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THE MICROMORPHOLOGY OF THE GLANDS OF THE INFRA-ORBITAL CUTANEOUS SINUS OF THE STEENBOK (*RAPHICERUS CAMPESTRIS*)

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ABSTRACT

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The infra-orbital cutaneous sinus produces a black secretion which is the combined secretion of melanaceous, branched, alveolar, sebaceous and enlarged, coiled apocrine glands. The micromorphology of these glands is described with special emphasis on the sebaceous glands and melanin transfer. The secretion, which may be used for unintentional territorial demarcation, is possibly produced as small black granules and is most likely important for short range communication.

Résumé

MICROMORPHOLOGIE DES GLANDES DU SINUS CUTANÉ INFRA-ORBITAL CHEZ LE STEENBOK (Raphicerus campestris)

Le sinus cutané infra-orbital produit une sécrétion noire qui résulte de l'excrétion conjointe de glandes mélanacées, ramifiées, alvéolaires, sébacées et de glandes apocrines agrandies et enroulées.

On décrit la micromorphologie de ces glandes en détaillant particulièrement les glandes sébacées et le transfert de la mélanine. On pense que la sécrétion, qui peut servir à une délimitation territoriale involontaire, est produite sous forme de petits granules noirs. Elle semble être surtout importante pour les communications rapprochées.

INTRODUCTION

Various modified cutaneous glands or glandular regions occur in different wild animal species and, although reference has been made to a black secretion (Tinley, 1969; Gosling, 1972), melanin has never been positively identified in secretions of these animals. In an investigation of the cutaneous glands of both male and female steenbok (Cohen & Gerneke, 1976), the glands of the infra-orbital sinus of this species contained what appeared to be black pigment in all of the 40 animals examined. A histological examination was therefore undertaken to determine the nature of the black secretion.

MATERIALS AND METHODS

Tissue from the infra-orbital cutaneous sinus of both male and female animals was fixed in 10% formalin, Zenker's and Bouin's fixatives and processed by standard histological techniques. Paraffin sections were cut at 4 microns and stained with haematoxylin and eosin. Frozen sections were also cut from the formalin-fixed tissue and stained with Sudan IV. The samples for electron microscopy were treated as described elsewhere (Gerneke & Cohen, 1978).

RESULTS

The infra-orbital sinus is situated about 10 mm ventral to the medial angle of the eye. The cavity has an epidermal lining (Fig. 1) with its opening directed laterally. In the deeper regions there are enlarged sebaceous and apocrine glands which open into the cavity through several large common ducts (Fig. 2). These ducts represent the enlarged openings of the sebaceous glands in which the ducts of the apocrine glands open distally on one side and a small hair follicle branches from the opposite side, but more proximally (Fig. 2). The sebaceous glands beyond the rim of the sinus are free of melanin pigment. At the rim of the sinus half or part of a gland may be pigmented and the remainder unpigmented.

The most striking feature of the sebaceous glands is that the glandular cells in the alveoli are filled with oval black melanosomes (melanin granules) (Fig. 3). These granules are synthesized by the large stellate melanocytes arranged between the basal cells on or near the basal lamina (Fig. 3) and their processes penetrate between the immature sebaceous gland cells (Fig. 3, 4, 5 & 6) where they are often encountered in oblique or transverse section. Clumps of melanosomes accumulate in the distal ends of these processes and are transferred as such to the immature sebaceous gland cells by the process of cytocrine secretion. (Fig. 3, 4 & 8).

The melanocytes present between the immature cells are easily distinguishable from the latter because their melanin granules are evenly dispersed throughout the cytoplasm except in the distal ends of their processes (*vide supra*), whereas the melanosomes taken up by the immature sebaceous gland cells initially remain as clumps and become evenly dispersed only as the cells gradually mature (Fig. 3, 4 & 5). More melanin appears to be taken up than is present in the mature cells. This melanin apparently has no suppressive influence on lipoid formation because lipoid droplets increase in number and size as the cells mature (Fig. 6).

The melanocytes contain a relatively large nucleus with one or more distinct nucleoli (Fig. 6). In their cytoplasm there are ribosomes and polyribosomes, oval to elongated mitochondria, a Golgi apparatus and numerous evenly distributed pre-melanosomes and mature melanosomes (Fig. 6, 7, 8 & 9). Strands of granular endoplasmic reticulum are also present (Fig. 7). The melanocytes do not have any desmosomes or microvilli (Fig. 6), except that an occasional minute villus-like projection from the cytoplasm is encountered touching the plasmalemma of the sebaceous gland cell.

The dendritic processes of the melanocytes can apparently be extended and retracted as the need arises for melanosomes to be transferred to the sebaceous gland cells. Before transfer, the melanosomes tend to become clumped, especially in the more distal parts of the processes which then have a beaded appearance (Fig. 3 & 4).

