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Specialized Mucocutaneous Junction Glands of the Indian Musk Shrew

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Histological and histochemical studies on the specialized skin glands found in the mucocutaneous junctions of the Indian musk shrew have been conducted. Oral lip and angle regions of both sexes possess highly specialized sebaceous glands, whereas the perineal region has well developed sudoriferous glands with a few enlarged sebaceous glands. The specialized sebaceous glands at the oral lip and angle are characterized by relatively larger size and proliferation of the glandular follicles. The secretory product is predominantly lipoid in nature. The specialized perineal glands are comprised of follicles of sudoriferous glands. Their main ducts transport the predominantly acid mucopolysaccharides and glycoprotein secretions to the anal cleft.

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INTRODUCTION

Considerable literature is available on the histophysiological features of the specialized skin glands of quite a few species of mammals (M yk y t o w y c z, 1970). Most of these glands are hypertrophied sebaceous or sudoriferous, being lipid secretory in nature, whereas a few void proteinaceous with carbohydrate and mucopolysaccharide materials (G o o dr i c h & M y k y t o w y c z, 1972). However, relatively scant data are available on the distribution and physiology of the specialized skin glands at the mucocutaneous junctions of mammalian body regions. The report of coiled, tubular glands of the apocrine sudoriferous type in the angulus oris of three genera of microtine rodents (Q u a y, 1962) led to a survey of specialized sudoriferous, sebaceous and mucous glands (Q u a y, 1963; 1965) in many rodent species.

Recent behavioural observations on the musk shrew, Suncus murinus viridescens (Balakrishnan & Alexander, 1974a) revealed the possibility of functionally significant glandular structures occurring at the

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oral lip and perineal regions of this animal. The main objective of this investigation is to elucidate the histology and certain histochemical aspects of these glands.

MATERIALS AND METHODS

Shrews were trapped live from the fields around the University Campus, Kariyavattom and brought to the laboratory. The sexes were identified and body weights were recorded. After administering an excess ether anaesthesia to adult healthy shrews (10 males and 10 females), hair from the oral lip and perineal regions (Fig. 1) was removed by a sharp blade. Skin from these regions was quickly removed, blotted dry on a filter paper, cut into manageable strips and fixed in the following fixatives for histological and histochemical studies: 1) Bouin's fluid, 2) formol calcium, 3) ice-cold alcoholic picro-formol, 4) ice-cold alcoholic silver nitrate and 5) ice-cold acetone-ethanol mixture. Routine processing of tissues fixed for histology and histochemical tests for protein, ascorbic acid, glycogen

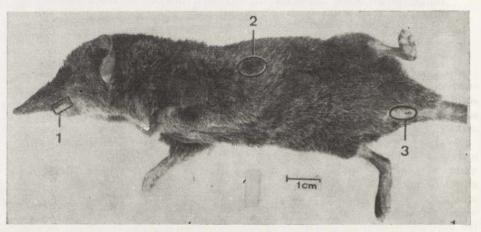


Fig. 1. Specialized glandular zones of the shrew, *Suncus murinus viridescens*. 1. Oral lip and angle. 2. Flank gland. 3. Perineal glandular region.

and alkaline phosphatase was carried out after 24—48 hours. Tissues were embedded in paraffin wax and sections were cut at 5—10 μm . Those tissues fixed for lipids were washed well after 24 hours, embedded in gelatin and were sectioned at 8—12 μm on a model CTD-International-Harris-Cryostat at $-20^{\circ}C$. Cryostat sections of perineal gland were also used for the histochemical localization of acid mucopolysaccharides. Freshly dissected frozen sections were also used for the demonstration of alkaline phosphatase.

As for histological investigations, sections were stained with haematoxylin and eosin, and with Mallory's triple stain. Measurements were carried out in an Olympus binocular microscope by a Leitz micrometer.

Histochemical studies were conducted employing Sudan black-B, and fettrot for lipids; bromphenol blue for protein; *PAS* and Best's carmine techniques for glycogen; alcian blue, silver nitrate and modified calcium cobalt methods for acid mucopolysaccharides, ascorbic acid and alkaline phosphatase respectively. Routine processing of control sections was also carried out (Pearse, 1968).

