Onderstepoort J. vet. Res. 45 (2), 67–74 (1978)

THE MICROMORPHOLOGY OF THE APOCRINE GLANDS OF THE INTERMANDIBULAR REGION OF THE STEENBOK (*RAPHICERUS CAMPESTRIS*)

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ABSTRACT

GERNEKE, W. H. & COHEN, M., 1978. The micromorphology of the apocrine glands of the intermandibular region of the steenbok (*Raphicerus campestris*.) *Onderstepoort Journal of Veterinary Research* 45 (2), 67–74 (1978).

The histological structure of the newly-discovered intermandibular glandular region in male and female steenbok is described. This region consists of enlarged sebaceous and apocrine glands which secrete a substance used for demarcating grazing territories and for marking females. Migratory lymphoid cells in the epithelium of the apocrine glands resemble Langerhans cells of the epidermis and forestomach epithelium but do not contain Langerhans cell granules. Cells, which are probably of lymphoid origin and resemble sebaceous gland cells, sometimes occur in the apocrine glands.

Résumé

MICROMORPHOLOGIE DES GLANDES APOCRINES DE LA RÉGION INTERMANDIBULAIRE CHEZ LE STEENBOK (Raphicerus campestris)

On décrit la structure histologique de la région glandulaire intermandibulaire qui vient d'être découverte chez le mâle et la femelle du Steenbok. Cette région se compose de glandes apocrines et sébacées agrandies dont la sécrétion sert à délimiter les territoires de pâture et à marquer les femelles. Des cellules lymphoïdes migratrices dans l'épithélium des glandes apocrines ressemblent aux cellules de Langerhans de l'épiderme et de l'épithélium de la panse, mais ne contiennent pas de granules de Langerhans. On trouve parfois dans les glandes apocrines des cellules qui sont probablement d'origine lymphoïde et ressemblent à des cellules de glandes sébacées.

INTRODUCTION

An intermandibular glandular region (Fig. 1) which has not been found in any other ungulate was recently described in the steenbok (*Raphicerus campestris*) by Cohen & Gerneke (1976). This region consists of enlarged sebaceous and apocrine sweat glands which are situated in the upper two-thirds of the dermis (Fig. 2). The glands produce a dirty white, almost flaky, odoriferous substance which clings to the hairs of the area and is easily rubbed off for demarcating territorial areas as well as for marking females during or after mating (Fig. 3). They are better developed in the male than in the female. Although the glands are enlarged, their openings in the hair follicles are small and not visible macroscopically. The hair over the area is shorter and coarser than hair elsewhere (Fig. 1).

The histological structure of this region was studied with both light and electron microscopes to determine whether these glands present any features different from the typical skin glands.

MATERIALS AND METHODS

Specimens of skin from the intermandibular region and other parts of two adult males and one adult female were fixed in 10% formalin and Zenker's and Bouin's fixatives, and processed by routine histological techniques. Paraffin sections were cut at 4 microns and stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and Mallory's phosphotungstic acid haematoxylin (MPAH). Frozen sections were also cut from the formalin fixed tissue and stained with Sudan IV.

Skin specimens, collected for electron microscopic studies from the intermandibular glandular region of a male animal shot in the Kruger National Park, were fixed in 4% glutaraldehyde in Millonig's phosphate buffer (pH 7,3) for 72 h at 4 °C in a portable coolbag, washed in the same buffer and post-fixed in 2% osmium tetroxide also in the same buffer (pH 7,3). The specimens were washed in two changes of buffer, dehydrated in ethanol and propylene oxide and embedded in Epon 812 in gelatin capsules for 48 h at 60 °C. Ultra-thin sections were cut with glass knives on a Reichert OMU 3 ultramicrotome, stained in a saturated solution of uranyl acetate (1 hour) and 0,2% lead citrate (4 min) and examined in a Philips EM 301 electron microscope.

RESULTS

The intermandibular glandular region was present in all 40 adult animals (*Raphicerus campestris*) which had been immobilized with a mixture of 10 mg Fentanyl* and 10 mg Rompun V** for ear tagging. An extraordinary phenomenon was that when the glandular region of an immobilized animal was touched, the animal jerked its head and kicked with all four legs. This happened every time the gland of an immobilized animal was touched, but not in the case of untreated animals.

Histologically the intermandibular glandular region consists of enlarged sebaceous and extensively coiled apocrine sweat glands (Fig. 2). Both glands opened into the hair follicles of the area but the openings of the coiled glands were above those of the sebaceous glands. The glandular area started 15 mm from the chin and extended caudally for 125 mm. Its general shape was oval with a width of 40 mm at its widest part (Fig. 1). These were the dimensions in a subadult male. Slight variations in the degree of development could be expected in mature and immature animals.

