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Catecholaminergic innervation of interscapular brown adipose tissue in the naked mole-rat (*Heterocephalus glaber*)

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ABSTRACT

The thermogenic potential of the interscapular brown fat pad in the naked mole-rat *Heterocephalus glaber*, that exhibits poikilothermic thermal responses to changing temperatures is reported. Histological and ultrastructural study of the brown fat pad showed that it consists of layers of skeletal muscle interposed between the layers of brown adipose tissue with both unilocular and multilocular adipocytes. Large numbers of mitochondria were present between and around the lipid droplets of these cells. Glyoxylic acid condensation, used to demonstrate catecholaminergic nerves, was evident in low concentrations in the connective tissue between the brown adipocytes. A 3-dimensional computer-aided reconstruction of the fat pad showed the extent and ramification of nerves and blood vessels between the adipocytes. These findings show that although the naked mole-rat is regarded as an endothermic poikilotherm, it possesses anatomical features usually found in homeothermic mammals, which are essential for thermogenesis.

Key words: Thermogenesis; sympathetic innervation; brown adipose tissue; naked mole-rat.

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INTRODUCTION

Naked mole-rats (*Heterocephalus glaber*) are thermoconformers rather than thermoregulators (Buffenstein & Yahav, 1991) in that, unlike other mammals, body temperature tracks ambient over the entire range of temperatures experimentally measured (12–36 °C). The relationship between oxygen consumption and ambient temperature, however, switches from a typical poikilothermic pattern to a homeothermic mode at 28 °C (Buffenstein & Yahav, 1991). Non-shivering thermogenesis in brown adipose tissue (BAT) of small mammals usually plays a pivotal role in this homeothermic metabolic response. The sympathetic nervous system regulates this process by direct noradrenergic innervation of BAT cells (Carneheim et al. 1984). This gives rise to heat generation by uncoupled mitochondrial metabolism. Furthermore, the sympathetic nervous system regulates the adaptational response of BAT to cold acclimatization as implied by the increase in catecholamines in associated nerve plexuses in rats (Cottle & Cottle, 1970). Mole-rats respond to pharmacological doses of noradrenaline in that both metabolic rate and body temperature are elevated (Hislop & Buffenstein, 1994) but nevertheless do not normally regulate body temperature. In the light of the above, we investigated the extent and ultrastructure of interscapular brown adipose tissue (IBAT) and its sympathetic innervation in the naked mole-rat, in relation to its thermogenic potential.

MATERIALS AND METHODS

Naked mole-rats were housed in the Central Animal Unit, University of the Witwatersrand, at 31 °C (as previously described by Buffenstein & Yahav, 1991; Hislop & Buffenstein, 1994). Five animals were killed with 0.5 ml of 6% pentobarbitone injected intraperitonealy and the entire pad of IBAT was removed from each animal. The pads were immediately frozen in isopentane cooled in liquid nitrogen and stored at −70 °C. Sections 12–15 μm thick were cut at −30 °C and placed on glass slides at room temperature. Catecholamines were demonstrated by a sucrose–potassium phosphate–glyoxylic acid (SPG) technique of De la Torre & Surgeon (1976) as modified by Cottle et al. (1985). The sections were viewed on a Nikon

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Optiphot fluorescence microscope using BP 340–380 and suppressor LP 430 filters. These sections were subsequently stained with haematoxylin and eosin for light microscopy.

IBAT from an additional mole-rat was removed, fixed in 10% buffered formalin and routinely processed for light microscopy. Wax embedded 5 μm serial sections were stained alternately with haematoxylin and eosin or Palmgren's silver stain for 3-dimensional reconstruction using a Kontron computer-assisted image analysis system. Pieces of similar tissue from a further mole-rat were removed and placed in 2.5% glutaraldehyde for 4 h, washed in phosphate buffer and postfixed in osmium tetroxide. After further processing, the material was 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

Histological observations

The bilobed fat pad extended from the cervical region to the lower thoracic region, covering the dorsal extent of the thoracic girdle. Reconstruction of serial haematoxylin and eosin stained sections showed that the interscapular pad consists of 'tile-like' layers of skeletal muscle interposed between layers of brown adipose tissue. Glandular tissue embedded in the lateral neck region of the IBAT consisted of deeply stained serous cells with basophilic nuclei arranged in numerous acini. Amongst the serous acini were large striated ducts lined by columnar cells. Histologically the structure of this tissue was similar to that of the parotid salivary gland.

