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Ultrastructure of Epidermal Sensory Receptors in *Amphibolurus barbatus* (Lacertilis: Agamidae)

Shirley Maclean

School of Anatomy, University of New South Wales, Sydney, Australia

Summary. The histological and ultrastructural organisation of the epidermal sensory organs in *Amphibolurus barbatus* has been described with respect to their position and possible functions. The sensory organs, located at the scale's edge, are most numerous in scales of the dorsal surface of the head. Most other scales of the body surface have two receptors located laterally to the spine or keel of the scale. In the imbricate scales of the ventral body region, the receptors lie just beneath the reinforced scale lip. Scanning electron microscopy has revealed the surface of the organ to be a crater lacking any surface projections. These sensory organs have a dermal papilla consisting of a nerve plexus and loose connective tissue. The nerve fibres arising from the plexus, pass to the epidermal columnar cells, where some form nerve terminals at the base of the cells, while others pass between them to form nerve terminals embedded in a superficial layer of cuboidal cells. The superficial terminals are held against the overlying α keratin by masses of tonofilaments. The β keratin is thickened to form a collar around the periphery of the organ but is only about 0.5 µm thick immediately above it. Mechanical deformation of the scale's spine or reinforced scale lip may initiate stimulation of the nerve terminals described.

Key words: Ultrastructure – Mechanoreceptor – Skin – Reptile.

The histology of cutaneous sensory organs in reptiles has been described by Schmidt (1917, 1918, 1920), Cohn (1914) and Jaburek (1926). More recently, a morphological classification of these organs has been proposed (Landmann 1975; von Düring and Miller 1979), primarily based on observations by light microscopy, but with some reference to ultrastructural studies (von Düring 1973a, 1974; Hiller

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Send offprint requests to: Shirley Maclean, School of Anatomy, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia

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1976a, b, 1977). In addition, von Düring and Miller (1979) have emphasised the important potential contribution of ultrastructural studies with the improvement of fixation techniques. The ultrastructure of epidermal receptors in Gekkonids has been described by Hiller (1976a, b).

While there have been several descriptions of the cutaneous sensory organs in Agamidae (Schmidt 1920; Miller and Kasahara 1967; Landmann 1975), no ultrastructural studies have been reported. The present study supplements the ultrastructural descriptions of receptors in Gekkonid lizards with an examination of the ultrastructure of epidermal receptors in an Agamid lizard, *Amphibolurus barbatus*.

Materials and Methods

Electron Microscopy

Perfusion of several animals showed no appreciable advantage in fixation. The material presented was obtained by taking skin samples from 12 live animals using a gauge 2 dermal punch. These samples were cut into 50 µm sections using a tissue chopper or were trimmed by hand before fixation in 3% gluteraldehyde. The tissue was post-fixed in 1% buffered osmium tetroxide, and after dehydration, embedded in Spurr's resin. Ultrathin sections were cut using glass knives on a Huxley ultramicrotome. The sections were stained in lead citrate and uranyl acetate before examination in a Siemens Elmiskop IA.

Scanning Electron Microscopy

Skin samples 1 cm square were taken from 5 animals killed with an overdose of Nembutal (pentobarbitone). Two of these were in the process of shedding skin while the others were at the resting stage of the sloughing cycle (Maderson 1965). Some skin samples from the sloughing animals were taken and processed for transmission electron microscopy. The skin samples were pinned out on cork before fixation in 3% glutaraldehyde, dehydration in graded alcohols, and final air drying. They were coated with approximately 50 nm of gold in an Edwards coating unit before examination in a Cambridge stereoscan.

Light Microscopy

Semithin sections approximately 1 µm thick were taken from blocks prepared for electron microscopy. The sections were stained in 1% toluidine blue in 1% borax. To determine the distribution of the sensory organs the β keratin of scales of the animals used for scanning electron microscopy was removed with a dissecting needle and examined with a light microscope. The sensory organs were most easily recognised in β keratin from animals which had recently sloughed.