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Received 20 December 1977—Editor

* Fentanyl citrate, Ethnor Laboratories (Pty) Ltd, P.O. Box 1934, Johannesburg 2000
** Xylocine HCl 2%, Bayer Pharmaceuticals, P.O. Box 10233, Johannesburg 2000

FIG. 1 The region of the intermandibular gland with its white, coarser hair indicated by three arrows and a pencil. These hairs are covered with a dirty-white, flaky secretion. The position of the infra-orbital gland with its lateral opening is indicated ventro-medial to the eye. (Photograph: H. Braack)

FIG. 2 A section near the periphery of the intermandibular region of a male steenbok shows the distribution of the deeply situated apocrine glands and the more superficial sebaceous glands. ×25

FIG. 3 A male steenbok marking its female with the intermandibular glandular region

FIG. 4 Section showing the abrupt transition from the secretory to the conducting portion of the apocrine gland. ×800

FIG. 5 The basket-like network formed by the myoepithelial cell is shown after staining with Mallory's phosphotungstic acid haematoxylin (MPAH). ×410

FIG. 6 Sebaceous-like cells (s), lymphoid cell (l), myoepithelial cells (my) and the slightly granular content of the columnar secretory cells of the apocrine glands are clearly shown. Stain: Sudan IV and Haematoxylin. ×1 025

FIG. 7 A sebaceous-like cell (s) seen in the epithelium of the apocrine glands (HE). ×1 025

FIG. 8 Section showing karyorrhexis of the nucleus of a sebaceous-like cell (s) in the epithelium of an apocrine gland as well as some physiological desquamation. ×800

FIG. 9 Physiological desquamation seen in the apocrine glands as well as a number of sebaceous-like (s) and lymphoid cells (l). ×640

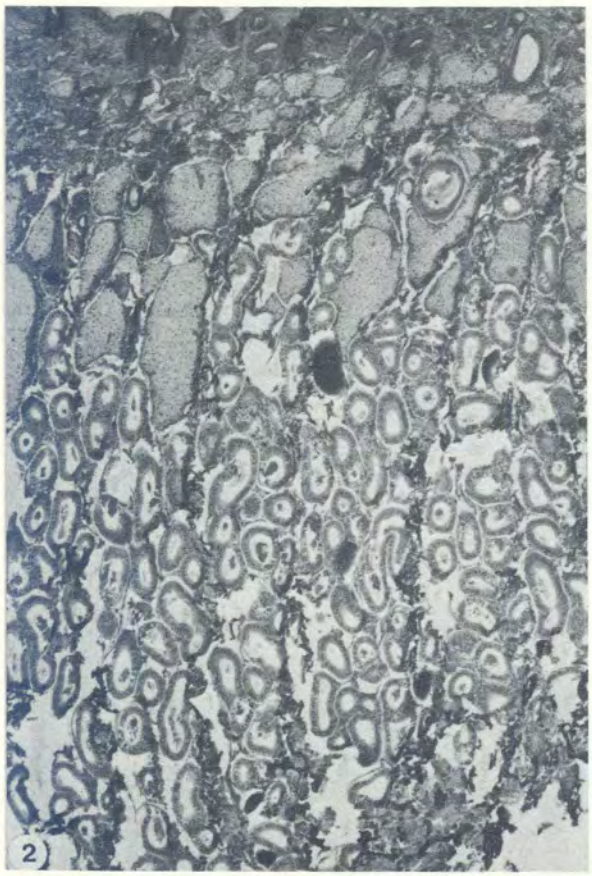
FIG. 10 Electron micrograph showing the double cuboidal nature of the duct epithelium of the apocrine glands. The proximal layer of cells resting on the basal lamina (b) becomes changed to myoepithelial cells lower down in the secretory portion. Tonofibrils (t), desmosomes (d) and microvilli (v) are also present. ×10 750

