Further observations on the ultrastructure of the flank gland of the musk shrew, *Suncus murinus viridescens* (Blyth)

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Abstract. The ultrastructure of the flank gland tissue of both sexes of the Indian musk shrew, *Suncus murinus viridescens* was investigated. The undifferentiated and differentiated sebaceous secretory cells in the glands of both sexes possess 1–8 mitochondrial dense osmophilic inclusions. A few inclusions of medium electron density, and clear vacuoles were also seen in the mitochondria. The muscle fibres in the glandular complex were devoid of these characteristic mitochondrial inclusions. Secretory activity of the well differentiated sebaceous cells was characterised by the presence of a number of large secretory granules. Mitochondria and polyribosomes were found in close association with the secretory granules. The flank gland tubules of the shrew were highly vascularised.

Keywords. Indian musk shrew; *Suncus murinus viridescens*; flank gland; sebaceous tubules; ultrastructure; mitochondrial inclusions.

1. Introduction

Specialized integumentary glands play an important role in the mammalian social behaviour. Ethological and histological parameters concerned with mammalian olfaction have been the focus of attention of most of the investigations on mammalian chemoreception during the past few years (Müller-Schwarze 1983; Balakrishnan and Alexander 1985). Information on the fine structure of mammalian sebaceous glands has also accumulated in the past few years (Kurosuni 1961; Ellis and Henrikson 1963; Sansone et al 1970; Bell 1974; Gorgas and Völkl 1984; Jenkinson et al 1985; Sokolov et al 1986).

Although both sebaceous and sudoriferous glands of mammals have odour producing properties, these glands are widely different in their structural organisation, chemical composition and in the functional process of secretory activities (Mykytowycz 1970; Quay 1972; Strauss et al 1976). The specialized integumentary glands in the Indian musk shrew, *Suncus murinus viridescens* have been the subject of detailed investigation in recent years (Balakrishnan 1975; Balakrishnan and Alexander 1985). An investigation on the fine structure of the sebaceous cells of the flank gland of the male shrew was also made (Balakrishnan et al 1984). The present report represents the results of further detailed investigations on the ultrastructure of the flank gland of *S. m. viridescens*.

2. Materials and methods

Adult shrews of both sexes were trapped live on the University Campus, Kariavattom, and were kept in individual wire-mesh cages for 2–3 days on a regular supply of...
minced beef sprinkled with shark liver oil and tap water ad libitum. The fur of the flank gland area was shaved off using a fine blade and the glandular skin from both sides was dissected out from 4 males and 3 females under ether anaesthesia. Thin slices of the glandular tissue were fixed in 2·5% glutaraldehyde in 0·1 M phosphate buffer (pH 7·2-7·3). The fixed tissues were washed in the buffer for 2 h and post fixed in 1% osmium tetroxide in 0·1 M phosphate buffer. The samples were then carried to the USSR in the same buffer and dehydrated using alcohol and acetone in the ascending grades and embedded in Epon 812. Ultra-thin and 1 μm sections were cut using glass knives in LKB Ultrotomes III and IV. The ultra-thin sections were mounted on formvar-coated grids. These sections were stained with uranyl acetate and lead citrate as detailed by Reynolds (1963) and examined under a JOEL JEM 100 C Electron Microscope at an accelerating voltage of 80 kV. 1 μm sections were stained with toluidine blue and observed under a light microscope for cellular localisation of the secretory tubules and their pattern of arrangement.

3. Results

The flank gland tissue of S. m. viridescens is comprised of sebaceous and sudoriferous gland tubules. The latter are few in number and situated mainly on the glandular periphery. The undifferentiated sebaceous secretory cells were distinguished from the mature differentiated and differentiating cells by the absence of lipid secretory droplets in the former. The undifferentiated cells formed the basal layer of the secretory tubules whereas the inner lining of the tubular lumen was with well differentiated and disintegrating holocrine secretory cells. Disintegrating cellular structures were also seen in the sebaceous tubular lumen (figures 1 and 2). The differentiating and the differentiated sebaceous cells contained a few to several lipid droplets. Further, the differentiated cells were having comparatively larger granules.

