

Skin morphology and its role in thermoregulation in mole-rats, *Heterocephalus glaber* and *Cryptomys hottentotus*

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ABSTRACT

The skin structure of 2 Bathyergid rodents, the naked mole-rat (*Heterocephalus glaber*) and the common mole-rat (*Cryptomys hottentotus*) is compared, to investigate whether thermoregulatory differences may be attributed to different skin features. Histological and ultrastructural studies of the dorsal skin of these closely related species show morphological and structural similarities but differences in the degree of skin folding, thickness of the integument and dermal infrastructure were evident. The skin of the common mole-rat conforms with expected morphological/histological arrangements that are commonly found in mammalian skin. Many features of the skin of the naked mole-rat, such as the lack of an insulating layer and the loosely folded morphological arrangement contribute to poikilothermic responses to changing temperatures of this mammal. Further evidence for poikilothermy in the naked mole-rat is indicated by the presence of pigment containing cells in the dermis, rather than the epidermis, as commonly occurs in homeotherms. Lack of fur is compensated by a thicker epidermal layer and a marked reduction in sweat glands. Differences in skin morphology thus contribute substantially to the different thermoregulatory abilities of the 2 Bathyergids. The skin morphology is related to the poor thermoinsulatory ability of the animals while simultaneously facilitating heat transfer from the environment to the animal by thigmothermy and/or other behavioural means.

Key words: Epidermis; naked mole-rat; common mole-rat; poikilothermia; homeothermia; ectothermia.

INTRODUCTION

Mole-rats (Order Rodentia; Family Bathyergidae) lead a strict subterranean existence and thus encounter a very different environment to mammals dwelling above ground. While this subterranean milieu protects against predation and climatic extremes, it is not without disadvantage: foraging beneath the ground is energetically costly and both heat and gas exchange are impeded (Ar, 1977; Nevo, 1995). The Bathyergidae, like other subterranean mammals overcome some of the problems encountered below ground by having lower resting metabolic rates and lower body temperatures than above-ground dwelling mammals (McNab, 1979; Buffenstein & Yahav, 1991; Bennett et al. 1992). The naked mole-rat *Heterocephalus glaber* (Rüppell, 1842) and the common mole-rat *Cryptomys hottentotus* (Lesson, 1826), 2

hystricomorph rodents, differ markedly in other aspects of their thermoregulation. The common mole-rat is a good thermoregulator and maintains a constant body temperature over a wide range of ambient temperatures (i.e. homeothermic), whereas the naked mole-rat is poikilothermic in that its body temperature is dependent on and reflects ambient temperature (Buffenstein & Yahav, 1991).

The common mole-rat and the naked mole-rat differ markedly morphologically. The common mole-rat, like most mammals, is furred whereas the naked mole-rat is hairless. The dorsal skin of the latter species lacks sweat glands and hair follicles (Tucker, 1981). These unusual mammalian skin properties may reflect the warm humid underground environment of tropical Africa, in which this species resides.

The integument of an animal is in direct contact with its environment. It affords mechanical protection

of the soft tissues and also acts as a barrier against infection and physiological stresses encountered in its habitat. Skin properties of an animal may change in relation to the environment. Indeed skin morphology may be a better indicator of habitat and lifestyle than phylogeny (Hildebrand, 1988).

In this investigation we compare skin structure in 2 Bathyergid rodents (*Heterocephalus glaber* and *Cryptomys hottentotus*) to elucidate whether marked thermoregulatory differences in these closely related species may be attributed to different skin features.

MATERIALS AND METHODS

The skin from the dorsal region of 5 adult male naked mole-rats (*H. glaber*) (~ 30 g, aged 2 y) housed in the Central Animal Unit of the University of the Witwatersrand at room temperature (32 °C) and 2 field-caught male common mole-rats (*C. hottentotus*) (~ 120 g, age unknown) was removed. The tissue was fixed in 10% buffered formalin and routinely processed for light microscopy. Alternate sections were stained with haematoxylin and eosin and Masson's trichrome stain for examination by means of a Nikon Optiphot II light microscope. In addition, paraffin sections of skin were treated with the Masson-Fontana reducing method in order to demonstrate both formed melanin and melanin precursors (Bancroft & Stevens, 1990). Sections were treated with 10% ammoniacal silver nitrate overnight in the dark at room temperature, washed in several changes of distilled water and treated with 5% sodium thiosulphate. After washing in tap water the sections were counterstained with 1% neutral red (aqueous), dehydrated through a graded series of alcohols to xylene and mounted in DPX. Control sections of naked mole-rat, common mole-rat and human skin were bleached with either potassium permanganate and oxalic acid or hydrogen peroxide and counterstained in neutral red.

