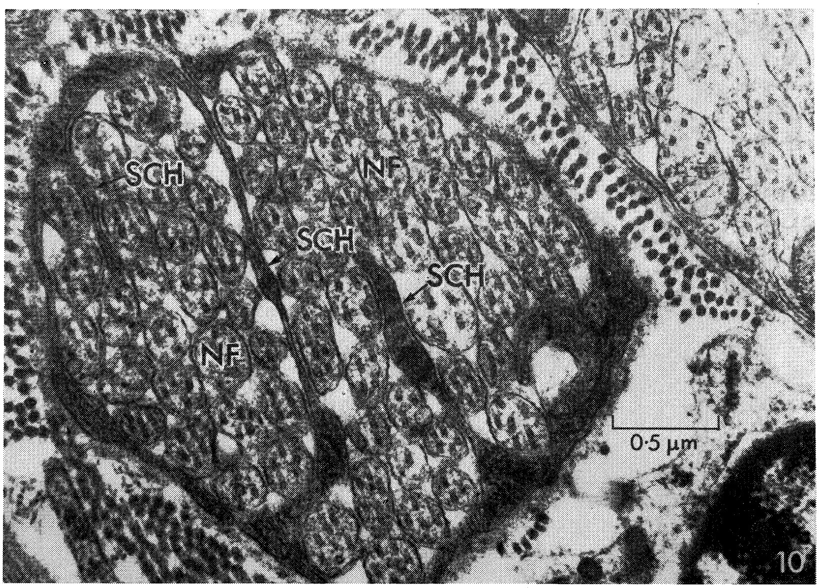


Fig. 8.—Surface of an atypical supporting cell. Microvilli (*MV*) are sparse; microtubules resemble those in olfactory cells. Formation of pinocytic vesicles is indicated by arrows.

Fig. 9.—Basal body of a cilium in apical cytoplasm of supporting cell.

Fig. 10.—A bundle of olfactory nerve fibres. Each fibre (*NF*) contains several microtubules. Thin processes (*SCH*) of darker Schwann cell cytoplasm partially subdivide the bundle. Collagen fibres surround the bundle.

of the supporting cell cytoplasm is characterized by extensive smooth endoplasmic reticulum. Mitochondria also are most numerous in the apical region; in structure they resemble those in the olfactory cells with dense matrix and relatively few narrow tubular cristae. No secretion droplets were observed in the apical region, but deeper in the epithelium supporting cells contain many dense bodies similar to those seen in the olfactory cells. A limited amount of rough endoplasmic reticulum is evident in the perinuclear cytoplasm and isolated clusters of ribosomes can be seen here and in the subapical area of the cells. Junctional complexes between neighbouring supporting cells and between supporting cells and olfactory cells are to be found just at the epithelial surface. Deeper in the epithelium occasional interdigitations occur between cells.

Some variations from the typical supporting cell structure were observed, the most frequent being variations in the electron density of the cytoplasm, so that there are dark and light types of supporting cells (Fig. 2). More rarely, some cells show features of both main cell types, sensory and supporting. This cell has relatively few microvilli, smooth endoplasmic reticulum is not as extensive as in the typical supporting cell, and numerous longitudinally orientated microtubules can be seen which are similar to those in the olfactory cells (Fig. 8). In a few instances an otherwise typical supporting cell can be seen with a basal body in the apical cytoplasm similar to those of the olfactory cilia (Fig. 9).