One to eight electron dense osmophilic inclusions were observed in the mitochondria of the secretory cells. Mitochondria also contained a few inclusions of medium electron density. Some of the mitochondria had 2-4 clear vacuoles (figure 3). Detailed observations have revealed that these were not artefacts. The mitochondrial inclusions were seen both in the undifferentiated and differentiated sebaceous secretory cells of the flank gland of both sexes of the shrew. These characteristic mitochondrial inclusions were absent in the adjacent muscle fibres. The mitochondria were in close association with the lipid droplets (figure 4). Many free ribosomes were seen throughout the cytoplasm of the secretory cells, and polyribosomes were situated around the periphery of the lipid droplets (figure 5). A large number of capillaries were found around the secretory tubules of the flank gland showing the high vascularisation of the gland (figure 3). Further, the invagination of capillaries into the sebaceous secretory tubules was evident even in light microscopic observations (figures 1 and 2). Some of the sebaceous cells were having a number of finger-like peripheral protrusions at the level of capillaries (figure 6).

4. Discussion

The flank gland of the Indian musk shrew consists of well developed sebaceous and a few sudoriferous secretory tubules (Balakrishnan and Alexander 1977a). No significant
Figures 1-2. 1. Light micrograph of part of a sebaceous secretory tubule of the flank gland of a male shrew showing undifferentiated and differentiated cells of several layers. Note the invagination of capillaries with erythrocytes at definite levels in the glandular tubule. Large secretory granules are seen accumulated towards the tubular lumen (×1875). 2. Light micrograph of part of a secretory tubule of the flank gland of a male shrew showing the undifferentiated epithelial cells at the tubular periphery and differentiated disintegrating cells towards the inner wall of the tubule. A few epithelial structures of the disintegrated cells are also seen in the lumen (×1500). (C, Capillary; LU, lumen of a sebaceous tubule).
Figure 3. Part of the sebaceous tubule of the flank gland of a female shrew showing an undifferentiated cell on the left bottom and parts of differentiated secretory cells on upper left and on bottom right-hand sides. Note the mitochondrial dense inclusions (I) and vacuoles (holes, H) in both undifferentiated and differentiated cells. On the upper right-hand side, a capillary with erythrocytes (E) is seen (× 25,000) (N, nucleus; L, lipid droplet).
Figure 4.
sexual dimorphism is discerned in the tubular pattern and in the structure of these secretory tubules. Lipid forms the major biochemical constituent of the flank gland sebaceous secretory material (Balakrishnan and Alexander 1977b).

Ultrastructural differentiation of the flank gland sebaceous secretory cells of both sexes of this species has now been elaborated. The undifferentiated sebaceous cells are characterised by the absence of any lipid droplets, whereas during the process of differentiation, small lipid droplets are developed and then fused to form large droplets as seen in well differentiated sebaceous gland cells. However, the presence of numerous mitochondria with characteristic electron dense inclusions (Balakrishnan et al 1984) in both undifferentiated and well differentiated cells of both sexes and their absence in the adjacent muscle fibres of this glandular tissue is of considerable interest. Presence of the mitochondrial inclusions thus forms a specific feature of the sebaceous glandular cells.

The size, number and nature of occurrence of the mitochondrial inclusions in the sebaceous gland tubules of male shrews have been discussed earlier (Balakrishnan et al 1984), and present observations revealed that the mitochondrial inclusions are similar in size, number and in the nature of occurrence in both sexes of the species. These results differ from the observations of Sansone et al (1970) on mice preputial gland only in the number of the mitochondrial inclusions. Balakrishnan et al (1984) found a few mitochondrial dense inclusions in the cytoplasm and in the ducts of the sebaceous tubules, probably as a result of their discharge from the mitochondria. The close association of mitochondria with lipid droplets in the differentiated gland cells suggest that the mitochondria not only have the function of energy production and release but also have a role in the lipid metabolism as suggested earlier (Bjersing 1967; Fawcett 1981; Jenkinson et al 1985). Fawcett (1981) also suggested that mitochondria involve in the metabolism of triglycerides and in the transport of some substrate to enzymes. The possible role of mitochondrial inclusions in the synthesis of the lipid droplets in specialized gland cells has already been suggested by Sansone et al (1970).