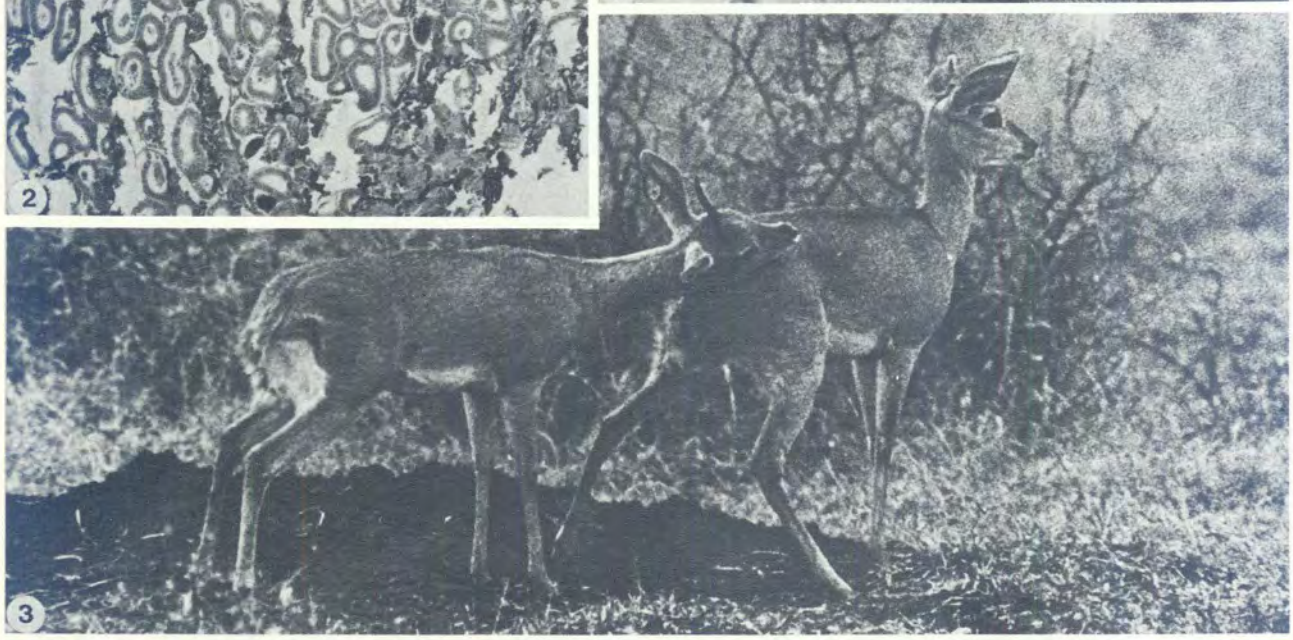
FIG. 11 A cell from the proximal layer of an apocrine gland duct showing a single cilium (c) and numerous tonofibrils (protein fibrils) (t). Desmosome=d, microvilli=v. ×19 600

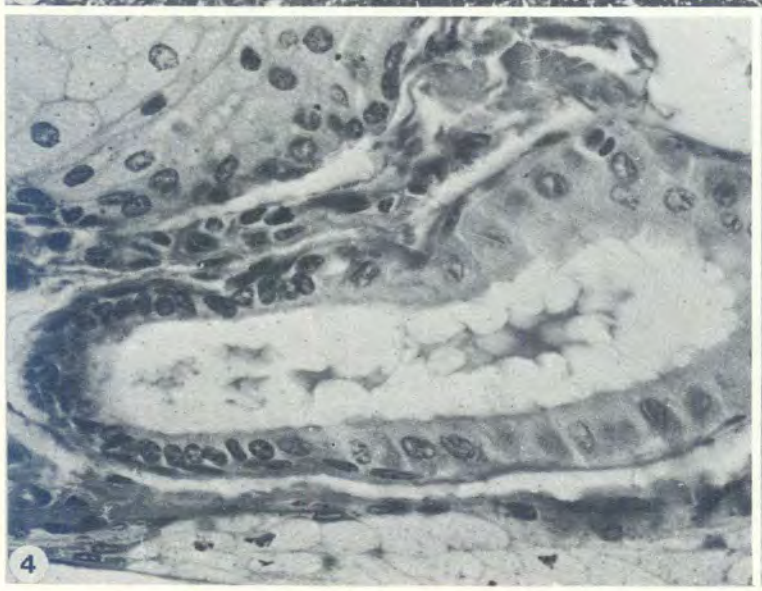
FIG. 12 Myoepithelial cells attached to the basal lamina by hemidesmosomes (h) and to each other and to the epithelial cells by desmosomes (d). Microvilli (v) are scarce or mostly absent from the myoepithelial cells but more numerous on secretory cells. ×32 500

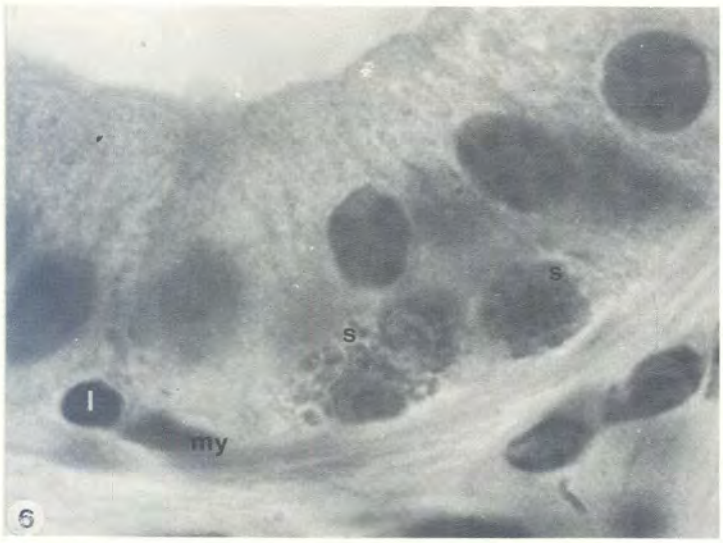
FIG. 13 Light and dark secretory cells with mitochondria (m), secretory granules and granular endoplasmic reticulum occurring in the apocrine glands. A lymphoid migrating cell (l) with granules of an unknown nature is seen between the myoepithelial (my) and secretory cells. ×6 530