The brown adipose tissue consisted of clusters of unilocular and multilocular adipocytes which had a variable number of lipid droplets in their cytoplasm. Unilocular adipocytes had peripherally located nuclei while the multilocular adipocytes usually had centrally placed nuclei with prominent nucleoli (Fig. 1). Between, as well as surrounding the clusters of adipocytes, were blood vessels of various diameters. Small capillaries coursed between the adipocytes. Nerve fibres accompanied the blood vessels. Nerve bundles were found both in the connective tissue between adipocytes as well as in the connective tissue septae separating lobules of adipose tissue.

Ultrastructurally both unilocular and multilocular adipocytes contained large numbers of mitochondria in their cytoplasm (Fig. 2*a*). In most multilocular adipocytes the nuclei were located centrally with the lipid droplets and mitochondria occupying most of the cytoplasm. Small capillaries were evident in the connective tissue between adipocytes (Fig. 2*b*). The endothelium of these capillaries was continuous and nonfenestrated, with numerous pinocytotic vesicles associated both with the inner and outer plasma membranes. Larger blood vessels with associated nerve fibres were also found between adipocytes and in the thicker connective tissue septae. Unmyelinated nerve fibres characteristically wrapped in single folds of a Schwann cell were found in the connective tissue septae, while single nerve fibres were often found associated with blood vessels. In section, the nerve processes contained numerous cytoplasmic vesicles and mitochondria (Fig. 2*c*).

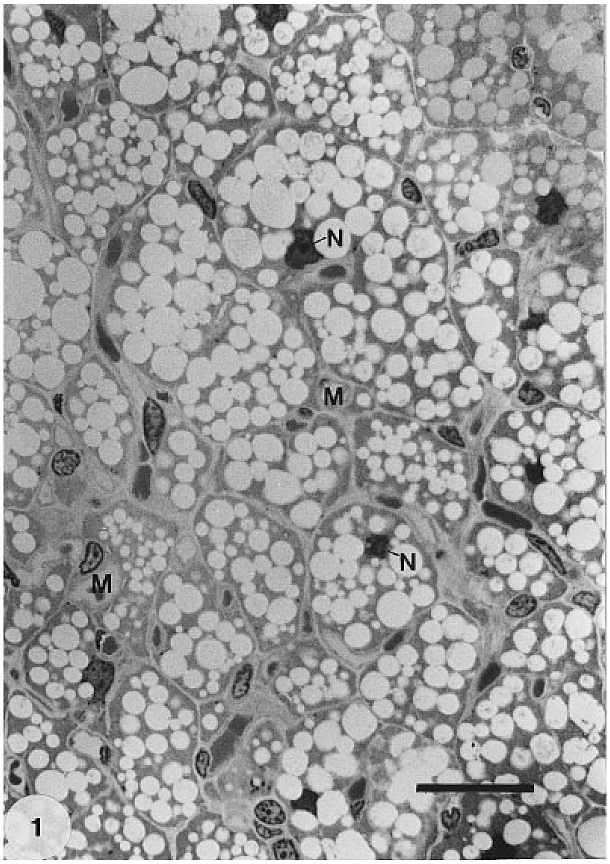

Fig. 1. Light micrograph of a toluidine blue stained resin section of interscapular brown adipose in the naked mole-rat. Some of the multilocular adipocytes have a centrally placed nucleus (N) with a prominent nucleolus. Microvessels (M) are found in the connective tissue between the adipocytes. × 1600. Bar, 10 μm.