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RESULTS

1. Oral Lip Glands

Table 1 gives data on size measurements of oral lip glands. The glandular area was not superficially discernible in both the sexes. Although these glands are apparently similar in structure to the normal sebaceous glands of skin of other body regions, they could be differentiated by their relatively larger size and abundance of the sebaceous lobes (Figs. 2, 3, 4 and 5).

These glands appeared to be radiating from the associated hair follicle in transverse sections, having a total mean diameter of 265 μ m in males and 245 μ m in females. Since hair was rather sparse at this zone, practically every follicle other than that of the vibrissae had a specialized oral gland. A maximum of up to 16 sebaceous lobules could be observed to be

Da	Data on measurements of the specialized oral lip glands of the shrew, Suncus murinus viridescens.						
Sex	Number of animals	Body weight, g	Diameter of glandular area associated with one hair follicle, µm	Length of sebaceous tubule, µm	Width of sebaceous tubule, µm		
Male	10	47 ± 2	265 ± 8	126 ± 5	51 ± 2		
Female	10	30 ± 0.7	245 ± 14	120 ± 7	46 ± 1		

Table 1

Data analysed statistically; means with \pm standard errors are given.

associated with each of the hair follicle concerned. These sebaceous lobes had a mean length of $126 \,\mu\text{m}$ and a width of $51 \,\mu\text{m}$ in males with the females having values of $120 \,\mu\text{m}$ and $46 \,\mu\text{m}$ respectively.

Histochemical studies revealed that the specialized oral lip glands were predominantly sudanophilic. The glandular tissues stained deeply with Sudan black-B (Fig. 6). The lipophilic secretory materials were pushed towards the lumen of each sebaceous tubule and they proceed to the exterior through the associated hair follicle. The sebaceous secretory tubules were only slightly stained for protein (Fig. 7) and it was relatively indistinct as compared to the surrounding tissues. The associated hair follicle was devoid of any protein stained material. The secretory lobes of the gland did not exhibit any Best's carmine and *PAS*-positive material. Contrarily, the interglandular connective tissue and the basal membrane had some carbohydrate granules (Fig. 8). The secretory materials were also observed devoid of glycogen content. Silver nitrate-

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positive materials could be seen in the form of granules in the specialized sebaceous glands of the oral lip and angle (Fig. 9). Further, hair follicles were also stained with silver nitrate, indicating the possibility of ascorbic acid contents in the secretory materials. The peripheral layer of each sebaceous follicle was stained for alkaline phosphatase. It could be observed that the enzyme activity was concentrated only at the outer regions of that layer of follicle cells along with the basal sheath (Fig. 10).

2. Perineal Glands

Table 2 shows data on measurements of histological parameters of the specialized perineal glands of shrews. The perineal region of Suncus murinus viridescens is a glandular complex of enlarged sebaceous glands and specialized sudoriferous glands. A few of the sebaceous glands of this region were observed to be comparatively well developed with the normal structural and secretory patterns (Figs. 11 and 12).

Data on measurements of the specialized perineal glands of the shrew, Suncus murinus viridescens.							
Sex	Number of animals	Body weight, g	Width of the glandular area, µm	Diameter of the sudoriferous tubules, µm	Height of the follicle cells, µm		
Male	10	50 ± 1	932 ± 16	77 ± 3	15 ± 0.5		

 914 ± 66

 102 ± 5

 14 ± 0.6

Table 2

Data analysed statistically; means with \pm standard errors are given.

 31 ± 1

10

Apart from the sebaceous glands, two types of sudoriferous glands were discernible. The coiled, tubular, sweat glands of apocrine type, which compare favourably with those reported by Dryden & Conaway (1967) behind the ears and throat of Suncus murinus, were localized in the perineal region at the lower aspect of the sebaceous follicle (Figs. 13 and 14). These glands drain to the exterior by special constricted ducts (Fig. 11). Another type of apocrine, tubular glands was observed at a slightly deeper level in the skin being confined specifically to the perineal region of this species. This perineal gland of S. murinus was quite well developed in both sexes. It is comprised of clusters of highlydeveloped sudoriferous glands encircling the perineal region (Fig. 15). The epithelial wall of each tubule was a single layer of cuboidal and columnar cells (Fig. 16). The glandular area, surrounding the anal cleft had a width of 932 µm in males and 914 µm in females. The sudoriferous tubules were comparatively larger in female shrews with 102 um, where-