Pieces of skin tissue were also placed in 2.5% glutaraldehyde for 2 h, washed in phosphate buffer and postfixed in 1% osmium tetroxide. After further processing the tissue was vacuum embedded in Epon-Araldite and sectioned on a Reichert-Jung Ultracut ultramicrotome. Thin sections (60–80 nm) were stained with uranyl acetate and lead citrate and viewed in a JEOL 100S transmission electron microscope at 80 kV.

RESULTS

The skin of both species of mole-rat is loosely folded but this is more pronounced in the naked mole-rat (Fig. 1a, b). Marked interspecies differences in skin

histology of both the epidermal and dermal layers were evident.

Epidermis

Histological and ultrastructural studies of the epidermis of the naked mole-rat shows it to be a relatively thick layer when compared to that of *C. hottentotus* (Figs 2, 3). The junction between the dermis and epidermis is regular with no dermal papillae or epidermal ridges. The epidermis is approximately 8–12 cells in thickness with large cuboidal cells resting on a prominent basement membrane making up the stratum basale. The cells have large rounded sometimes indented nuclei with 1 and occasionally 2 nucleoli. The cell borders are irregular with short cytoplasmic processes projecting into narrow intercellular spaces making contact with neighbouring cells. The processes are characterised by having numerous desmosomes. Cells of the stratum spinosum do not appear spindle-shaped in paraffin or resin embedded sections for light microscopy but ultrastructurally exhibit tonofilaments and tight junctions between the cells. The stratum granulosum forms an incomplete layer of flattened cells with oval to round nuclei. The cells of the granular layer have a reduced number of organelles, desmosomes that show marked thickening of the intercellular contact layer and more densely staining cell membranes. Above the stratum granulosum a prominent lucent layer is clearly visible in resin embedded sections (Fig. 2). If this is indeed a layer of cells then they lack nuclei and have a clear cytoplasm. However, no equivalent layer or a structured stratum lucidum could be identified in either paraffin-embedded sections or sections for ultra-microscopy. Ultrastructurally the stratum corneum has several layers of flattened keratinised cells.

In *C. hottentotus* the epidermis is approximately 4–8 cells thick (Fig. 3). The large cuboidal cells which make up the stratum basale, appear similar to those found in the naked mole-rat, with large rounded, sometimes indented, nuclei and 1 or 2 nucleoli. The division between the dermis and the epidermis is even and lacks dermal papillae and epidermal ridges. The stratum granulosum is discontinuous in sections examined under the light microscope. Above this layer and similar in appearance to that found in *H. glaber*, is a prominent lucent layer of cells in resin embedded sections. As in *H. glaber*, this layer could not be confirmed in either paraffin-embedded sections or sections for ultramicroscopy. The stratum corneum although also made up of flattened keratinised cells appeared to be thicker in the skin from the common