Histofluorescence observations

SPG-treated cryostat sections of the IBAT revealed fluorescent yellow–green monoamine-containing

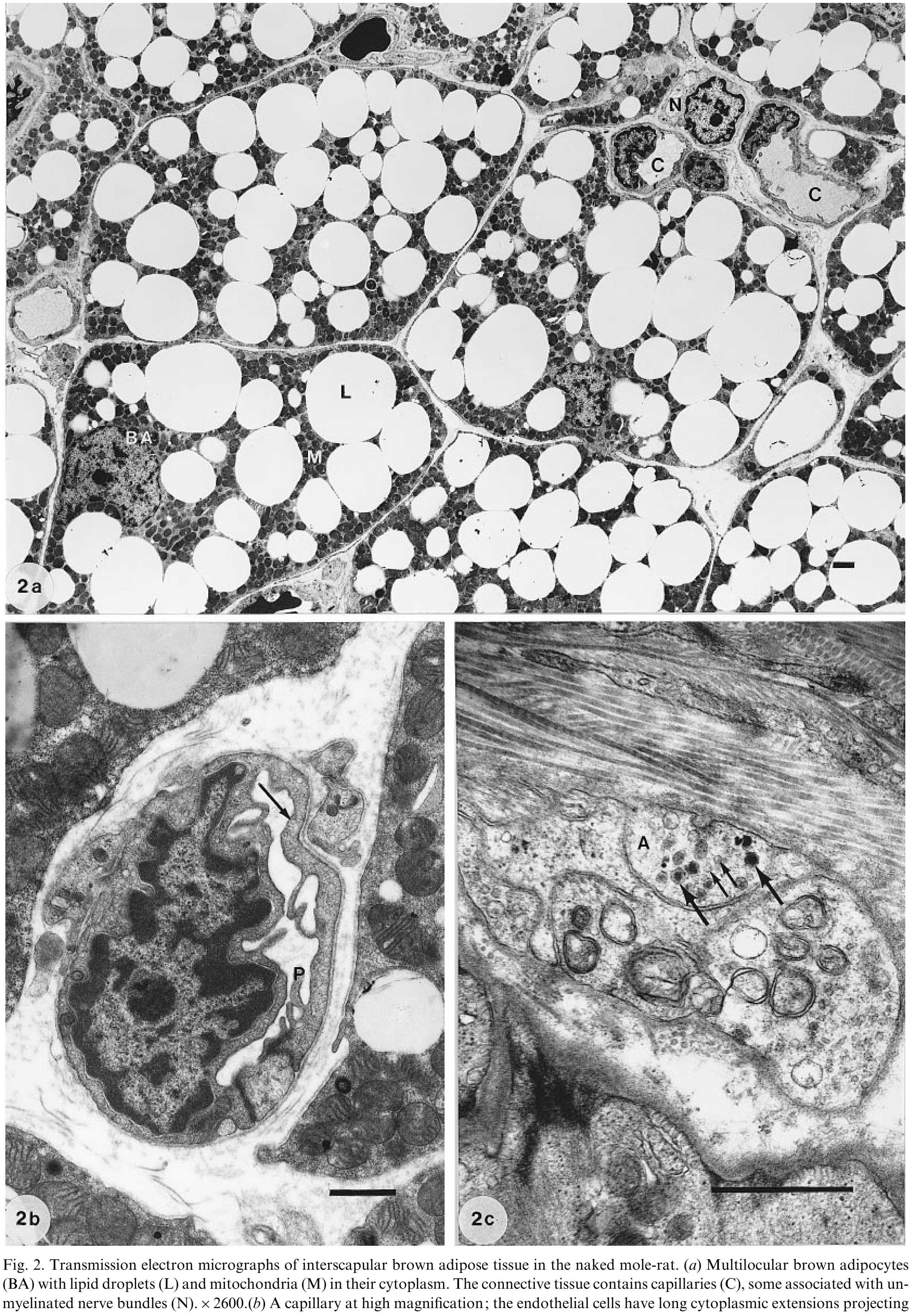

Fig. 2. Transmission electron micrographs of interscapular brown adipose tissue in the naked mole-rat. (*a*) Multilocular brown adipocytes (BA) with lipid droplets (L) and mitochondria (M) in their cytoplasm. The connective tissue contains capillaries (C), some associated with unmyelinated nerve bundles (N). × 2600.(*b*) A capillary at high magnification; the endothelial cells have long cytoplasmic extensions projecting (P) into the lumen and their cytoplasm is filled with pinocytotic vesicles (arrow). × 12000.(*c*) Unmyelinated nerve bundle in the intercellular connective tissue space. The axons (A) contain both dense-cored vesicles (heavy arrows) and clear vesicles (light arrows). × 26000. Bars, 1 μm.

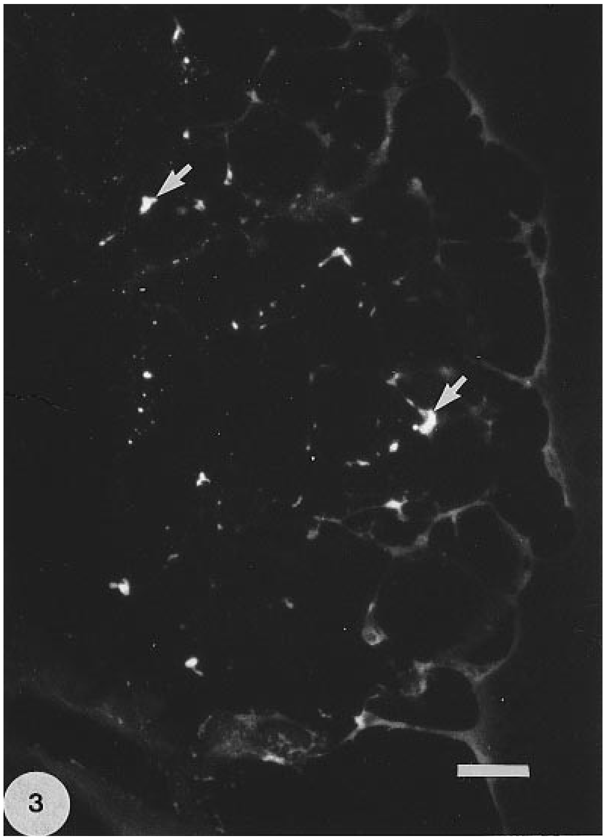

Fig. 3. Fluoresence micrograph of glyoxylic acid-treated interscapular brown adipose tissue from the naked mole-rat. Note the beaded fluorescent catecholaminergic axons (arrows) in the connective tissue between the brown adipocytes. × 1000. Bar, 10 µm.

nerve fibres (Fig. 3). The nerves were seen as fluorescing particles and groups of particles in the connective tissue between the adipocytes. Sections from the middle thoracic region of the IBAT produced the most extensive fluorescent reaction, although areas of fluorescence were relatively sparse. In sections from the cervical and lower thoracic regions, fluorescing particles were fewer in number and more solitary. This was especially evident in lower thoracic sections with only infrequent clumps of fluorescence in the cervical region of the pad. On subsequent staining of these cryostat sections with haematoxylin and eosin the presence and positions of the nerves were confirmed by light microscopy and corresponded to the fluorescing particles.

Three-dimensional reconstruction

The distribution of interposing fat and muscle was clearly noted in the specimen used. Figure 4*a* shows serial sections of the fat pad which are staggered over a distance (i.e. the images are not directly apposed) so that the entire fat pad and structures within the fat pad may be viewed as a whole. Figure 4*b* shows a dorsal view of a longitudinal section in the coronal plane of the IBAT such that all internal structures of the tissue are visible. The computer generated 3-dimensional images reinforced observations noted in the microscopic examination of the tissue, i.e. the blood vessels and nerves appeared to lie along the longitudinal axis and, in many instances, in close association with each other. Although both myelinated and unmyelinated nerves (as differentiated by Palmgren's silver stain) were traced, it was impossible to estimate the number of myelinated axons innervating the striated muscle as opposed to unmyelinated nerve fibres supplying the brown adispose tissue as only the larger nerves and blood vessels could be traced.