Results

Distribution of Epidermal Receptors

The epidermal sensory organs are found over the whole body surface. The number of organs per scale is greatest in the larger flattened scales on the dorsal surface of the head, where 12–15 are located around the periphery of the scale. Labial scales

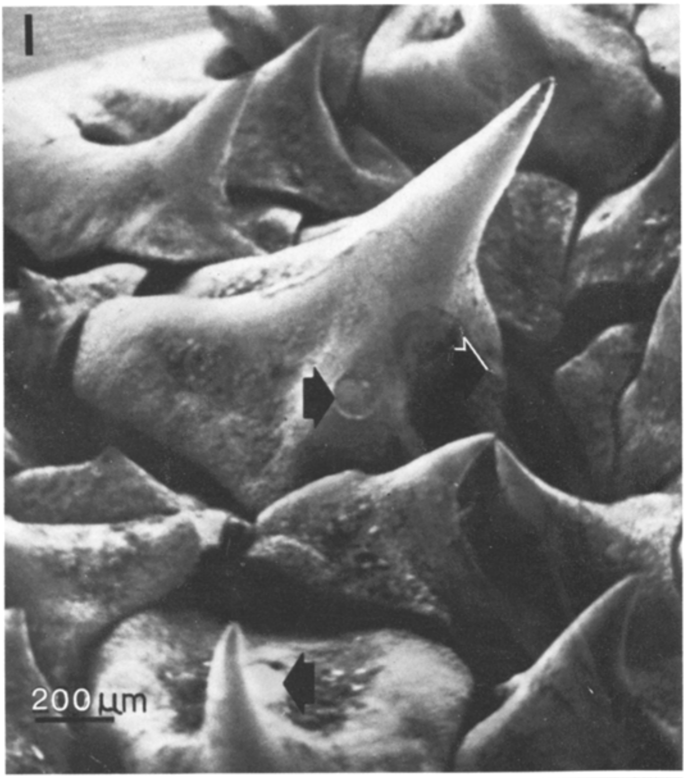


Fig. 1. Scanning electron micrograph of dorsolateral trunk scales. Sensory organs indicated by *arrows*

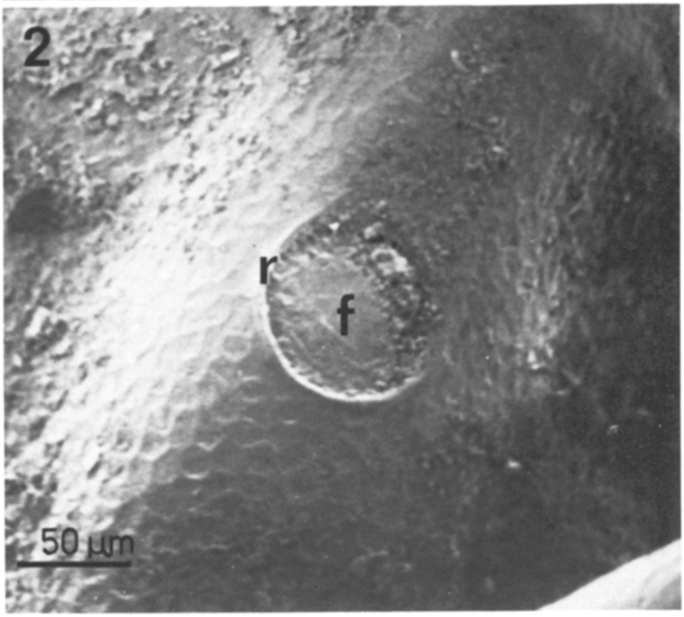


Fig. 2. Higher magnification of the sensory organ in Fig. 1. The surface of the sensory organ appears as a crater with a distinct rim (*r*) and floor (*f*)

have 5–8 organs with the number being greater on the upper lip than on the lower. In the dorsal neck region three receptors are located on the free edge of the scales; one centrally placed below the spine and one on either side. In the dorsal body region, the large spinous scales have a sensory organ located on either side of the spine (Fig. 1), while the smaller scales between these either lack sensory organs or have one to two located laterally. Ventrally, the large spinous scales of the beard show no evidence of sensory organs and histological sections failed to clarify their presence or absence conclusively. The imbricate scales of the ventral surface have one to two organs located laterally from the central ridge and spine on the free edges of the scales, just below the reinforced lip of the scale edge.

The Morphology of Epidermal Sensory Organs

The sensory organs appear on the skin surface as craters measuring 60–100 μm in diameter (Fig. 1). The edge of the receptor is sharply delineated and the rim is concave on the inner surface. The floor of these sensory pores is flat and does not have any surface projections. The surrounding surface of the scale shows a microornamentation of ridges and pits which is absent in the sensory pore (Fig. 2).

In section, the epidermal component of the sensory organ consists of a basal layer of columnar cells with a single layer of cuboidal cells which contain the nerve terminals covering them. Superficial to the cuboidal cells is a layer of α keratin, the mesos layer, and finally a thin layer of β keratin in the region corresponding to the floor of the sensory pore. Beneath the rim of the sensory pore the β keratin is thickened to surround the cellular component of the organ (Figs. 3, 8).