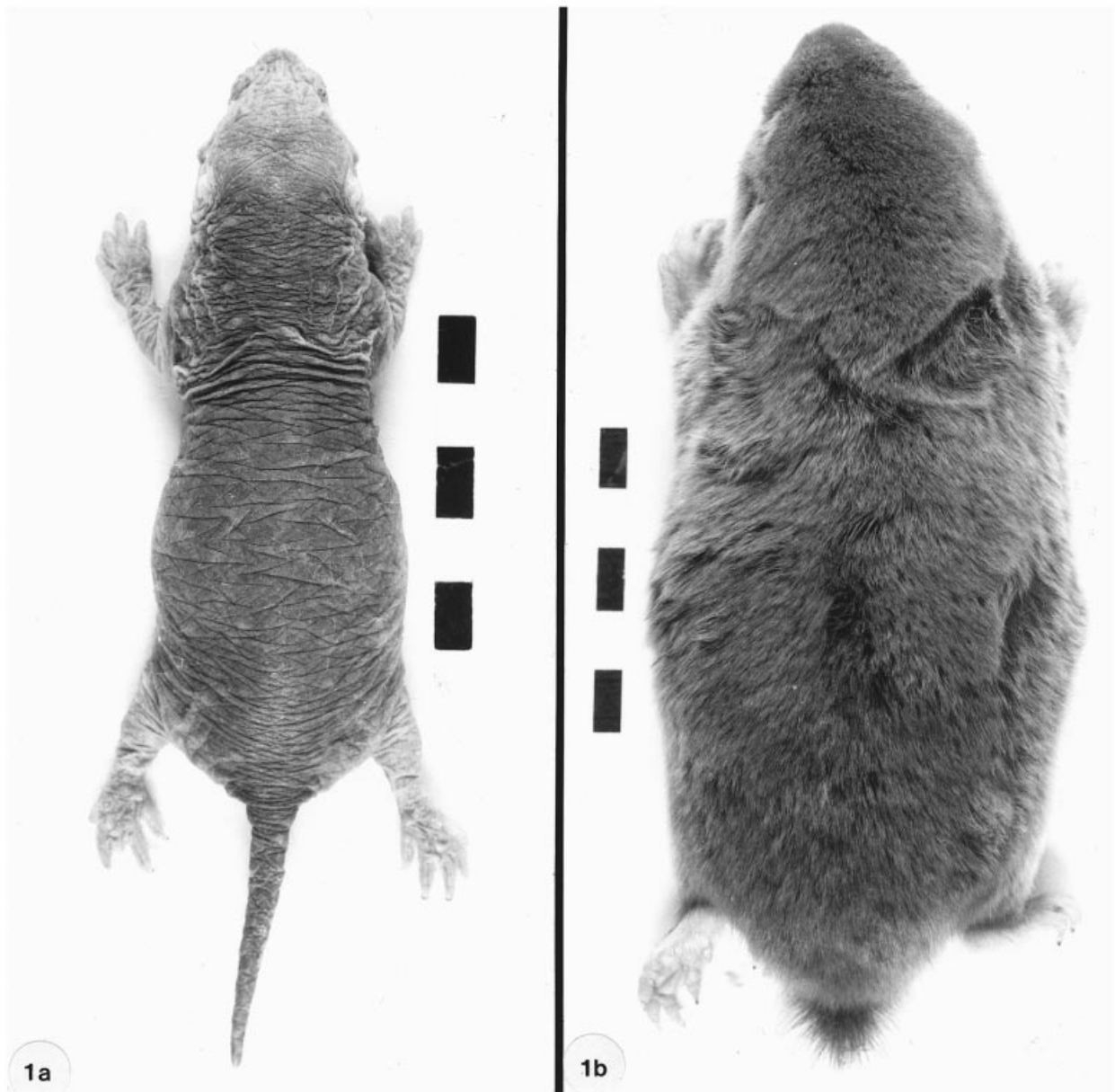


Fig. 1. (a) Dorsal surface of the naked mole-rat (*Heterocephalus glaber*) showing extensive folding of the skin especially in the neck and on the lateral sides of the body. (b) The common mole-rat (*Cryptomys hottentotus*) is furred over the entire surface of the body other than the feet which are covered by short coarse hairs on their dorsal surface. Bars, 5 cm.

mole-rat. This may either be due to a greater number of cells in this layer or may be artifactual, owing to the partial removal of this layer in the naked mole-rat during processing.

Dermis

In *H. glaber*, the underlying connective tissue dermis contained large bundles of irregular collagen fibres and associated fibroblasts. This layer showed a characteristic absence of hair follicles, associated sebaceous glands and sweat glands. Although the

dermis appeared to be well innervated with unmyelinated nerve fibres readily identifiable even in the superficial layers, microvessels other than capillaries and larger vessels were concentrated mainly in the basal layers of this dense connective tissue layer. Blood capillaries were numerous in the superficial layers of the dermis. Ultrastructurally, the capillaries were composed of a single layer of endothelial cells resting on a basal lamina. The capillary most frequently encountered was of the continuous type, with only a small proportion of fenestrated vessels. Pigment containing cells were found forming an