DISCUSSION

The brown adipose tissue of *Heterocephalus glaber* is organised into a bilobed structure situated in the interscapular region. Although brown adipose tissue may be present in other anatomical sites in rodents, e.g. perirenally (Norman et al. 1988), the interscapular brown fat pad is the largest accumulation of this tissue type in naked mole-rats. The arrangement of the various tissue types within the IBAT was that of interposed skeletal muscle and brown fat layers separated by connective tissue septae. In addition to muscle and brown fat, the parotid salivary gland was incorporated into the lateral neck region of the pad. In all sections of brown adipose tissue both unilocular and multilocular adipocytes were evident. This result is consistent with observations made by Cunningham et al. (1985) and Lever et al. (1986), in human perirenal brown adipose tissue.

The computer generated 3-dimensional images reinforced observations noted in the microscopic examination of the tissue. Blood vessels and nerves, at times closely associated in any particular plane of section, were seen to lie along the longitudinal axis of the pad in both the brown adipose tissue and the skeletal muscle. The course of the nerves in the connective tissue of the fat was confirmed by histological examination. These appeared to be more sparsely distributed than those of typical homeothermic mammals (Carneheim et al. 1984; Norman et al. 1988; Lever et al. 1989).

The demonstration of catecholaminergic nerves within brown adipose tissue is significant with respect to thermogenesis. Brown adipocytes are said to receive direct sympathetic innervation (Lever et al. 1989), a