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FIG. 1 A vertical section through the infra-orbital sinus of the steenbok showing the large melanaceous branched alveolar sebaceous glands below the stratified squamous epithelium (e) of the sinus. Below the sebaceous glands small coiled apocrine glands are visible. ×16

FIG. 2 In the infra-orbital sinus the sebaceous gland duct (b) becomes the major structure with the hair follicle (f) and sweat gland duct (s) minor accessories. ×20
e=stratified squamous epithelium r=striated muscle

FIG. 3 A stellate melanocyte (c) with its numerous beaded processes extending between the immature cells of the sebaceous gland seen in a horizontal section. The "beads" are formed by clumps of melanosomes which in turn are phagocytosed by the immature sebaceous gland cells (is) seen higher up from the tips of such processes. As the sebaceous gland cells mature the clumps of melanosomes are dispersed (p) as seen here in a transverse section through an alveolus. ×410

FIG. 4 In the proximal regions of the alveoli of the melanaceous sebaceous glands, the melanocytes (c) with their evenly dispersed melanosomes and the immature sebaceous gland cells (is) with clumps of melanosomes are easily distinguishable. The individual melanosomes are minute oval black granules. ×640

FIG. 5 A melanaceous alveolus showing a melanocyte (c) proximally and a layer of immature sebaceous gland cells (is) with clumps of melanosomes more distally. The mature cells have evenly dispersed melanosomes. The vacuoles in the sebaceous gland cells are formed by lipoids dissolving during the preparation of the specimen. ×410

FIG. 6 Sebaceous gland cells with desmosomes (d) and microvilli (v) between adjacent cells and containing mitochondria (m), melanosomes (o) and vacuoles where lipoids have been dissolved out. Two melanocytes without any desmosomes or microvilli, numerous black melanosomes and processes penetrating into the intercellular spaces, are shown. Some melanosomes have shifted in position or have fallen out during preparation of the sections. ×8 400
bl=basal lamina n=nucleus

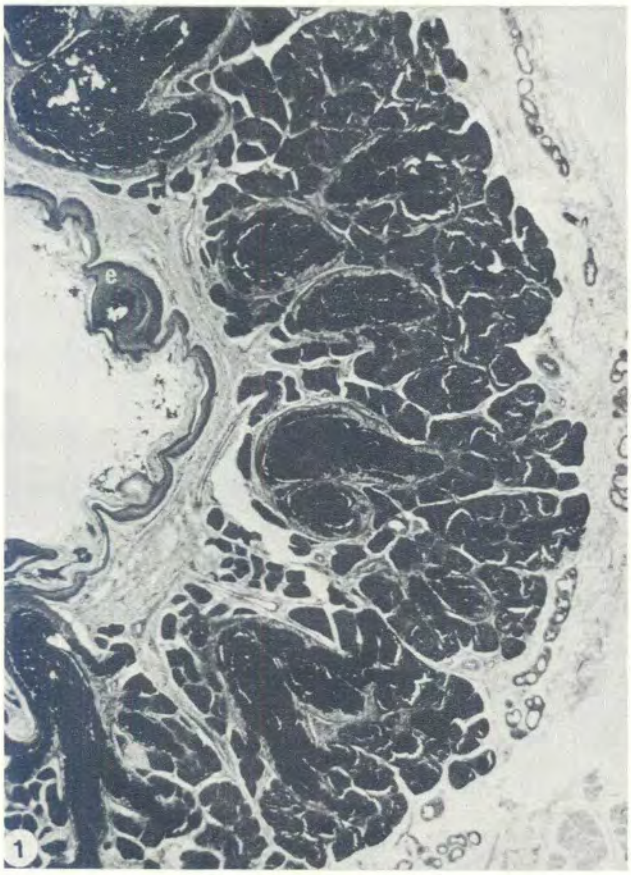
FIG. 7 A melanocyte, showing granular endoplasmic reticulum, ribosomes, nucleus (n), a mitochondrion (m) and a premelanosome (pm). ×26 950

FIG. 8 A sebaceous gland cell is seen engulfing a clump of melanosomes by pushing out two cytoplasmic veils. At o two melanosomes are seen within a sebaceous gland cell with a cytoplasmic film probably from the melanocyte still around them. Melanocytic processes with ribosomes are present between the sebaceous gland cells. ×15 000
d=desmosome n=nucleus v=microvilli

FIG. 9 Melanosomes in various stages (1–8) of development in a melanocyte. ×56 000

FIG. 10 An immature sebaceous gland cell of the infra-orbital sinus revealing numerous vacuoles and ribosomes, some microvilli (v) and desmosomes (d) and numerous mitochondria (m), some with a peculiar vesicular structure of unknown significance. ×28 600