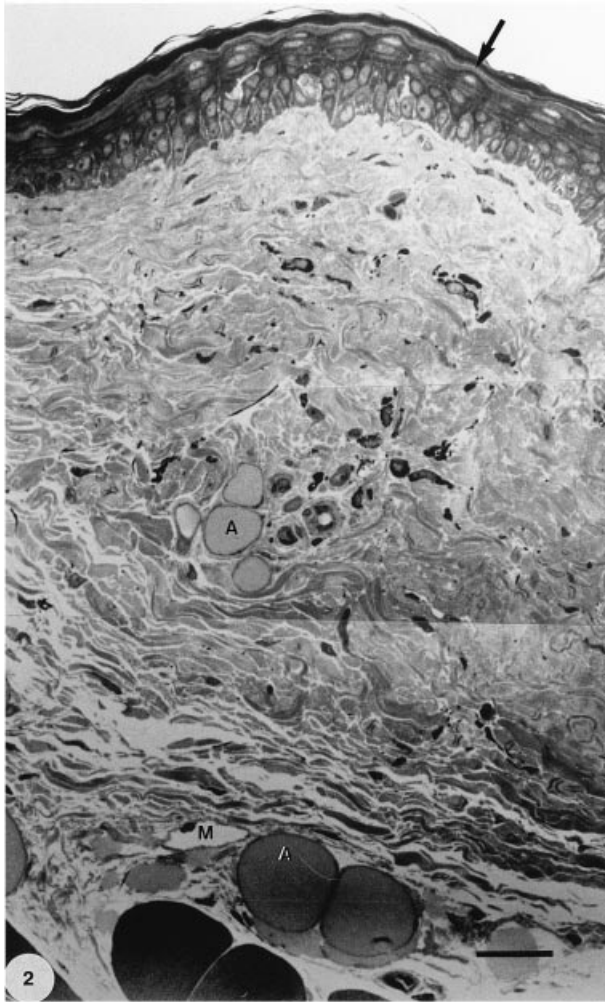


Fig. 2. Light micrograph of toluidine blue stained resin section of skin from the dorsal surface of the naked mole-rat. Note the lucent layer (arrow) in the epidermis and the isolated adipose cells (A) and microvessels (M) in the basal layers of the dermis. Bar, 20 μ m.

incomplete layer/stratum approximately midway within the dermis (Fig. 4a). Ultrastructurally these cells were spindle-shaped with long cytoplasmic processes (Fig. 5). The membrane bound, electron dense pigment containing granules were distributed in the cytoplasm both around the nucleus and in the processes. Melanin and melanin precursors have the ability to reduce the ammoniacal silver nitrate used in the Masson-Fontana method to metallic silver without an external reducing agent. However, in order to eliminate the possibility of the action of other reducing agents such as argentaffin and lipofuscin, two negative controls using bleaching methods were used in addition to a positive control which identified pigment in human skin. In the basal layers of the dermis, associated with blood microvessels, were unilocular adipocytes, either isolated or in groups. The adipocytes did not always form a distinct layer nor were they sufficiently clustered so as to form adipose