Axons measuring 0.5–1.0 μm in diameter pass between the columnar cells of the ventral scales, which are joined closely to each other by desmosomes (Fig. 5). The columnar cells of sensory organs on the dorsal surface are more loosely arranged with dermal melanophore processes and nerves passing between them. A discontinuous basal lamina lies beneath these cells. The cuboidal cells are attached to the columnar cells by desmosomes. The layer of cuboidal cells extends as far as the apex of the thickened ring of β keratin surrounding the organ. At this point they form a continuous layer with the keratinocytes of the epidermis (Fig. 8). The tonofilaments from the desmosomes are particularly condensed in the cuboidal cells where they form supportive pedestals which hold the terminals up against the overlying α keratin (Fig. 4). The membrane of the terminal is separated from the α keratin by a distance of 285 nm. This space is occupied by fine fibrillar material arranged to form five to eight rows running parallel to the α keratin (Fig. 7). The terminals, which measure 2.7–3.5 μm in diameter, are round but the border closest to the keratin is flattened. They contain few mitochondria (Fig. 4), large vesicles, and large quantities of glycogen granules (Fig. 7).

In the resting stage of the sloughing cycle (Maderson 1965) a dermal papilla is formed beneath the epidermal part of the sensory organ. This papilla contains fibrocytes interspersed with connective tissue and nerve terminals. The fibrocytes show no apparent orderly arrangement (Fig. 3). The nerve terminals are located either immediately beneath the columnar cells (Fig. 6), or embedded into the bases of these cells. The internal structure of the terminal in this region appears to be