FIG. 11 A melanophore below the basal lamina (bl) in the dermis with mitochondria (m), granular endoplasmic reticulum and numerous phagocytosed melanosomes. ×11 800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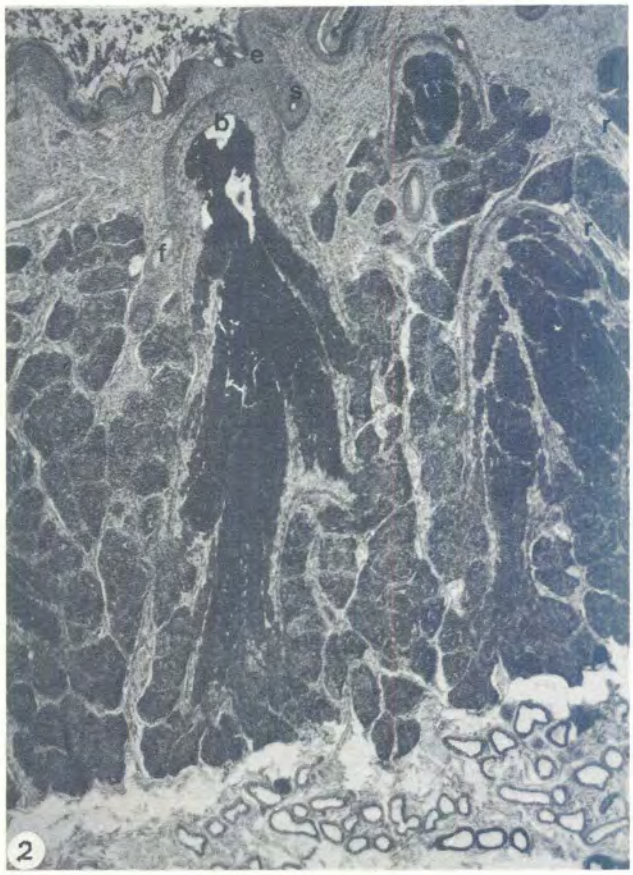
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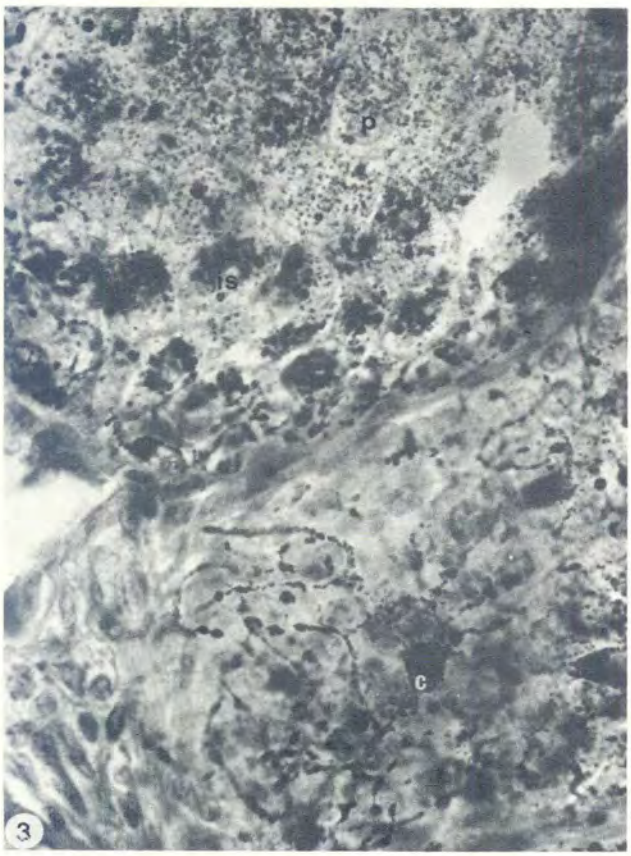
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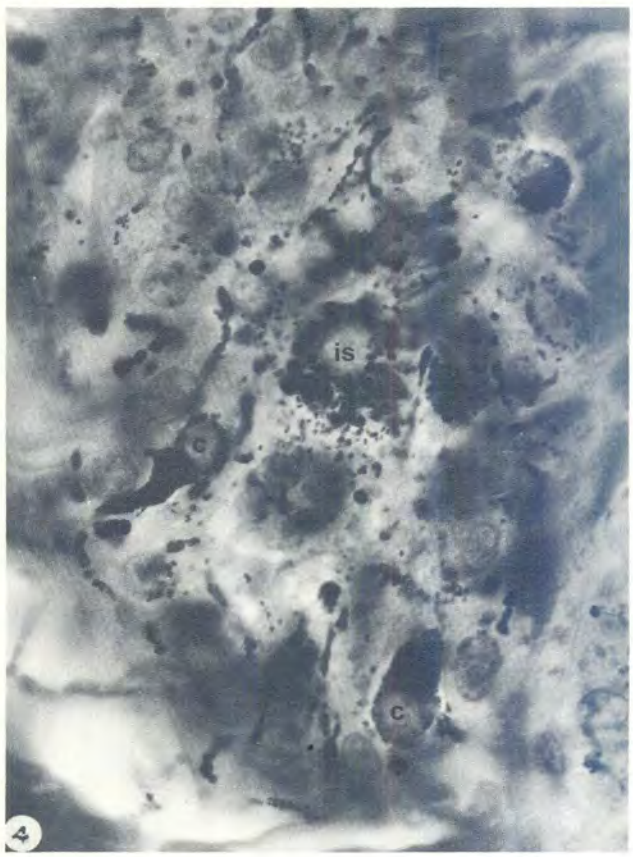
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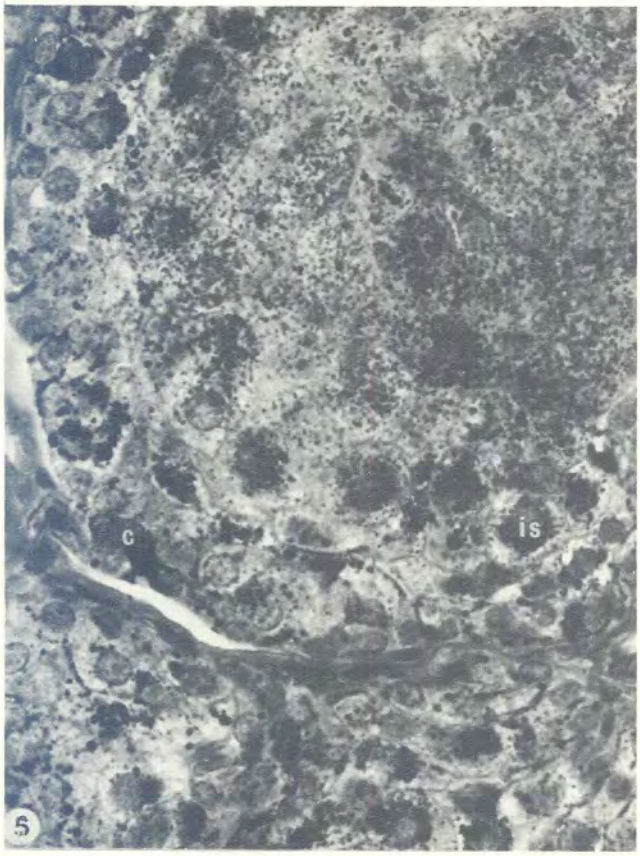