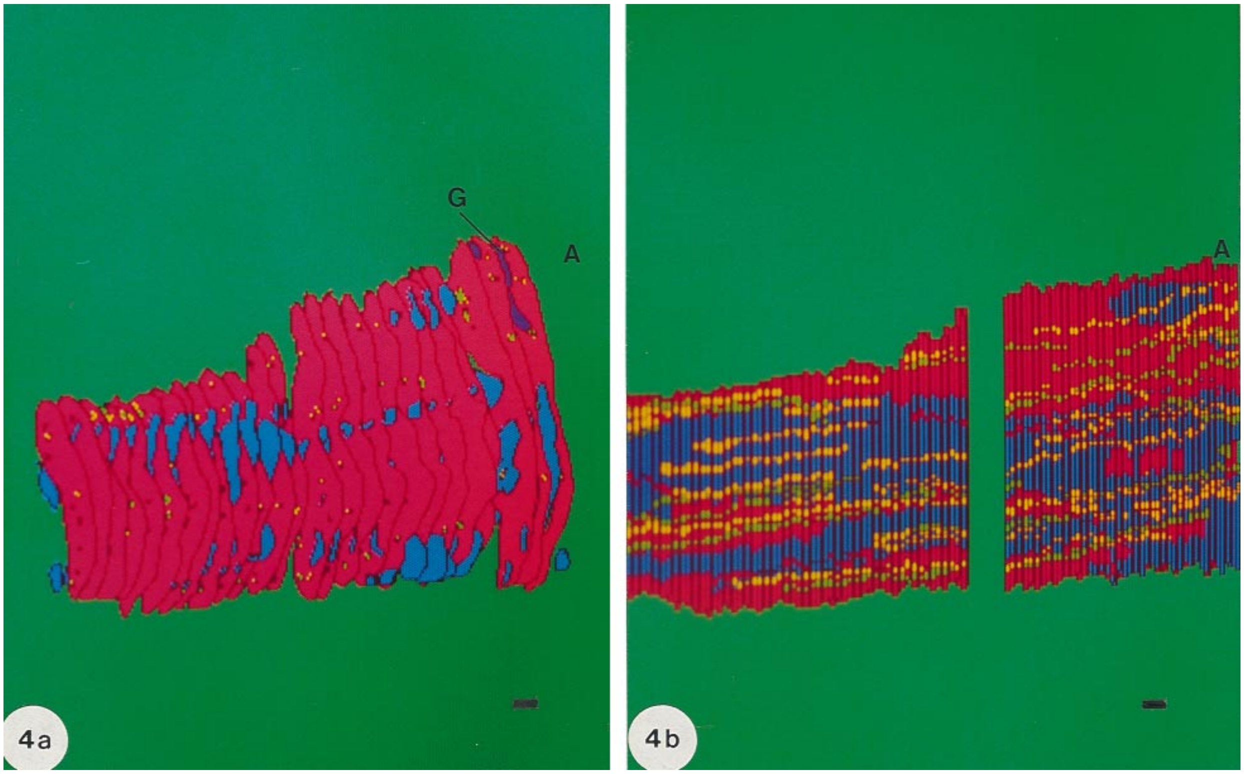

Fig. 4. Computer generated 3-D reconstructions of the interscapular brown adipose tissue of the naked mole rat. Colouration: red, fat; blue, muscle; yellow, nerve; green, blood vessels. A, anterior. (*a*) Superodorsal view of the entire fat pad. Note the relation of the muscle to the fat. Part of the parotid gland (G) can be seen in the anterolateral part of the pad. × 3. (*b*) Dorsal view of a longitudinal section in the coronal plane of the fat pad showing the internal relationship of the nerves and blood vessels. Note sections 183–219 are missing. × 3. Bars, 1 mm.

factor essential for the mechanism of thermoregulation. The vascularisation of the fatty component of the pad appeared extensive. The arrangement is significant as the smaller capillaries which surround the brown adipocytes facilitate the transport of metabolites to and from the cells, while the larger vessels which course through the fat play a role in protecting the animal from cold by transferring heat. Smith (1964) noted an intimate relationship between brown adipose tissue and returning venous drainages in the Wistar rat. This close contact facilitates the direct transfer of metabolic heat produced in the brown adipocytes into the blood stream. These returning vessels transfer the heat to vital organs in the thoracic region, preventing undue cooling of these organs in times of cold stress (Smith, 1964). The close association of the nerves and blood vessels may be important for the transport of chemical messengers from the blood stream to nerve plexuses surrounding the vessels (Lever et al. 1989).

The naked mole-rat is neither completely poikilothermic, nor is it a true homeotherm. While body temperature closely tracks ambient, there is some metabolic evidence of attempted thermoregulation at temperatures greater than 28 °C (Buffenstein & Yahav, 1991). These authors attributed the inability of the naked mole-rat to maintain a constant body temperature to various factors such as the poor insulative covering of loosely folded skin and a limited amount of subcutaneous fat. These factors, coupled with the naked mole-rat's high surface area to volume ratio, results in the loss of any endogenously produced heat through passive transfer to the environment. Therefore, no heat is retained and the animal cannot maintain a constant body temperature. An alternative reason for the atypical mode of thermoregulation present in the naked mole-rat may be that this animal has lost the ability to produce a sufficient amount of endogenous heat. This is implied by its attempt to thermoregulate at temperatures exceeding 28 °C. This latter hypothesis is refuted for mole-rats can and do respond to gram positive pyrogens by increasing body temperature (Urison et al. 1993) and can induce a nonshivering thermogenic response with pharmacological doses of noradrenaline (Hislop & Buffenstein, 1994). As such the naked mole-rat is capable of endogenous heat production.

The positive fever and nonshivering thermogenic response is typical of endothermic animals. However, despite being able to generate endogenous heat and therefore having the potential to thermoregulate, the naked mole-rat is not successful in maintaining a constant body temperature by endothermic means alone. Although no extraction of the 32 kDa uncoupling protein in the mitochondria of brown adipose tissue was attempted in this study, the demonstration of catecholaminergic nerves justifies the thermogenic potential of these animals.