(*e*) *Basal Cells*

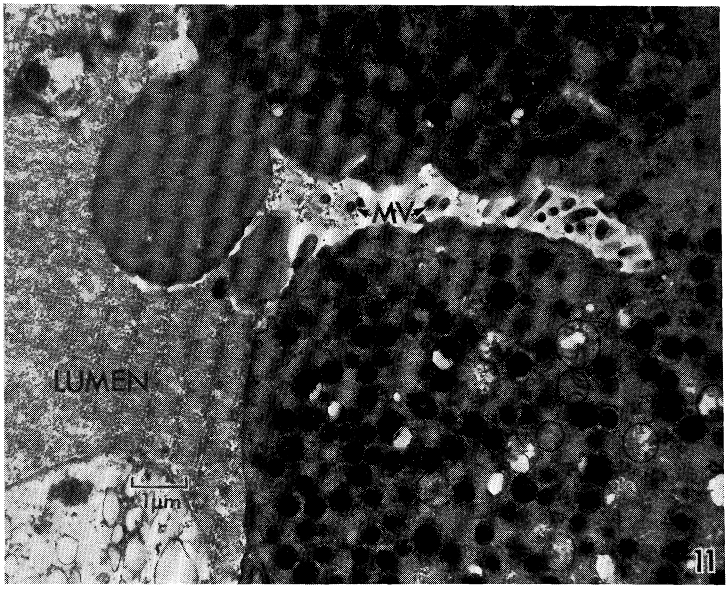
These cells provide an irregular and interrupted basal stratum to the epithelium. Their cytoplasm is irregularly extended into thin processes (Fig. 6) which interdigitate with neighbouring cells and form sheaths for the proximal extensions of the olfactory cells as they extend into the lamina propria. The cytoplasm of the basal cells has a greater electron density than supporting or olfactory cells and includes the dense bodies already noted in olfactory and supporting cells. The basal cells abut onto a thin basement membrane which is interrupted at intervals where groups of proximal processes of olfactory cells leave the epithelium to join the fila olfactoria.

(*f*) *Subepithelial Structures*

Below the epithelium, two other distinctive features of the olfactory mucosa deserve comment. They are the bundles of olfactory nerve fibres and the distinctive Bowman's glands. Proximal extensions of the olfactory cells pass through the basal level of the epithelium, decrease rapidly in diameter as they extend through glandular tissue, and aggregate to form bundles of olfactory axons surrounded by the sparse cytoplasm of Schwann cells (Fig. 10). Axon diameters within these bundles are about 2 μm and each axon contains about 2–7 darker microtubules. Subunits of as many as 30 axons are wrapped in a single process of the Schwann cells, and each may be in close connection with as many as six surrounding axons. Large groups of these bundles of unmyelinated fibres are the characteristic feature of the deep layer of the olfactory lamina propria.

(*g*) *Bowman's Glands*

Cells of these simple tubular glands have their apical cytoplasm filled with round secretion droplets of varying electron density. Some are uniformly electron



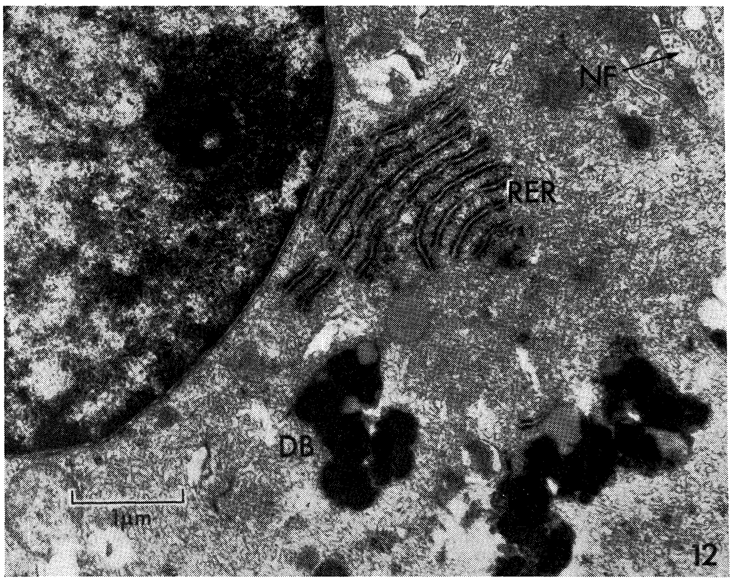


Fig. 11.—Apical cytoplasm of Bowman's gland cells. Dark secretion droplets are surrounded by a single membrane. Smooth endoplasmic reticulum and mitochondria are abundant. Small microvilli (*MV*) project into the lumen.