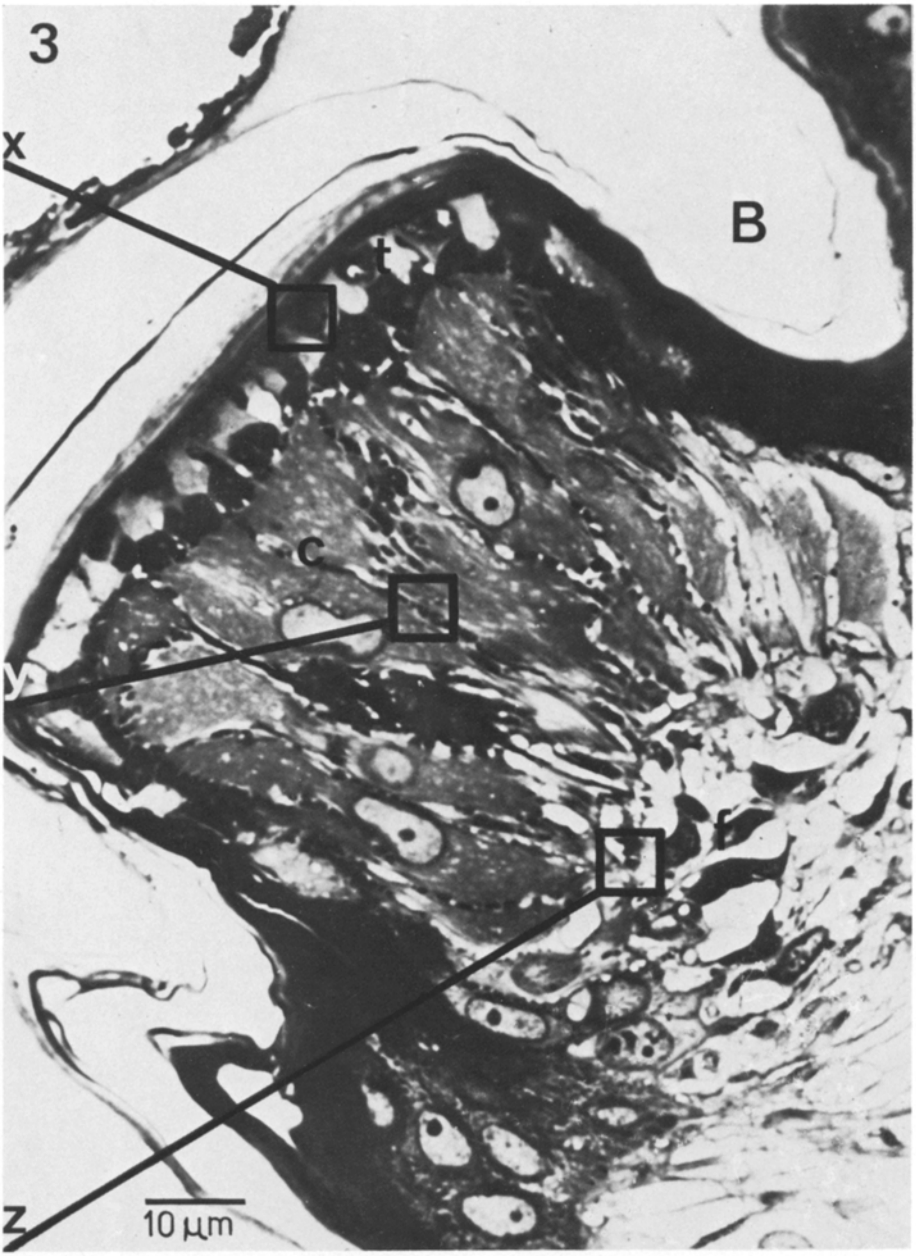
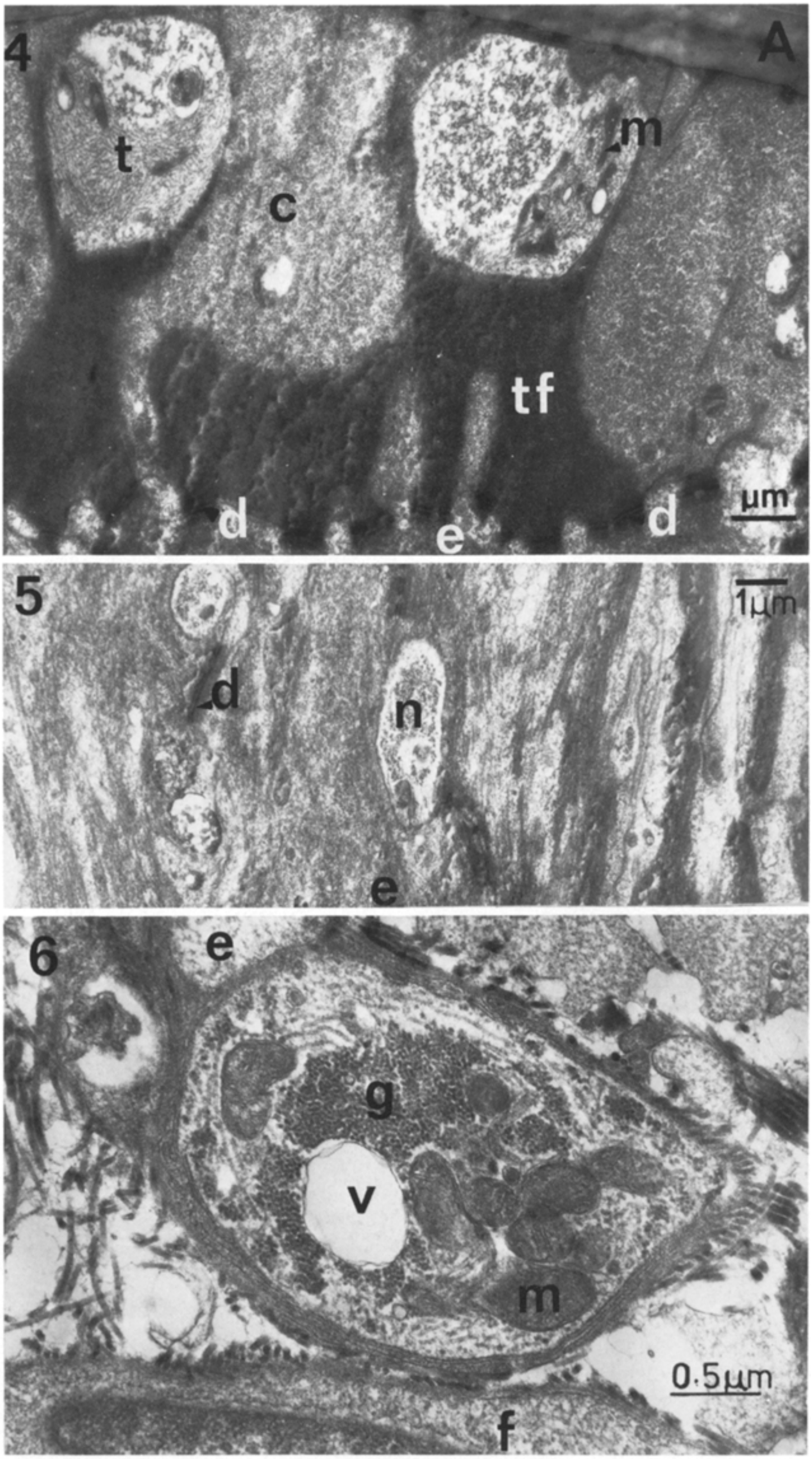