FIG. 14 A migrating lymphocyte with ribosomes, mitochondria (m), granular endoplasmic reticulum and dendritic processes in its typical intercellular position between myoepithelial (my) and secretory cells. Granular endoplasmic reticulum, mitochondria and electron dense granules (e) are present in the secretory cells. ×17 000





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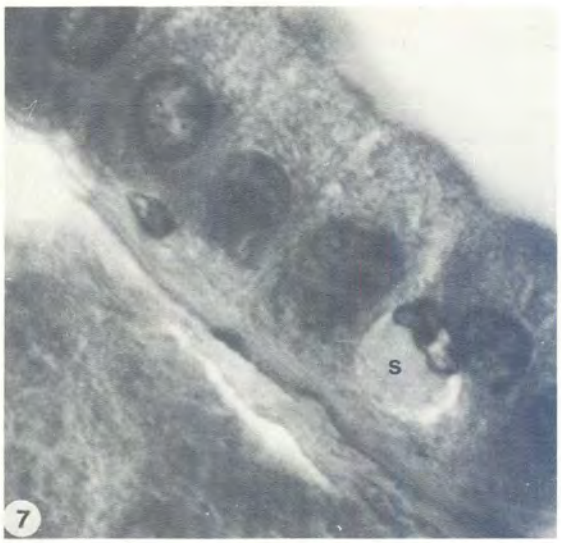
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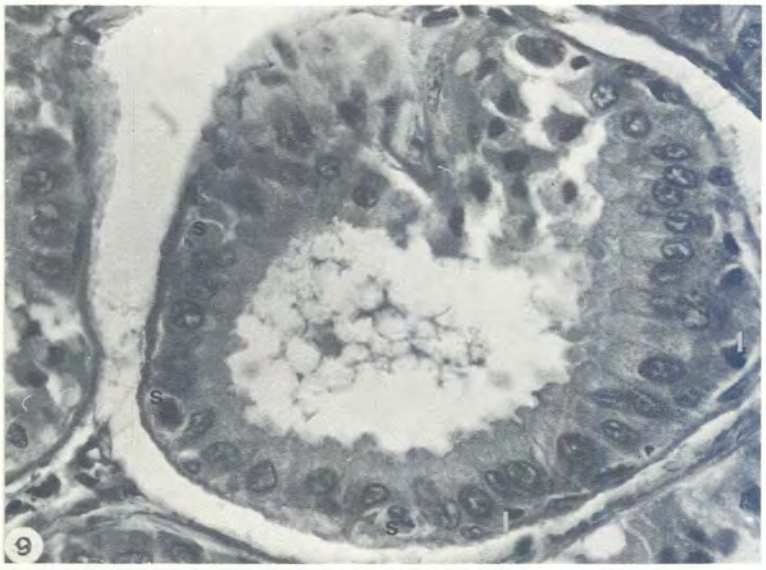
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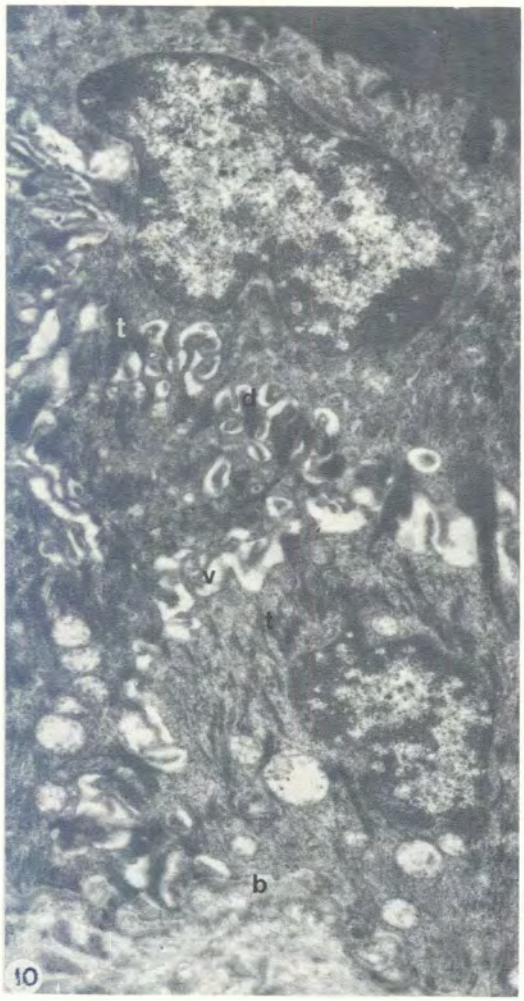
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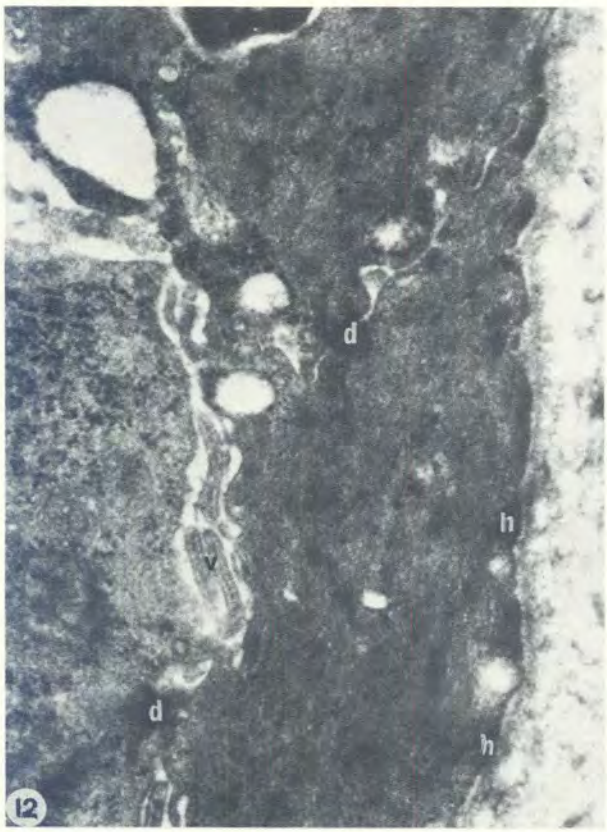