Fig. 12.—Basal cytoplasm of Bowman's gland cells. Parallel stacks of rough endoplasmic reticulum (*RER*) occur near the nucleus, and dark bodies (*DB*) of varying density without a clear limiting membrane lie below the nucleus. There is a small bundle of olfactory nerve fibres (*NF*) in close proximity to the gland cell. Smooth endoplasmic reticulum is abundant.

dense, some little darker than the surrounding cytoplasm, and some include a dark area within a paler zone. Mitochondria are abundant in this region, especially where there is a large proportion of dense secretion bodies (Fig. 11). The perinuclear cytoplasm contains abundant smooth endoplasmic reticulum very similar to that seen in the supporting cells. In addition, there is a distinctive stack of parallel rows of rough endoplasmic reticulum near the nucleus or towards the base of the cell. In the same area clusters of dark irregular dense bodies occur which resemble the irregular dark bodies in olfactory, supporting, and basal cells (Fig. 12).

Bowman's glands discharge their secretion onto the surface of the epithelium. Dark cells line this surface part of the gland; their cytoplasm is filled with light or clear vesicles and scattered mitochondria which are more numerous near the apex of the cell.

IV. Discussion

Since the cilia of the olfactory rod extend the area of the receptor cell membrane which is exposed on the surface of the mucosa, they have received considerable attention as the probable initial site of olfactory reception. Descriptions of very long cilia (up to 200 μm) were reported in the frog by Reese (1965), who also described a dilation of its outer membrane to form a vesicular section. Descriptions of olfactory cilia in mammals suggest shorter length, though authors emphasize that it is difficult to measure length in thin sections. Okano, Weber, and Frommes (1967) stated that the longest seen in the olfactory membrane of the dog was $8 \cdot 3$ μm, and Frisch (1967) gave 4 μm as the longest individual cilium followed in mouse olfactory epithelium, but stated they were undoubtedly much longer. In the present study, no individual cilium could be followed for more than 2 μm, but since the cilia project in many directions from the rod, most thin sections will show only a limited portion of each. Up to this length the cilia retained the 9+2 array of filaments. Sections of cilia among the microvilli did not show any of the membrane dilations described by Frisch (1967), but many showed a reduction of filaments. Cross-sections of cilia containing 6, 4, 2, or 1 filaments were found. A longitudinal section of a terminal part of a cilium with a single central filament measured $1 \cdot 4$ μm. The organization of basal bodies of olfactory cilia differs considerably from those of motile cilia in nearby respiratory mucosa which have well-developed rootlet fibres with marked periodicity, orientated unidirectionally in the cytoplasm. Olfactory cilia lack this type of rootlet fibre, though some sections show dense areas radiating in a wheel-spoke pattern from the filaments of the basal bodies. The absence of orientated rootlet fibres, the multidirectional spread of cilia from the olfactory rod, and the dense mesh of microvilli surrounding them all suggest that any movement of olfactory cilia must be very limited in extent.

The number of cilia on an olfactory rod seems to vary widely in different mammalian species, perhaps as widely as the variations in total extent of the olfactory area. Estimates range from 1 to 6 for the mole (Graziadei 1966) to 100–150 for the dog (Okano, Weber, and Frommes 1967). In the sheep each rod carries about 40–50 cilia, estimated from longitudinal and cross-sectional views. Personal observations on a number of other species, including several ground-dwelling rodents, indicate that the sheep has fewer olfactory cells in relation to supporting cells in the olfactory epithelium, and each cell carries a smaller number of cilia than those animals which rely more on olfaction in feeding and breeding behaviour.

The cytoplasm of the olfactory cell is neuronal in character but lacks a well-developed Golgi apparatus. Olfactory cells are frequently in contact with other receptors in the epithelium (Fig. 5), contrary to de Lorenzo's (1963) report that supporting cells ensheath all receptors except at the olfactory rods. The proximal parts of the cells are often in contact with several of their fellows before being loosely invested by the basal cells. Once gathered into bundles of the olfactory nerves, each may be in close association with as many as six other axons. The distal or dendritic parts of the cells seem to be separated from one another by the cytoplasm of the supporting cells and each olfactory rod is completely surrounded and separated from neighbouring rods by the rounded apices of supporting cells and their fringe of microvilli.