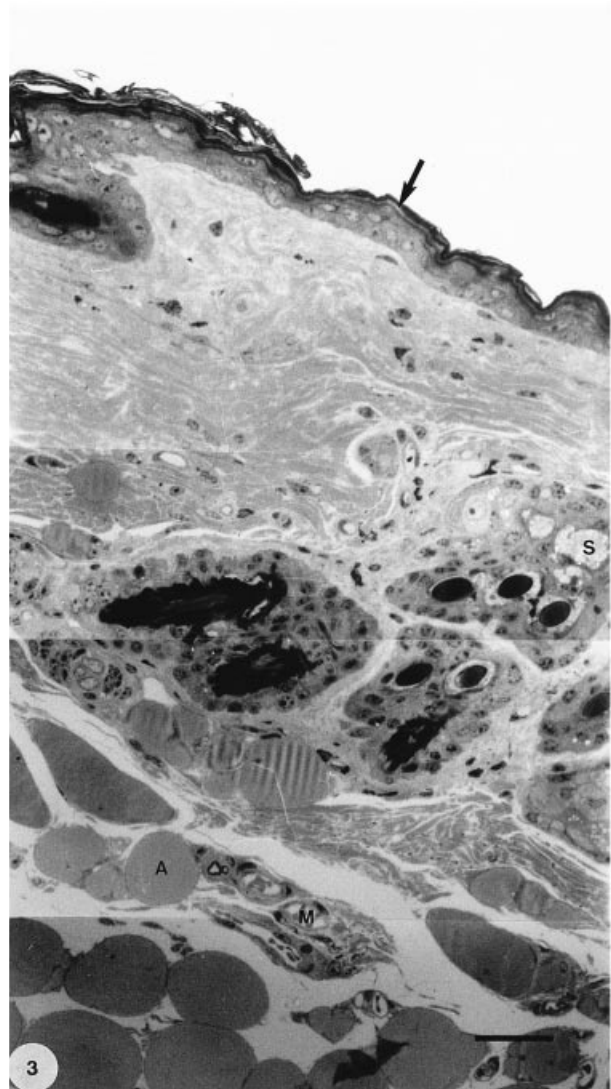


Fig. 3. Light micrograph of a toluidine blue stained resin section of skin from the dorsal surface of a common mole-rat. A lucent layer is apparent in the epidermis (arrow) and the adipose cells (A) in the basal layers of the dermis are associated with microvessels (M). Hair follicles and associated sebaceous glands (S) are evident in the middle layers of the dermis. Bar, 20 μ m.

connective tissue characteristic of a typical hypodermis. Fibres of striated skeletal muscle, the panniculus carnosus, were evident directly below the basal layer of the dermis.

The dermis of *C. hottentotus* was of comparable extent to that of the naked mole-rat and contained numerous epithelial derivatives. Hair follicles were associated with simple branched alveolar sebaceous glands. Sweat ducts and glands were present in the dorsal region of *C. hottentotus* but were absent in that of the naked mole-rat. Structurally the dermis of the common mole-rat was similar to that of the naked mole-rat, with microvessels located in the basal strata. Pigment was found associated and incorporated into the hair shaft in hair follicles (Fig. 4b). This pigment