Female

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as in males it was only 77 μ m. The height of the secretory epithelial cells of the sudoriferous tubule of male shrews was 15 μ m, whereas it was 14 μ m in females. Each glandular cluster was ensheathed by connective tissue and each secretory tubule had a thin basement membrane. The ducts of the secretory tubules of each cluster joined together constituting a large duct having a dilation at its base and drain to the anal cleft (Fig. 17) by distinctive duct pores. Blebs of secretory materials could be observed in the tubules and filling the dilated portions of the main duct also.

The perineal glands and their secretions were sudanophobic in nature. No trace of lipid could be observed in the secretory tubules, ducts or in the dilated portion of the duct, although normal and specialized sebaceous glands found in this region were highly sudanophilic (Fig. 18). The glandular structure as well as the secretory material found inside the tubules were moderately stained for protein with bromphenol blue (Fig. 19). The sudoriferous perineal gland and its secretory products exhibited a very intense staining reaction for PAS-positive materials. The concentration was relatively more intense toward the interior of each tubule (Fig. 20). However, only a partial reduction was observed in the concentration of PAS-positive materials of these glandular tubules after digestion with diastase. Contrarily, these tubules were fairly well stained with alcian blue (Fig. 21). The secretory materials found in the tubular lacunae were also stained for acid mucopolysaccharides. The specialized sebaceous glands of this region were devoid of any Best's carmine and PAS-positive materials. Ascorbic acid granules could be noticed in the tubular cells of the perineal gland (Fig. 22). These small granules were uniformly distributed throughout the glandular tissues. The coiled apocrine sudoriferous glandular tubules of the perineal region had a comparatively high content of ascorbic acid granules. The inner lining of these tubules had a row of deeply stained granules with the secretory blebs attached alongside to the tubule. The lumina with secretions were also stained for ascorbic acid, although at a lower intensity. Alkaline phosphatase could be noticed in the epithelial wall of the secretory tubules in the form of fine granules (Fig. 23). The secretory materials found attached to the epithelial layer also exhibited the enzyme activity.

DISCUSSION

Despite the considerable attention recently focussed on mammalian skin glands, studies on specialized skin glands of insectivores have been relatively few (Pearson, 1946; Dryden & Conaway, 1967). The flank glands have been reported to be present in most of species of shrews

(Pearson, 1946: Dryden & Conaway, 1967; Balakrishnan & Alexander, 1974b), whereas the ventral gland (Coues, 1896; Eadie, 1938; Pearson, 1946) has not been observed in some species, e.g., Suncus murinus (Balakrishnan & Alexander, unpubl.). In fact, Dryden & Conaway (1967) reported that the postauricular sweat glands of apocrine sudoriferous type were responsible for odour production in S. murinus. The present study has revealed the occurrence of highly specialized sudoriferous glands at the perineal region of $S. m. v_i$ ridescens of both sexes which have not yet been reported for any shrew species to the best of our knowledge. Further, organoleptic panel technique employing petrolatum-coated cotton tufts rubbed on the perineal region of this species have indicated that in addition to the postauricular glandular regions, the perineal region also possess the typical musky odour of shrews (Balakrishnan & Alexander, unpubl.). The data obtained indicate the occurrence of odour producing glands of the type described by Dryden & Conaway (1967) localized at the perineal region of S. m. viridescens.

Structurally, the sebaceous glands of the oral lip and angle of S. murinus are quite comparable to those of the normal but for their larger size and preponderance of sebaceous lobes draining into each hair follicle. Despite the slightly larger size in male shrews, no significant sexual dimorphism could be seen in this glandular structure. The oral lip and angle of many rodents possess specialized sebaceous and sudoriferous glands (Q u a y, 1962; 1965). Q u a y's (1965) investigations on 79 species of rodents revealed that diverse compound, tubular, tubulo-alveolar mucous and mixed sero-mucous glands extend into the oral lip and angle area. Although the sebaceous glands of rodents are comparable to those of S. murinus both structurally and in mode of secretion, the musk shrews do not exhibit any specialized sudoriferous glands in their oral lip and angle regions.