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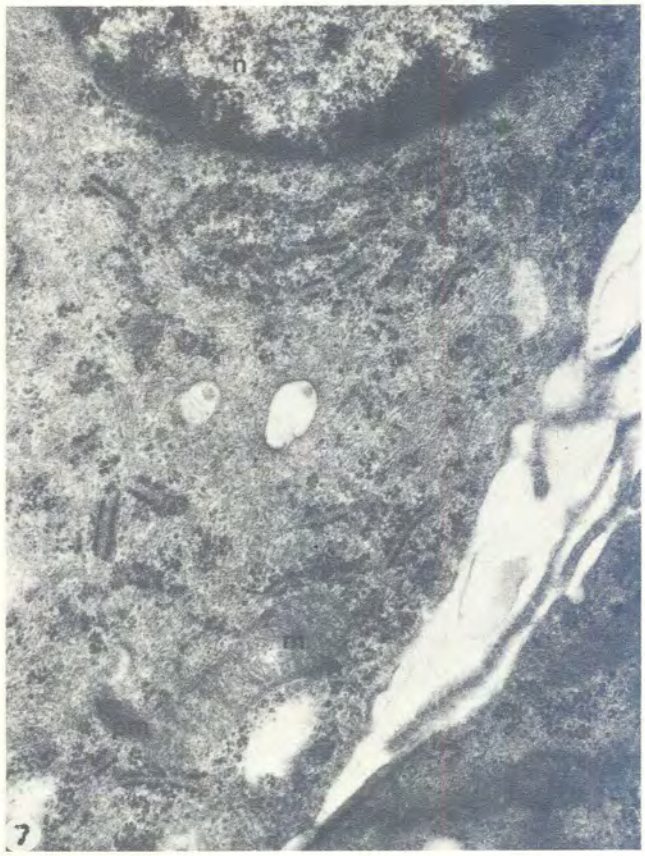