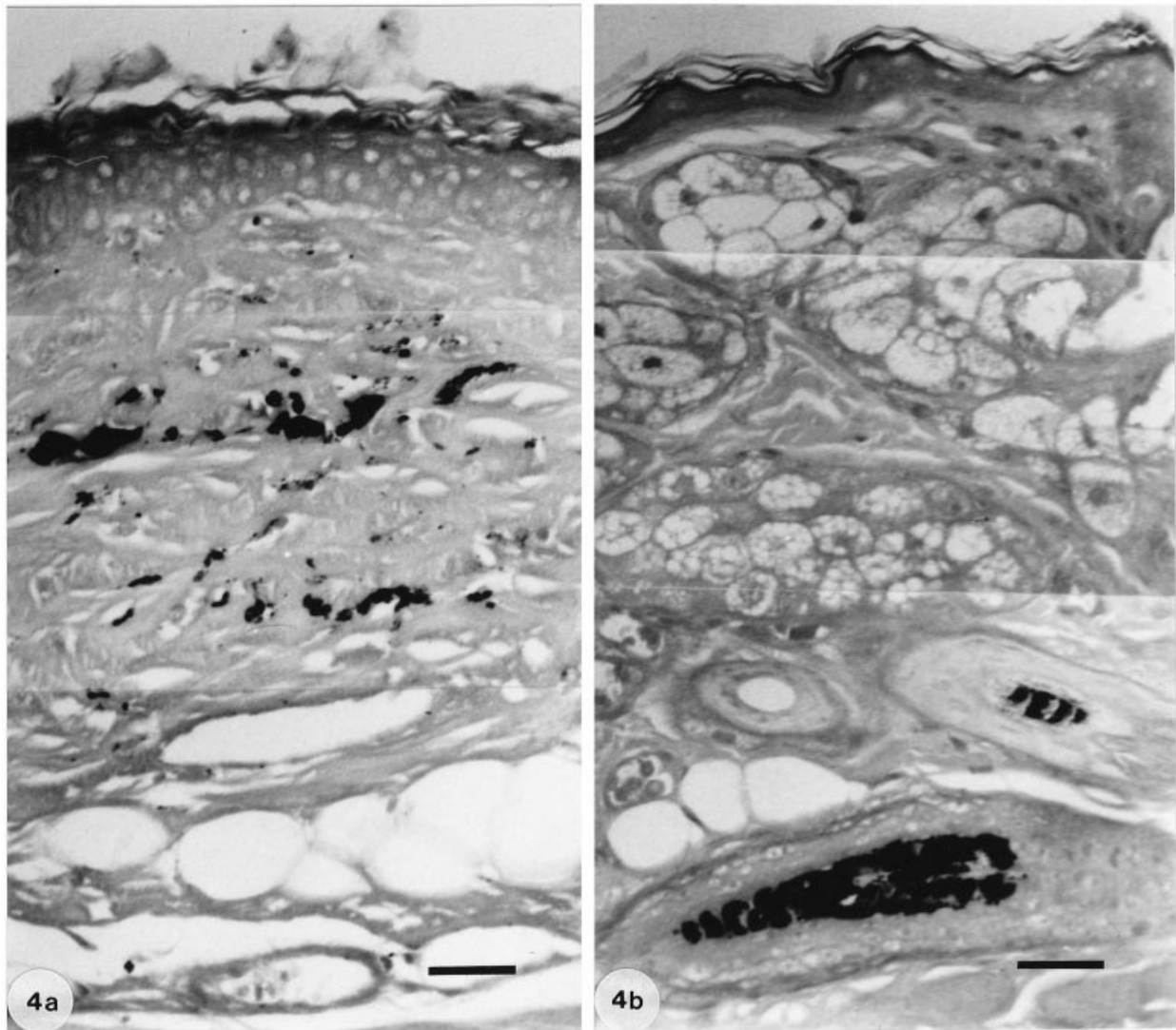


Fig. 4. Light micrographs of skin sections treated with the Masson-Fontana method for the identification of melanin in (a) the naked mole-rat and (b) the common mole-rat. Melanocytes occur as a noncontinuous layer in the middle reaches of the dermis interspersed among fibroblasts and dense irregular connective tissue in the naked mole-rat. In the common mole-rat melanin is only found associated with the cortical layers of the hairs. Bars, 20 µm.

could only be removed by hydrogen peroxide treatment over a 24 h period. No pigment containing cells were evident either in the epidermis or in the dermis.

DISCUSSION

Morphologically, while the skin of both the naked mole-rat and the common mole-rat are both loosely folded and although they share many similarities, marked differences in the degree of folding, thickness of the epidermis and dermal infrastructure were evident. The skin of *C. hottentotus*, is in many ways similar to that of the rat and is thus typical of a homeothermic mammal, while many of the features of the skin of the naked mole-rat could contribute significantly to the poikilothermic traits of this mammal (Buffenstein & Yahav, 1991).

Looseness of skin appears to be a common adaptive feature of a subterranean existence, present in a diverse array of animals ranging from fossorial worm-like reptiles (*Amphisbaenia*; Tucker, 1981), and fossorial insectivores (golden moles; G. Bronner, personal communication) to the subterranean rodents (*Bathyergidae*). The loose skin reduces friction and allows the body to move within the integument. The degree of folding is most pronounced in the naked mole-rat. Here, unlike other mammals, folding occurs over the entire body. Folding in this hairless mammal may play a greater role in protection of the underlying body compartments and would also assist in the prevention of excessive moisture loss through uncontrolled passive evaporation. Lack of sebaceous and sweat glands also reduces sebum and moisture loss.