Observations of Sansone et al (1970) that the sebaceous cells of the preputial glands of immature mice also contained similar mitochondrial inclusions support the present findings on the presence of mitochondrial inclusions in the undifferentiated sebaceous cells of the flank gland of the shrew. The secretory activity of the flank gland of S. m. viridescens initiates when they are at about 9 days old (Balakrishnan and Alexander 1977b), far before their sexual maturity. This indicates the need for further ultrastructural investigations on the flank gland tissue of immature shrews, to confirm the probable role of the mitochondrial inclusions in the secretory function. The relationship between the electron dense inclusions, electron medium dense inclusions and the vacuoles are still unknown. However, we would suggest that the vacuoles in the mitochondria may be formed as a result of the metabolic activities of the electron dense inclusions or due to the discharge of these inclusions from the mitochondria.

Figure 4. Part of a differentiated sebaceous cell of the flank gland of a female shrew showing the lipid droplets, mitochondria (M), short cisternae of rough endoplasmic reticulum (R), many free ribosomes and polyribosomes (P) in the cytoplasm. Note the electron dense inclusions, medium dense inclusions and clear holes in the mitochondria (H) (× 25,000). A mitochondrion with electron dense inclusions is seen in the inset. The mitochondrion is situated in close contact with a lipid droplet (L) (× 30,000).
Ultrastructure of shrew flank gland

Figures 5–6. 5. Part of the cell of the flank gland of a female shrew showing the close association of mitochondria with lipid droplets. Polyribosomes are also found close to the periphery of the lipid droplets (L). Large number of free ribosomes are seen in the cytoplasm (×25,000). 6. Part of the sebaceous secretory tubule of the flank gland of a female shrew showing a capillary at the upper portion, below which is part of an undifferentiated gland cell with finger-like peripheral protrusions (F) (×30,000).
The capillaries lying on the peripheral boundary of the sebaceous secretory tubules invaginate at definite levels in the tubule, facilitating better vascularisation of the sebaceous glandular cells to maintain a higher metabolic rate. Further, the peripheral finger-like protrusions of the secretory cells at the level of capillaries would provide a larger area of absorption of materials brought through the capillaries, which may also be required for the secretory activities of these cells. The polyribosomes found near the periphery of lipid droplets may contribute necessary enzymes for the synthesis and development of the lipid droplets, which ultimately form the glandular secretion of considerable behavioural relevance (Balakrishnan and Alexander 1980).

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References

Balakrishnan M 1975 *Studies on mammalian behavour: Aspects of ethology of the Indian musk shrew, Suncus murinus viridescens* (Blyth); Ph.D. thesis, University of Kerala, Trivandrum

Balakrishnan M and Alexander K M 1977a *Histology and histochemistry of the flank gland of the Indian musk shrew; Acta theriol* 22 241–249

Balakrishnan M and Alexander K M 1977b *A study of physiology and biochemistry of the flank gland of the musk shrew, Suncus murinus viridescens* (Blyth); *T.I.T.J. Life Sci.* 7 13–21


Balakrishnan M and Alexander K M 1985 *Sources of body odour and olfactory communication in some Indian mammals; Indian Rev. Life Sci.* 5 277–313


Bjersing L 1967 *On the ultrastructure of granulosa lutein cells in porcine corpus luteum with special reference to endoplasmic reticulum and steroid hormone synthesis; Z. Zellforsch.* 82 187–211


Gorgas K and Völkl A 1984 *Peroxisomes in sebaceous glands. IV. Aggregates of tubular peroxisomes in the mouse Meibomian gland; Histochem. J.* 16 1079–1098


Quay W B 1972 Integument and the environment: Glandular composition, function and evolution; Am. Zool. 12 95–108
Strauss J S, Pochi P E and Downing D T 1976 The sebaceous glands; Twenty five years of progress; J. Invest. Dermatol. 67 90–97