The holocrine sebaceous glands secrete a predominantly lipid secretory material which proceeds to the exterior through the associated hair follicle. Histochemical observations on the glandular secretions revealed neither protein nor carbohydrate materials. The structural proteins could be discerned in each glandular cells, but glycogen was restricted only to the connective tissue constituting the basal membrane of each sebaceous lobe. Ascorbic acid was observed in comparatively larger amounts in the lipophilic sites suggesting its functional relationship with fat metabolism (Banerjee & Ganguli, 1962). Alkaline phosphatase was restricted to the peripheral layer of each sebaceous tubule, with the enzyme activity also being confined to basal membranes.

The specialized perineal glands of S. murinus are apocrine in nature

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and are not related to the sweat glands of the general body surface. Structurally, the perineal glands of Suncus could possibly be compared with that of the anal gland of rabbit, which is comprised of two clusters of many apocrine lobes with their ducts joining to form a few ducts which open into the anus at the junction of mucus membrane of the rectum and the epidermis of the skin (Mykytowycz, 1966). No significant sexual dimorphism could be observed in the perineal glands of Suncus, whereas in many mammals the specialized skin glands at the posterior quarters were reported to be sexually dimorphic (Mykytowycz, 1966; Clarke & Frearson, 1972; Harmsen & Sittig, 1973). This glandular tissue of shrews in general was lipophobic and the secretory product was devoid of any lipid materials. Both the glandular tissues as well as its secretion exhibited fairly good amount of protein, and were rich with PAS-positive granules. These were comprised of predominantly acid mucopolysaccharides and a smaller proportion of glycogen. Ascorbic acid granules could be seen in the glandular tissue being linked with carbohydrate metabolism (Banerjee & Ganguli, 1962). Alkaline phosphatase was also observed in the perineal glands having a relatively uniform distribution throughout the glandular tubules.

The deployment of various specialized skin glands in mammalian communication systems and territorial marking have been well investigated only in a few species of mammals (Schultz-Westrum, 1965; Mykytowycz, 1965; 1968; Thiessen, 1968; Thiessen, Owen & Lindzey, 1971; Müller-Schwarze, 1971; 1972 for reviews, Mykytowycz, 1970; Ralls, 1971; Eisenberg & Kleiman, 1972; Johnson, 1973).

Although the oral lip glands are not employed in direct marking, shrews of both sexes groom occasionally at a high frequency during exploration in a fresh environment. This specific grooming behaviour of S. m. viridescens may possibly help in transferring the secretions of the sebaceous glands at the oral lip and angle area to the ground through the forepaws. A similar behavioural significance had been reported in some rodents having specialized skin glands in the oral lip and angle regions (Q u a y, 1965). Pearson (1946) and Dryden & Conaway (1967) have reported that shrews rub their sides along the cage wall, and throat and belly on the floor of the cage. During the course of the present investigation, no specialized glands could be observed on the belly of this species and in fact the lowering of the belly could be to facilitate active marking by the perineal glands. During exploratory behaviour, they also sniff at the ground and at objects which they encounter.

The marking behaviour and the functional role of these glands of S. m. viridescens will be published elsewhere.

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WYSPECJALIZOWANE GRUCZOŁY SKÓRNE U SUNCUS MURINUS

Streszczenie

Przeprowadzono histologiczne i histochemiczne badania nad wyspecjalizowanymi gruczołami skórnymi u *S. murinus viridescens* (Ryc. 1). Wargi i okolice kątowe pyska obu płci posiadają wysoko wyspecjalizowane gruczoły łojowe, natomiast okolica kroczowa ma dobrze rozwinięte gruczoły potowe z kilkoma gruczołami łojowymi. Gruczoły łojowe na wargach i w okolicy kątowej charakteryzują się stosunkowo większymi rozmiarami i proliferacją pęcherzyków gruczołowych (Tabele 1, 2; Ryc. 2—5). Wydzielina jest przeważnie natury lipoidowej. Wyspecjalizowane gruczoły kroczowe składają się z pęcherzyków gruczołów potowych. Ich główne przewody uchodzą do *rima ani* i transportują wydzieliny zawierające głównie kwaśne mukopolisacharydy i glikoproteiny (Ryc. 6—10).