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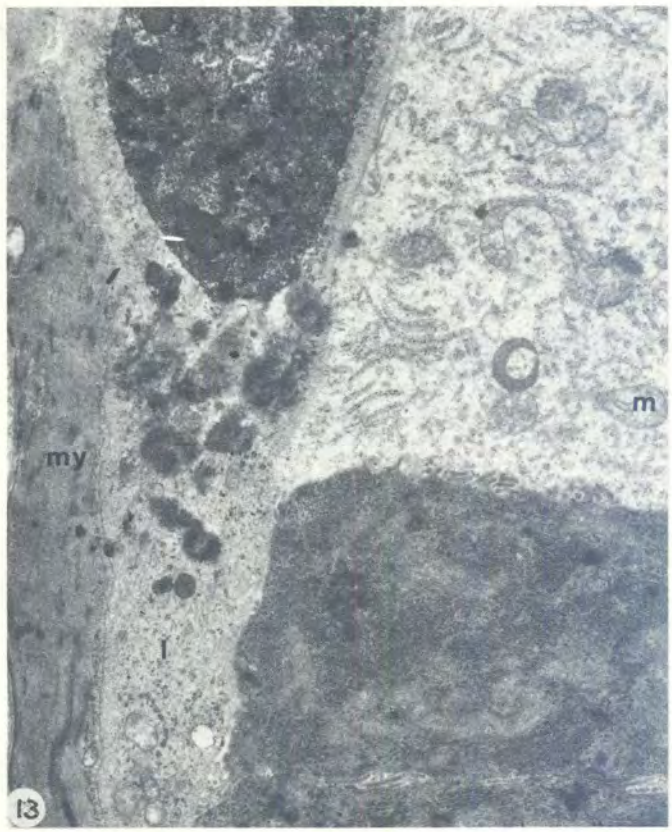