The increased skin folds may contribute to the poor

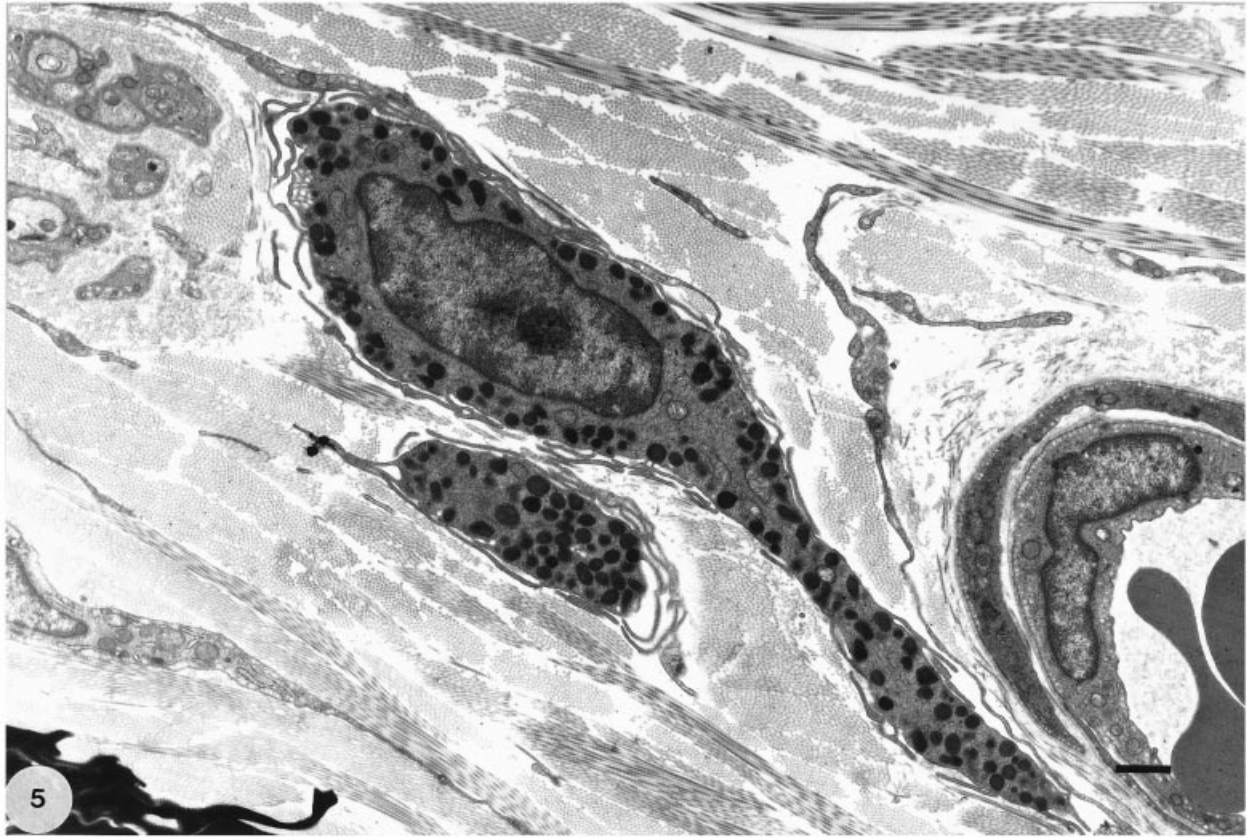


Fig. 5. Ultramicrograph of a pigment containing cell in the dermis of the naked mole-rat. The cell shows long cytoplasmic extensions between the collagen fibres of the matrix that harbour numerous oval-shaped melanosomes containing pigment of varying densities. In addition, the cytoplasm appears to be drawn out into thin tendril-like secondary extensions which do not contain pigmented melanosomes. Bar, 1 μ m.

thermoregulatory ability of this mammal (Buffenstein & Yahav, 1991). The increased skin area caused by greater skin folding increases the surface area for heat loss and may have a potentially cooling effect. However, folding in loose skin may also reduce heat loss by altering the microenvironment between folds and thus affect the thermal and water vapour gradients for heat transfer. The skin folds may be an adaptation to a disturbed thermoregulation, like those found in mutant hairless rodents, where a loose skin is probably the chief prerequisite for the formation of folds (David, 1934).

In the naked mole-rat the epidermis of the dorsum is thicker than that of the common mole-rat. There was no evidence of external epidermal papillae and the epidermal/dermal junction was smooth. The smoothness of this junction is attributed by Tucker (1981) to a reduction in epidermal pullulations of pilosebaceous, adenogenic (related to apocrine and eccrine sweat glands) and papillary buddings (vertical stratification). While the thickness of the epidermis may be attributed to a reduced number of horizontal layers, i.e. germinative, spinous, granulated, lucid and keratinised strata (horizontal stratification), in our

study of the dorsal skin of *H. glaber*, no disorder of the vertical and horizontal (stratification) migration of cells was evident nor found to be related to the lack of hair follicles. Hairlessness is attributed to a marked genetically determined reduction in the number of hair follicles, such that only scattered sensory hairs remain. Hair is an adjustable insulator and evaporation of glandular secretions provides a cooling effect (Romer & Parsons, 1977). This is evident in the common mole-rat while the naked mole-rat relies on its immediate environment and behavioural thermoregulation for control of much of its heat loss. The burrows are humid and maintain a more or less constant temperature between 32 °C and 34 °C. In addition, the members exhibit huddling behaviour in an effort to conserve heat (Jarvis & Bennett, 1991; Yahav & Buffenstein, 1991).