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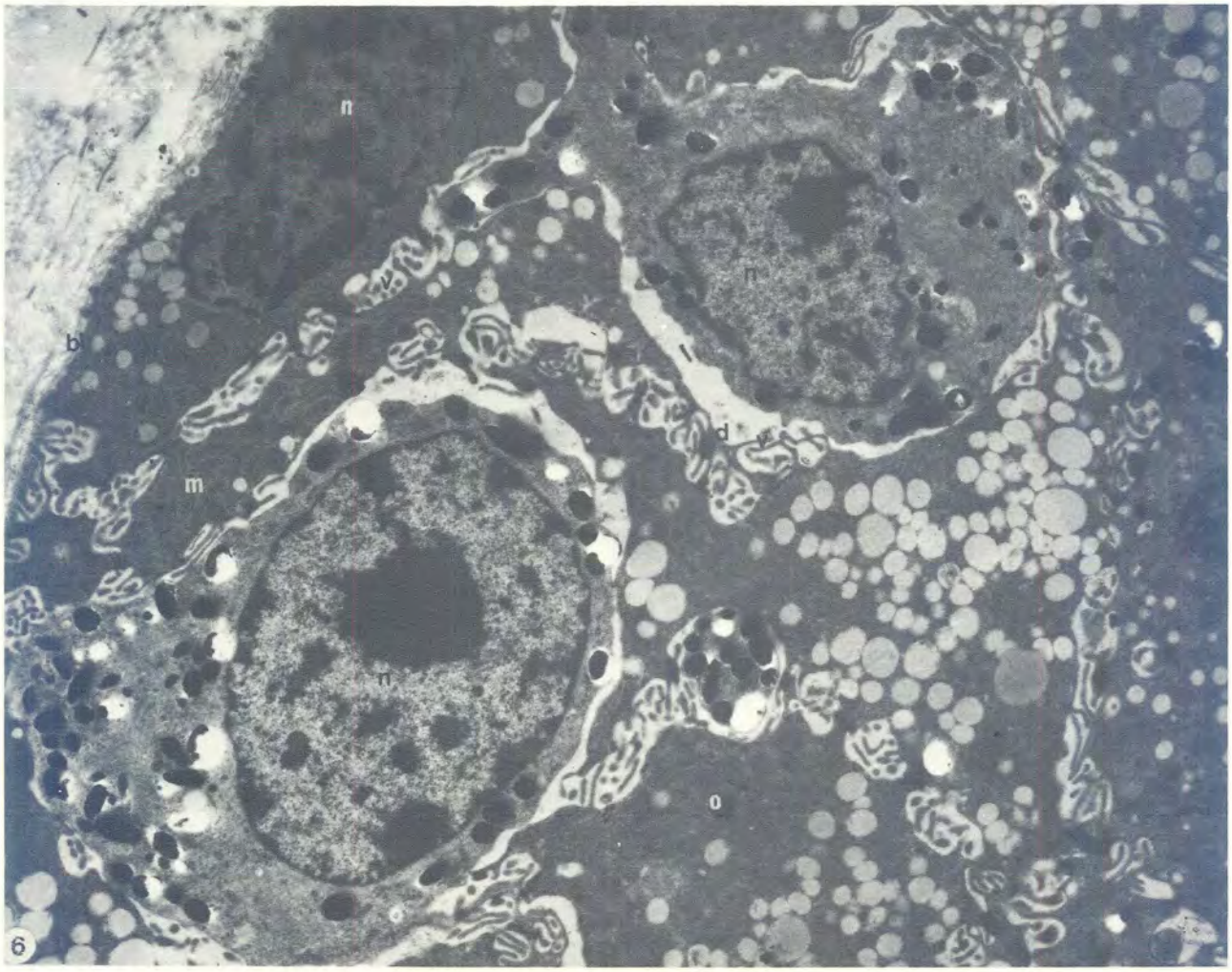

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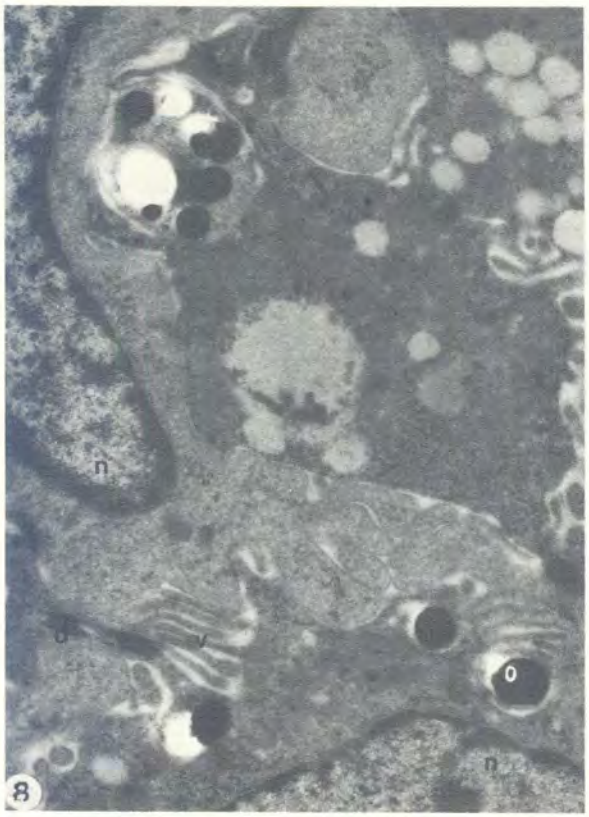