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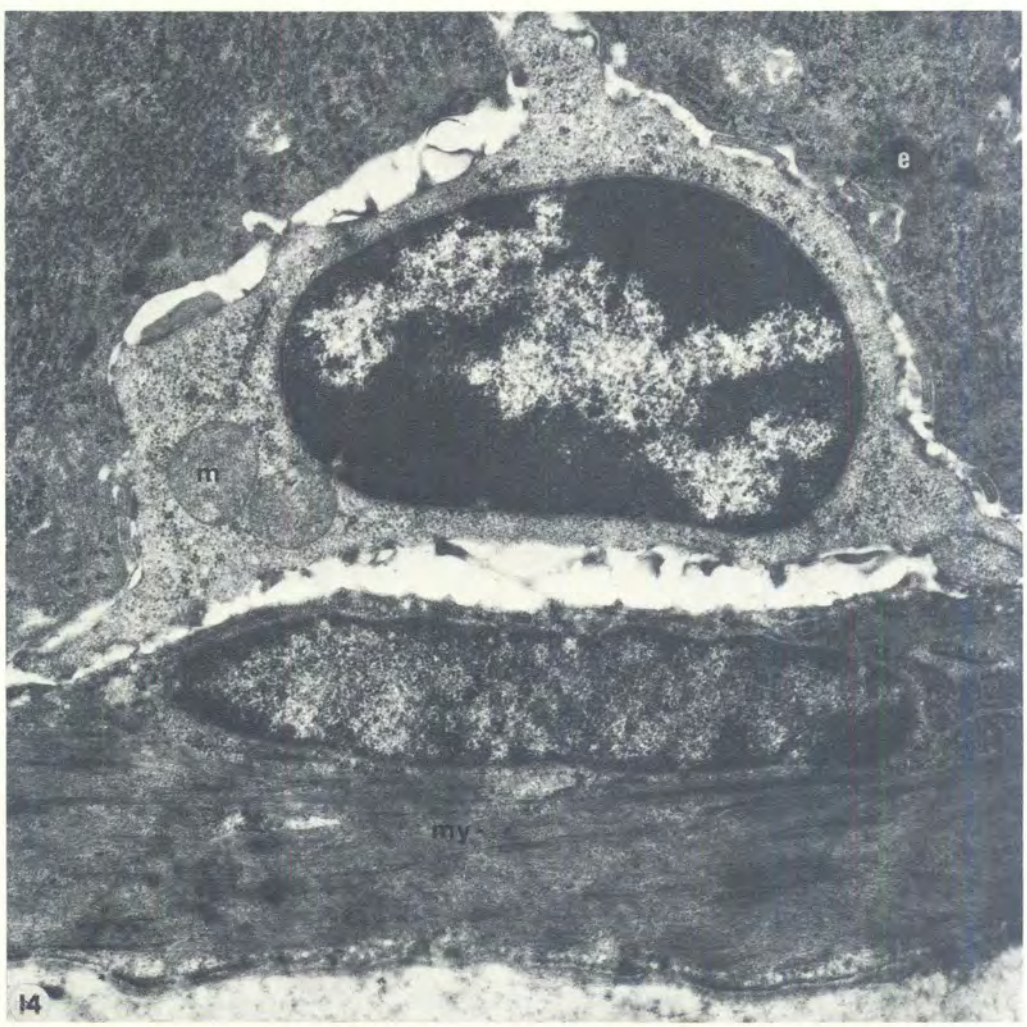

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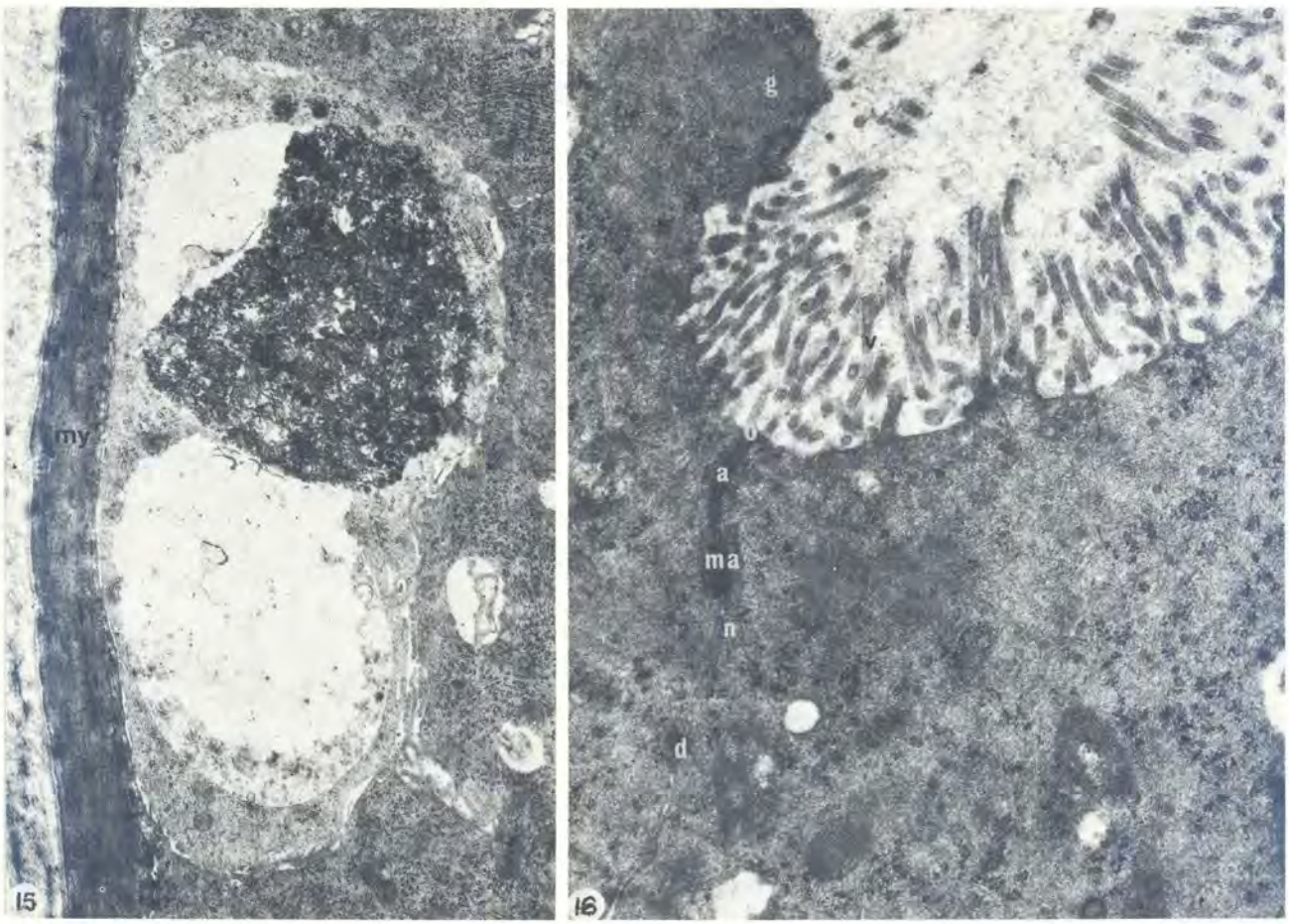