The dermis of the naked mole-rat while structurally similar to that of *C. hottentotus*, is typical of that of rodents in that it is composed mainly of dense irregular connective tissue with the predominant cellular component being fibroblasts. Although no discernible papillary and reticular layers were evident in the naked mole-rat or the common mole-rat in this

study, the existence of a stratum papillare of fine fibres running parallel to the surface of the epidermis and a stratum reticulare of heavier fibres in *H. glaber* has been reported previously by Thigpen (1948). In mammals, the deeper layers of the dermis are a major site for the deposition of adipose tissue or fat cells (panniculus adiposus). This layer functions in insulation and is generally present in hairless mammals. In both *H. glaber* and *C. hottentotus* the adipose tissue consists mainly of unilocular adipocytes which are sparse in places and the tissue consists of either isolated or grouped cells forming a discontinuous layer. Although the adipose tissue in both species of mole-rats is associated with larger blood vessels found deep in the dermis, the layer is not developed as a functional hypodermis and its potential role in insulation and temperature regulation is presumably limited.

The striated skeletal muscle, presumably derived from the underlying body tissue, may serve as an attachment for the overlying skin (panniculus carnosus). This system may allow the skin of the naked mole-rat to be extremely flexible and mobile thereby reducing the potentially damaging effect of abrasive forces, a condition which is highly favourable in a subterranean social-burrow environment. The presence of 'large subcutaneous fluid bubbles in the head, neck and thoracic regions' as reported by Tucker (1981) were not found in the dorsal skin of either mole-rat. However, below the dorsal skin of the neck region of *H. glaber*, the interscapular brown adipose tissue which functions in thermogenesis (Daly et al. 1997) is covered by connective tissue with unilocular adipose cells that accumulate under the skin and are apparent in histological section.

Unlike common mole-rats, naked mole-rats have a translucent pink coloured skin. The colour of skin results mainly from the presence of melanin and the number of blood vessels coursing through it. Melanocytes found in the epidermis of homeothermic mammals do not hold the melanosomes themselves. They are transported to the surrounding keratinocytes, through the extensive cytoplasmic processes that course between the keratinocytes, by cytokine secretion (Fawcett, 1986). In the naked mole-rat, melanocytes were detected only in the dermis while Tucker (1981) failed to find melanin granules in the epidermis using a silver impregnation technique. The dermal destination in the naked mole-rat appears to be developmentally similar to patterns found in poikilothermic vertebrate classes (reptiles and amphibians), which show the presence of melanophores in the dermis. The melanophores, although

providing some form of pigmentation in the dermis appear in most mammals also to be responsible for pigmentation in the epidermis by sending out long, slender processes which make contact with the epidermal cells and so allow for the transfer of pigment (Romer & Parsons, 1977). In the naked mole-rat the melanocytes do not appear to be associated with the cells of the epidermis and are located seemingly midway in the dermis. This location provides some speculation as to their activity as melanocytes and, as with their developmental similarity, they may be functionally similar to these cells found in reptiles or amphibians. *Heterocephalus glaber* has conserved the presence of melanocytes in its dermis whereas there appear to be no melanocytes in the skin of *C. hottentotus*. The functional significance of this is unknown but may relate to different thermal strategies and/or some reproductive function.

In vertebrates with a thick epidermis, the epidermal/dermal surface is usually irregular and forms an indented border due to epidermal ridges and invaginating dermal papillae. This arrangement assists in bringing capillaries closer to the surface and subsequently a closer relationship with the epidermis. However, in *H. glaber* the epidermis is thin with a regular epidermal/dermal interface which lacks epidermal ridges and dermal papillae. Larger blood vessels and nerves in *H. glaber* are found in the lower dermis which appear to break up and form a web of capillaries in the upper dermal layers. These vascular networks are developed in mammals that lack thick fur, in naked regions of hairy mammals and in egg-incubating brood patches of birds. The presence of arteriovenous anastomoses between the arterioles and venules allow the cooling of the blood due to the close contact of the capillary network with the skin surface. However, as the ambient temperature drops and the skin is chilled, blood flow to the skin would be reduced due to constriction of the capillaries. Heat would be conserved by the countercurrent exchange provided by the close association of arterioles and venules (Pough et al. 1996). This countercurrent system may well be functioning in *H. glaber*, in an attempt to conserve heat.

CONCLUSIONS

The marked thermoregulatory differences in the 2 Bathyergid rodents are reflected in skin morphology. The skin features of the common mole-rat (*C. hottentotus*) conform with those expected of a homeothermic mammal. The lack of a thermoinsulatory

layer and the loosely folded morphological arrangement presumably contribute to the inability of the naked mole-rat (*H. glaber*) to thermoregulate by endogenous means and instead facilitate ectothermic heat transfer. Furthermore, the presence of pigment containing cells in the dermis rather than in the epidermal layer further conforms with the poikilothermic nature of these mammals.

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