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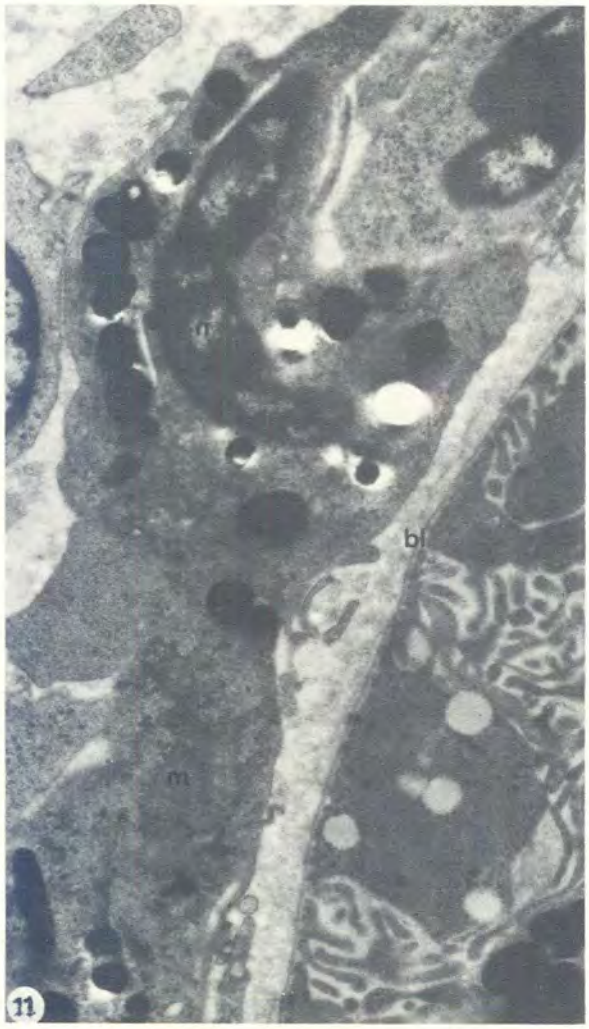

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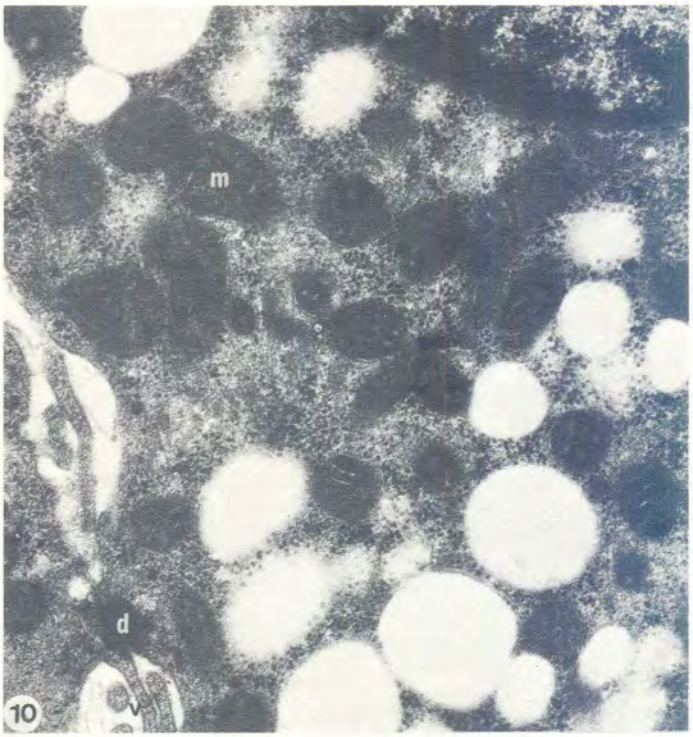