FIG. 15 A lymphoid cell, problably degenerate, with a granular mass and vacuoles between myoepithelial (my) and secretory cells. The vacuoles are caused by lipoids that have been dissolved out. ×8 600

FIG. 16 The distal end of the secretory cells show microvilli (v), a *zonula occludens* (o), a *zonula adherens* (a), a *macula adherens* (ma) various *nexes* (n), ribosomes, secretory granules (g) and desmosomes (d). ×21 670

(a) *The coiled glands*

In the rostral part of the glandular region just caudal to the chin the glandular region was subdivided by vertically arranged strands of striated muscle. These strands which were probably derived from the *M. mylohyoideus* became fewer towards the cauda and eventually disappeared. Below the glands was a fibro-elastic layer with its fibres arranged parallel to the surface. Some of the muscle fibres extended from this layer to the papillary layer of the dermis.

The coiled glands, being modified sweat glands, had numerous myoepithelial cells situated between the relatively thick basal lamina and the cuboidal to columnar secretory cells (Fig. 5, 6, 12, 13, 14 & 15). The ducts were very narrow, passed upwards alongside the hair follicles through well-developed arrector pili muscles and opened into the upper parts of the hair follicles. At approximately the height of the hair bulb there was an abrupt transition from a flattened non-secretory cuboidal epithelium lining the duct to a simple columnar epithelium lining the coiled secretory portion (Fig. 4). The coiling was serpentine rather than glomiform. A single cilium was present in one of the proximally situated duct cells (Fig. 11). The secretory cells had a finely granular eosinophilic cytoplasm but no distinct secretory granules were visible with light microscopy (Fig. 6 & 7). Proximally, the cells stained lightly basophilic. The secretion present in the comparatively large lumen was weakly PAS-positive. The nuclei were vesicular with a folded nuclear membrane which in electron micrographs showed numerous nuclear pores filled with a granular substance.

Electron micrographs clearly proved the epidermal origin of these secretory and myoepithelial cells by revealing the presence of numerous tonofibrils and desmosomes attaching adjacent cells to each other (Fig. 10, 11, 12 & 16). Between the desmosomes were numerous microvilli which practically filled the intercellular spaces (Fig. 10, 11 & 12). These microvilli were less evident on the myoepithelial than on the secretory cells (Fig. 12 & 15). In comparison with the keratinocytes of the epidermis the desmosomes were less numerous between adjacent secretory cells and even fewer between myoepithelial and secretory cells.

The myoepithelial cells, containing longitudinally arranged actin filaments, were attached to the basal lamina by numerous hemidesmosomes and to the epithelial cells by occasional larger desmosomes (Fig. 12). Between adjacent myoepithelial cells were numerous smaller desmosomes. Microvilli were mostly absent.

The secretory cells were covered by numerous microvilli on their distal surfaces while disto-laterally they had the typical juxta-luminal junctional complex consisting of a *zonula occludens*, a *zonula adherens* and a *macula adherens* (Fig. 16). Gap junctions (*nexes*) were present below these complexes which in the light microscope were visible as terminal bars. Both light and dark staining secretory cells were discernible (Fig. 13).

The proximal region of the secretory cells was rich in granular endoplasmic reticulum (Fig. 14), which in active cells was swollen and had a granular content. Secretion granules, free ribosomes and polyribosomes were visible distally (Fig. 16). Granular to rod-shaped mitochondria, a Golgi apparatus distal to the nucleus, a centrosome near the distal border and some lysosomes were present. Some epithelial cells rich in granular endoplasmic reticulum contained electron-dense, flakelike masses which revealed a fine fibrillar structure at 100 000 × magnification. Their fibrillar appearance resembled the appearance of the masses of actin filaments present in the myoepithelial cells.