The role of the supporting cell in the olfactory process has aroused considerable speculation ranging from nutritional, secretory, and mechanical supportive functions to a role in the production of the electro-olfactogram recordings (Tucker and Shibuya 1965). The complex mesh of microvilli surrounding the olfactory cilia and the junctional complexes between supporting cells and receptors at the neck of the olfactory rod may combine their undoubted sustentacular role with sites for nutritional exchange or maintenance of membrane potential. The striking feature of the supporting cell is the extensive development of smooth endoplasmic reticulum throughout the cytoplasm. Only the apical border of the cell and the fringe of microvilli are free of the fine tubular array of membrane. This abundance of smooth endoplasmic reticulum recalls the structure of cells engaged in steroid metabolism as, for example, the interstitial cells of the testes (Christensen and Fawcett 1966). In some species supporting cells may contribute to the overlying layer of mucus. In the mouse, Frisch (1967) describes secretory granules in those supporting cells which displayed stacked lamellae of smooth endoplasmic reticulum. In the sheep, however, no stacked lamellae were seen and there was no evidence of mucus secretion.

Two infrequent and atypical types of supporting cells deserve comment since they have features which are more characteristic of the olfactory cell. The more numerous of these are the cells with sparse, short microvilli and numerous microtubules. They resemble the fourth cell type described in the canine olfactory epithelium by Okano, Weber, and Frommes (1967). These authors suggest that they may be precursors of the more typical supporting cells. However, in the sheep olfactory epithelium they occur too infrequently to make a significant contribution to the replacement of supporting cells, nor are there any cells intermediate in structure between them and the typical form. Instead, this intermediate type of cell seems to suggest a failure to differentiate decisively along sensory or supporting cell lines. A somewhat similar sharing of characteristics is seen in those supporting cells which have a basal body in their apical cytoplasm. While no free cilia were observed in these cells, the fine structure of the basal bodies resembles those of the olfactory cilia. Otherwise, these cells resemble typical supporting cells.

The presence of dark, irregular bodies in the cytoplasm of all the epithelial cell types and in Bowman's gland cells seems to be a feature of sheep olfactory mucosa which is not reported in other species, though Rhodin (1963) describes large dense granules lying at the base of the supporting cells. These structures seem to be peculiar to the olfactory area since comparable sections from the neighbouring respiratory epithelium did not show them, nor do they appear in the cells of secretory alveoli in the respiratory mucosa. They are of varying size and electron density, sometimes incorporating what seem to be the remnants of cell organelles; usually no limiting membrane can be seen. Their appearance is in some ways similar to lipofuscin deposits in neurons (Fawcett 1966). In paraffin sections, granules in comparable positions in the cell are positive to PAS and Sudan black B, a reaction which is consistent with a lipofuscin composition (Pearse 1961). While it is not possible to determine the nature of these dark bodies from the present study, it seems possible that they may be the site of the pigment which gives the characteristic colour to the fresh olfactory mucosa of the sheep. If this is indeed the case, then it is worth noting the occurrence of these bodies in the olfactory receptors themselves as well as in Bowman's gland and supporting cells. Conflicting reports occur in the literature as to the function and composition of olfactory pigment and its presence or absence in the receptor cells (Moulton and Beidler 1967). Species differences have been reported in the occurrence and nature of the pigment, and they may also exist in its distribution in the cells. In the sheep, when these dark bodies occur they are always situated towards the basal part of the cell, not the apical portion. This is particularly evident in the cells of Bowman's glands. Here aggregations of the dark, irregular granules occur at the nuclear level of the cytoplasm, below the apical region of the cell with its typical secretory droplets. It seems probable that such granules represent a by-product of cell activity rather than an active constituent of cell secretion.

V. Acknowledgments

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