Plate X

Fig. 2. Specialized sebaceous glands of the oral lip region of the musk shrew. A large number of sebaceous lobes join one hair follicle. Haematoxylin and eosin,

100×.

Fig. 3. Enlarged view of specialized sebaceous glands of the oral lip region. Haematoxylin and eosin, $400 \times$.

Fig. 4. Specialized sebaceous glands of the oral angle. Haematoxylin and eosin, $100 \times$. Fig. 5. Normal sebaceous glands from the nearby region for comparison. Haematoxylin and eosin, $100 \times$.

Abbreviations: HF, hair follicle; ICT, interglandular connective tissue; OA, oral angle; SE, sebaceous gland.

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Plate XI

Fig. 6. Intense sudanophilia of oral lip glands, $100 \times$.

Fig. 7. Sebaceous glands exhibit a light homogeneous staining nature for protein. Bromphenol blue, $100 \times$.

Fig. 8. *PAS* positive materials could be seen as small granules localized along the interlobular connective tissues of the gland. The sebaceous structures are *PAS* negative, $100 \times$.

Fig. 9. Ascorbic acid is distributed more or less homogeneously in the sebaceous glandular tissues, $400 \times$.

Fig. 10. Alkaline phosphatase could be seen along the border of each of the sebaceous lobes, $400 \times$.

Abbreviations: HF, hair follicle; HS, hair shaft; ILC, interlobular connective tissues; SE, sebaceous gland.

Plate XII

Fig. 11. Enlarged sebaceous glands along with a few apocrine sweat tubules of the perineal region. Ducts of the apocrine sweat glands drain directly to the exterior. Haematoxylin and eosin, $100 \times$.

Fig. 12. Sebaceous glands of the perineal region. Haematoxylin and eosin, $100 \times$. Fig. 13. The apocrine sweat glands of postauricular region reported by Dryden & Conaway (1967) are discernible in the perineal region of *S. murinus viridescens*. Note the abundance of these glands around the anal cleft. Haematoxylin and eosin, $40 \times$.

Fig. 14. Enlarged view of the apocrine sweat glands of perineal region. Haema-toxylin and eosin, $100 \times$.

Fig. 15. Accumulations of perineal sudoriferous glands of the shrew, S. murinus viridescens encircling the anal region. Haematoxylin and eosin, $400 \times$.

Fig. 16. An enlarged view of perineal sudoriferous glands. Haematoxylin and eosin, $400 \times$.

Fig. 17. Formation of the main duct which leads to the anal cleft. The ducts of each tubule within a gland unite to form a vesicle from which the duct leads to the anal cleft. Haematoxylin and eosin, $100 \times$.

Abbreveations: AC, anal cleft; ASG, apocrine sweat glands; BPSG, bunches of perineal sudoriferous glands; DASG, duct of the apocrine sweat gland; HF, hair follicle; HS, hair shaft; PD, perineal gland duct; SE, sebaceous gland.

Plate XIII

Fig. 18. The perineal glandular area of S. murinus viridescens showing the sudanophilic nature of sebaceous tissues and sudanophobic nature of sudoriferous glands, $40 \times$.

Fig. 19. The perineal sudoriferous glands are stained positively for proteins. Bromphenol blue, $400 \times$.

Fig. 20. PAS-positive granules in the sudoriferous tissues. Note the higher concentrations of these granules towards the glandular lumen, $400 \times$.

Fig. 21. The *PAS*-positive materials are predominantly acid mucopolysaccharides. The secretory materials also could be seen stained with alcian blue, $100 \times$.

Fig. 22. Ascorbic acid granules in the perineal gland. The apocrine sweat glands also have a lining of silver nitrate positive materials, $100 \times$.

Fig. 23. Showing distribution of alkaline phosphatase in the perineal glands, $100 \times$. Abbreviations: ASG, apocrine sweat glands; BPSG, a bunch of perineal sudoriferous glands; SE, sebaceous gland.