In the intercellular space between the myoepithelial cells and the secretory cells were some cells which morphologically resembled the epithelial lymphocytes of the forestomach epithelium (Gerneke, 1977) (Fig. 6, 9, 14 & 15). They were easily distinguishable by the absence of tonofibrils and desmosomes and the presence of dendritic processes in which ribosomes and polyribosomes were the main organelles (Fig. 14). These presumably lymphoid cells contained an indented or oval nucleus, a few strands of granular endoplasmic reticulum, a few mitochondria, a small Golgi apparatus, lysosomes and a centrosome. In some of them were large vacuoles or electron-dense masses (Fig. 13). In Sudan IV stained sections some of these small lymphoid cells between the secretory cells and the myoepithelial cells were in what appeared to be various stages of either lipogenesis or lipophagocytosis (Fig. 6, 7 & 15). These cells to some extent resembled sebaceous gland cells. Some were small, without lipoid droplets and resembled small lymphocytes. As the lipoid granules increased, so the nucleus first became vesicular and then pyknotic and later even karyorrhectic (Fig. 8 & 9). In the latter case lipoid granules were numerous and completely filled the cell. Some other degenerate cells with karyorrhectic nuclei and compact eosinophilic cytoplasm, presumably regressive epithelial cells, were also seen.

In some segments of the coiled glands, areas of the secretory epithelium were completely sloughed off and were present practically intact, in the lumen (Fig. 8 & 9). These cells revealed pyknotic, shrivelled nuclei different from those of normal secretory cells. Some areas of the epithelium showed only shrivelled nuclei without desquamated cells. In some of the apocrine glands there were a few polyploid nuclei, probably preparing to undergo mitosis. However, no actual mitoses were found.

(b) *Sebaceous glands*

The sebaceous glands in the intermandibular region were richly branched, alveolar glands associated with hair follicles. They were about half as thick as the layer of coiled glands and, apart from their increased size, did not differ from the typical sebaceous glands of the skin (Fig. 2). As in the case of the coiled glands, their epidermal origin is revealed by the presence of desmosomes and microvilli.

Discussion

Since the mixed secretion from the coiled and sebaceous glands of the intermandibular glandular region is used for unintentional territorial demarcation and for marking females during mating (Fig. 1 & 3), it may be assumed that they have a cyclic activity which reaches a peak during the mating season. During the non-mating season, however, its activity cannot subside completely because unintentional territorial demarcating continues throughout the year. It is not known at present why the steenbok and no other species has this gland. The steenbok has no enlarged interdigital or inguinal glands. There is an interdigital sinus on the fore- and the hindfeet but the skin glands present have the same size and distribution as in the rest of the body. The only other gland which may play a role in territorial demarcating is the infra-orbital gland (Gerneke & Cohen, 1977) (Fig. 1).

Changes in the activity of the apocrine glands may be responsible for the sloughing off of sections of their epithelium (Fig. 8 & 9). In fact, during the histological investigations the impression was gained that this was a type of physiological desquamation and not merely due to post-mortem autolysis. Montagna & Parakkal (1974) also noted this sloughing off of epithelial cells in apocrine glands but failed to give any explanation for it. They found it remarkable that with so much cellular sloughing so few mitotic figures could be seen. It appears, therefore, to be a feature of apocrine glands in general.

According to certain recent concepts of immunology (Silberberg, 1971; Shelly & Juhlin, 1976) the small lymphoid cells noted in the epithelium must play an important role in the primary immune response of the body. They cannot at this stage be compared to Langerhans cells of the epidermis because no Langerhans cell granules have been found in them (Gerneke, 1977). Nevertheless, they resemble Langerhans cells in all other morphological aspects. The absence of desmosomes also indicates that they are migrating cells of extra-epithelial origin. No reference to them could be found in standard textbooks.

It is difficult to account for the fact that these lymphocytes accumulate lipoids. If it is assumed that they are present merely as antigen or allergen detectors, it is difficult to attribute to them simultaneously a phagocytic or even generative function unless there are two cell lines each with a different function. Unfortunately, because they are scarce and often degenerate (Fig. 15), examination of them is difficult. The degenerate cells may be epithelial cells undergoing regressive changes but none of them were seen in electron micrographs. This aspect will, however, be investigated should another opportunity occur.

The epithelial cells reveal considerable activity and are usually full of secretion granules in the distal parts of the cells (Fig. 16). Proximally, large amounts of granular endoplasmic reticulum responsible for producing the proteinaceous part of the secretion are present (Fig. 14). They therefore have features common to the majority of secretory cells. However, the significance of the light and the dark cells is not known.

The glandular area of an immobilized animal was found to be sensitive to touch. A possible explanation of this sensitivity may be that it is due to a reflex action associated with mating (Compare Fig. 3) which shows up while the higher brain centres are subdued by the drugs used. The untreated animal when held would be fearful and tensed and would obviously react differently.

Acknowledgements

The authors wish to express their sincere appreciation to Mr John Soley for his technical assistance and for developing and printing the micrographs, to Mrs

H. Rothman for developing and printing the micrographs and to Mrs S. Clarke for preparing the sections for light- and ultramicroscopy. Our sincere thanks are also due to Mr H. Braack for Figure 1 which was used by kind permission of the Editor of the Journal of the South African Veterinary Association (Cohen & Gerneke, 1976).

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Printed by and obtainable from The Government Printer, Private Bag X85, Pretoria, 0001