In its natural equatorial environment, *Heterocephalus glaber* is a highly social animal existing in colonies of up to 300 individuals (Jarvis & Bennett, 1991). Observed both in captivity and from field data (Jarvis & Bennett, 1991), these rodents exhibit huddling behaviour which is thought to play an adaptive role in thermoregulation in their natural habitat (Yahav & Buffenstein, 1991). Huddling not only reduces hypothermia but also reduces the metabolic cost of maintaining body temperature due to the sharing of body warmth (Yahav & Buffenstein, 1991). Furthermore, living in an underground habitat in equatorial Africa, temperatures within the deeper burrows are comparatively very stable. Mole-rats appear to regulate body temperature behaviourally by careful selection of burrows at the appropriate ambient temperature (Brett, 1991). This implies that (within their natural habitat) naked mole-rats are able to control their body temperature by a combination of ectothermic (behavioural) and endothermic mechanisms (albeit within a small temperature range), and can thus behave like homeotherms.

Although the naked mole-rat is classified as an endothermic poikilotherm, this study shows that it nonetheless possesses anatomical features usually found in homeothermic mammals which are essential for thermogenesis.

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REFERENCES

BRETT RA (1991) The ecology of naked mole-rat colonies: burrowing, food and limiting factors. In *The Biology of the Naked Mole-Rat* (ed. Sherman PW, Jarvis JUM, Alexander RD), pp. 97–136. Princeton: Princeton University Press.

BUFFENSTEIN R, YAHAV S (1991) Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *Journal of Thermal Biology* **16**, 27–232.

CARNEHEIM C, NEDERGAARD J, CANNON B (1984) β-Adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold. *American Journal of Physiology* **246**, E327–E333.

COTTLE MKW, COTTLE WH (1970) Adrenergic fibres in brown fat of cold-acclimated rats. *Journal of Histochemistry and Cytochemistry* **18**, 116–119.

COTTLE MKW, COTTLE WH, PERUSSE F, BUKOWIECKI LJ (1985) An improved glyoxylic technique for the histochemical localization of catecholamines in brown adipose tissue. *Histochemical Journal* **17**, 1279–1288.

CUNNINGHAM S, LESLIE P, HOPWOOD D, ILLINGWORTH P, JUNG RT, NICHOLLS DG et al. (1985) The characterization and energetic potential of brown adipose tissue in man. *Clinical Science* **69**, 343–348.

DE LA TORRE JC, SURGEON JW (1976) A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic technique: the SPG method. *Histochemistry* **49**, 81–93.

HISLOP MS, BUFFENSTEIN R (1994) Noradrenaline induces nonshivering thermogenesis in both the naked mole-rat (*Heterocephalus glaber*) and the Damara mole-rat (*Cryptomys damarensis*) despite very different modes of thermoregulation. *Journal of Thermal Biology* **19**, 25–32.

JARVIS JUM, BENNETT NC (1991) Ecology and Behaviour of the family Bathyergidae. In *The Biology of the Naked Mole-Rat* (ed. Sherman PW, Jarvis JUM, Alexander RD), pp. 66–96. Princeton: Princeton University Press.

LEVER JD, JUNG RT, NNODIM JO, LESLIE PJ, SYMONS D (1986) Demonstration of a catecholaminergic innervation in human perirenal brown adipose tissue at various ages in the adult. *Anatomical Record* **215**, 251–255.

LEVER JD, MUKHERJEE S, NORMAN D, SYMONS D, WHEELER MH, CONNACHER A et al. (1989) Catecholaminergic and peptidergic nerves in naturally occurring and pheochromocytoma-associated human brown adipose tissue. *Clinical Anatomy* **2**, 157–166.

NORMAN D, MUKHERJEE S, SYMONS D, JUNG RT, LEVER JD (1988) Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: a plurality of innervation. *Journal of Neurocytology* **17**, 305–311.

SMITH RE (1964) Thermoregulatory and adaptive behaviour of brown adipose tissue. *Science* **146**, 1686–1689.

URISON NT, GOELST K, BUFFENSTEIN R (1993) A positive fever response by a poikilothermic mammal, the naked mole-rat (*Heterocephalus glaber*). *Journal of Thermal Biology* **18**, 245–249.

YAHAV S, BUFFENSTEIN R (1991) Huddling behaviour facilitates homeothermy in the naked mole-rat *Heterocephalus glaber*. *Physiological Zoology* **64**, 871–884.