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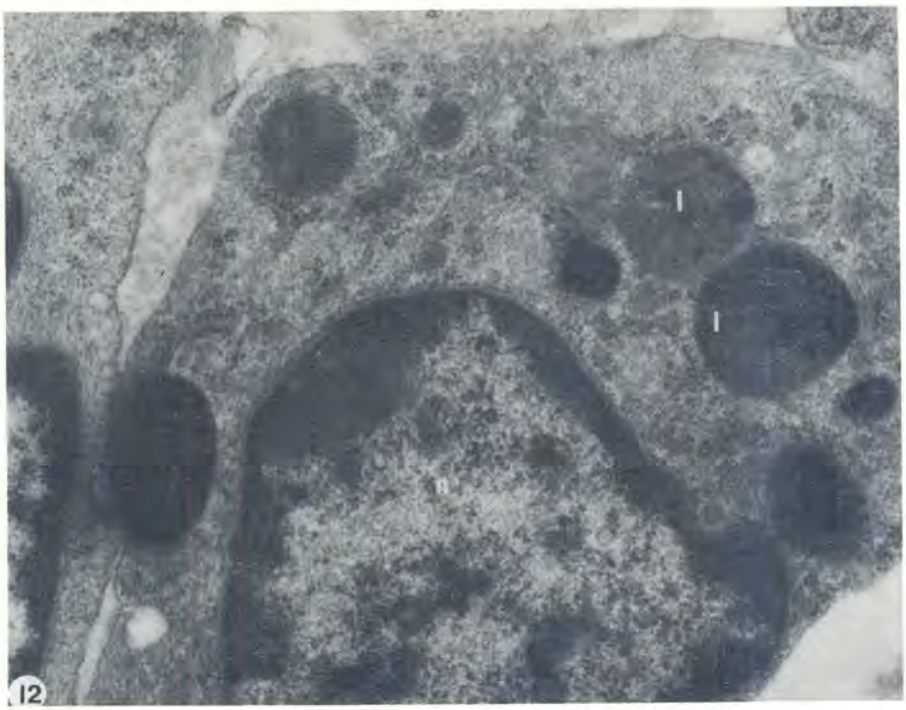

FIG. 12 A melanophore in the dermis of the infra-orbital sinus showing some melanosomes apparently being digested in lysosomes (l). ×26 000

Melanophores occur in the connective tissue between the sebaceous glands (Fig. 11 & 12). The melanophores phagocytose any melanosomes which go astray during transfer from the melanocytes to the sebaceous gland cells. Some melanosomes in the melanophores can also be digested by formation of phagosomes (Fig. 12). These melanosomes may be seen lying free in the dermal connective tissue. In the region subjacent to the sebaceous glands, a few melanocytes and sometimes a few melanosomes occur in the epithelium of the apocrine glands. The former are relatively small, are not very active and appear to be ectopic.

Besides the melanosomes the sebaceous gland cells contain a distinct vesicular nucleus, numerous granular to filamentous mitochondria, a few strands of granular endoplasmic reticulum, ribosomes, lipoid droplets of various sizes, a Golgi apparatus, numerous microvilli and an occasional desmosome between the adjacent cells (Fig. 6). Some mitochondria contain a peculiar vesicular structure which may be elongated (Fig. 10). The mature cells are filled with lipoid droplets, evenly dispersed melanosomes (Fig. 3 & 5) and a pyknotic nucleus. The immature cells sometimes show mitotic figures and have clumps of melanin that have been absorbed from the dendritic melanocytic processes (Fig. 3 & 8).

The apocrine glands below the melanaceous glands are not well developed. Their ultracytology is similar to that of the coiled glands of the intermandibular glandular region (Gerneke & Cohen, 1978). Between the myoepithelial and secretory cells are a few cells, presumably lymphoid, which resemble sebaceous gland cells in that they contain lipoid droplets. These cells are similar to the epithelial lymphocytes of the forestomach (Gerneke, 1977), but no Langerhans cell granules were found in them. Desmosomes and microvilli are absent from these lymphoid cells.

Strands of striated muscle pass up between the melanaceous sebaceous glands to the connective tissue of the *stratum papillare* (Fig. 2r). Contraction of these muscles should force the secretion out as small black granules. These muscles could be strands from either the *M. levator nasolabialis, M. malaris* or both.

The *arrector pili* muscle of the hair follicle is well developed and extends to the papillary layer alongside the sebaceous gland.

Discussion

The most remarkable finding of this investigation is the presence of large amounts of melanin in the sebaceous gland secretion (Fig. 1). The steenbok is the only species in which such a phenomenon has been described. Tinley (1969) states that the pre-orbital (=infra-orbital) glands of the dikdik produce a black tarry secretion which is rubbed onto vegetation to demarcate this animal's territory. A similar secretion has been described in the oribi (Gosling, 1972). Although it has not been verified histologically, the black colour of these secretions was probably due to melanin.

In the epidermis, melanosomes are produced by the melanocytes and passed into the keratinocytes by cytocrine secretion. In this cytophagocytic process, cytoplasmic veils from the keratinocyte enclose the dendritic endings containing groups of melanosomes (Fig. 8). The enclosed ending is subsequently broken up in the cytoplasm of the keratinocyte and the melanosomes scattered in its cytoplasm (Prunieras, 1969). Melanin transfer is apparently not dependent on the degree of melanization of the melanosomes as non-melanized premelanosomes pass into keratinizing cells in the hair follicles of albino mice (Parakkal, 1967). Swift (1964) found that single or groups of melanosomes are phagocytosed by the cortical cells

of human hair. These melanosomes were actually encased in the plasmalemma of the cortical cells and were always taken in from the tips of the dendritic processes. Some remained attached to the plasmalemma by double unit membranes which were in apposition.

Electron microscopic studies indicate that the sebaceous glands of the infra-orbital sinus of the steenbok obtain melanin from the melanocytes in the same sequence of events as in other epidermal derivatives. The small amounts of cytoplasm taken up by the sebaceous gland cells during melanin transfer is possibly of limited nutritional value after lysosome degradation.

Some melanin granules may also be digested by lysosome enzymes during the process of dispersion (*vide infra*). The presence of melanin either in clumps or dispersed does not interfere with lipoid production by the sebaceous gland cells (Fig. 3). There were no persistent unit membranes of the plasmalemma around the clumps of melanin granules probably as a result of lysosomal action. In keratinized cells, however, they do persist (Swift, 1964).

Melanosomes are generally considered very resistant bodies which are carried by the keratinocytes through the process of keratinization and are eventually discarded during desquamation of the epithelium. It has been shown recently, however, that melanosomes can be broken down by lysosomes and this phenomenon is responsible for hypopigmentation in the Chediak-Higashi syndrome (Zelickson, Windhorst, White & Good, 1967). Studies on tissue culture have also shown that after 3–4 days transferred melanosomes are located within single membrane-bound vesicles rich in acid phosphatase. Twelve to 15 days later the number of melanosome-containing cells was greatly reduced (Prunieras, 1969).

It may be concluded, therefore, that some melanin is broken down by lysosome action and these observations show this also occurs in sebaceous gland cells as well as in melanophores. Sufficient melanosomes, however, are retained to give the secretion a black colour. Further investigation is required to determine the nature of the peculiar vesicular structures present in the mitochondria.

Sebaceous glands have a holocrine secretion and therefore need a continuous supply of lipoid-forming cells. These are generated from the basal cells. This means that the sebaceous cells, as they develop and grow, continually pass the sedentary melanocytes and each time remove a piece of melanosome-filled cytoplasm from the melanocytic processes. These dendritic processes are continually extended and retracted, and so facilitate the passage of oscillating melanosomes along the processes (Prunieras, 1969). Because of this movement it would be completely unpractical for them to possess tonofibrils and desmosomes.

Preliminary observations indicate that these melanocytes are ideal for studying melanosome formation. Vesicles, which presumably arise from the Golgi apparatus, contain longitudinally arranged fibrils which become shortened and spirally twisted [Fig. 9 (4), 7 (pm)] and are gradually obliterated by deposition of melanin until homogeneous oval melanotic granules result (Fig. 9). These move along the processes and clump together to give a beaded appearance (Fig. 3 & 4). These "beads" are then phagocytosed by the sebaceous gland cells from the distal ends of the processes.

It has been shown that isolated melanocytes do not undergo mitosis (*in vitro*). They start multiplying and extending their processes only when in contact with keratinocytes (Prunieras, 1969). This would also apply to the sebaceous gland cells because with them the melanocytes are large, active cells. A certain degree of interdependence therefore exists between melanocytes and the sebaceous gland cells and this interdependence is restricted to the sebaceous glands of the infra-orbital sinus only, because, at the rim of the sinus, melanization of the sebaceous glands stops abruptly. Since this restriction is such that half a sebaceous gland may be pigmented while its other half may remain pigment free, "ectopic" melanocytes are rarely found. Although a few were encountered in the coiled glands subjacent to the pigmented sebaceous glands, they were small and appeared comparatively inactive.

The primary function of a melanotic secretion is, however, still unknown. Granules of black secretion may be passed out as a result of stimulation by androgens (Montagna & Parakkal, 1974) or by muscular contraction during chewing or sniffing operations. Although no dissections of facial muscles were made, the muscle fibres going into the gland could belong either to the *M. levator nasolabialis* or *M. malaris* or both. The intentional marking of twigs with the secretion of this gland observed in the dikdik (Tinley, 1969) and oribi (Gosling, 1972) has not been recorded in the steenbok. It is possible that this secretion may be used in territorial demarcation either to keep other herbivorous animals away or to restrict mating pairs to the same grazing area. It is apparent that the black secretion must owe its detection to its odoriferous content of pheromones as visual detection would be wellnigh impossible. Tinley, (1969) considered the glands of the infra-orbital sinus in the dikdik to be scent-producing. One may conclude from the available evidence that the glands of the infra-orbital sinus of the steenbok are used in short-range communication but this conclusion must be confirmed by further experimental observations and from analyses of their pheromone content before there can be a final consensus of opinion.

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