Fig. 3. Section of a sensory organ stained with toluidine blue. Regions x, y and z are shown in Figs. 4, 5 and 6 respectively. The β keratin (*B*) forms a thickened collar around the organ. Nerve terminals (*t*) are embedded in the superficial cuboidal cell layer, and columnar cells (*c*) lie beneath this layer. The dermal papilla of loose connective tissue contains fibrocytes (*f*)



Figs. 4–6. Electron micrographs of regions x, y and z of Fig. 3. Superficial nerve terminals (*t*) are embedded in the cuboidal cell cytoplasm (*c*) beneath the α keratin layer (*A*). They are supported by tonofilaments (*tf*). Nerves (*n*) from these terminals lie between columnar cells (*e*) joined by desmosomes (*d*). Deeper nerve terminals containing mitochondria (*m*) vacuoles (*v*) and glycogen granules (*g*). A fibrocyte (*f*) from the dermal papilla lies just beneath the terminal

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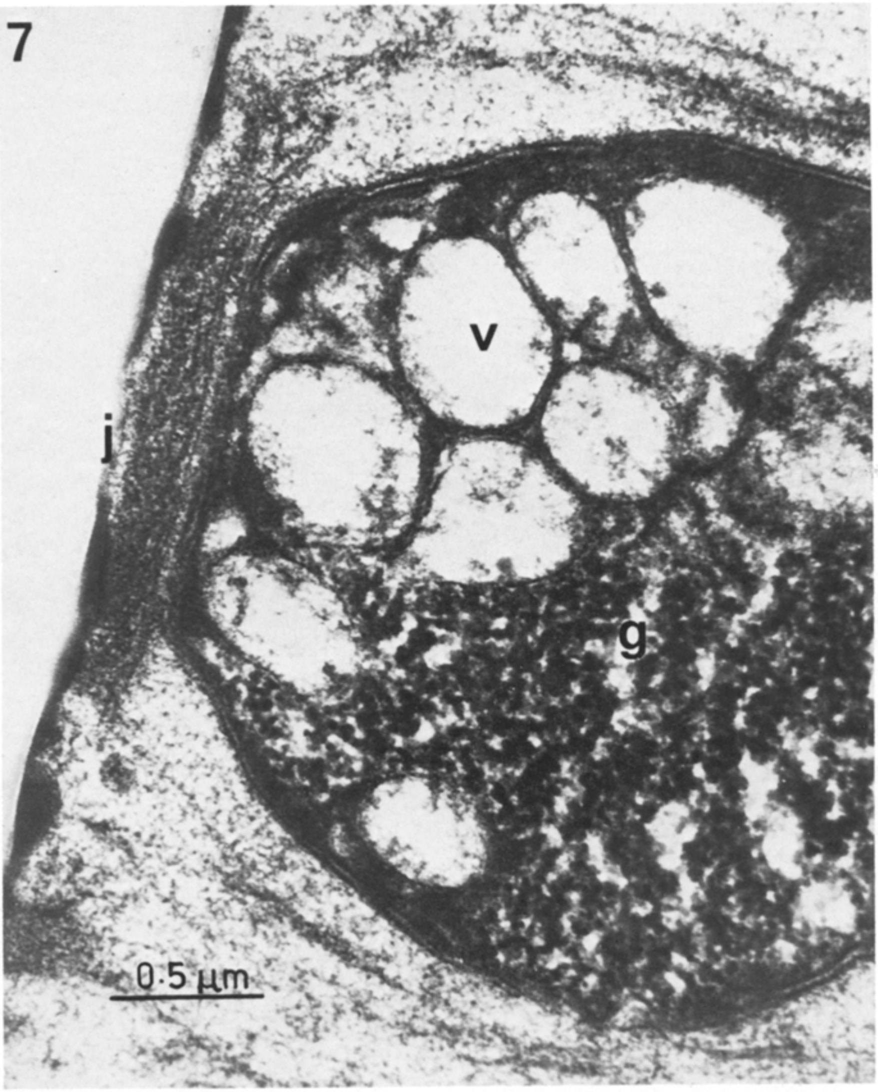

Fig. 7. Electron micrograph of a superficial nerve terminal. The α keratin has split away during processing but the junction (*j*) between it and the nerve terminal contains the fibrillar material in the cuboidal cell cytoplasm. The nerve terminal contains large vacuoles (*v*) and masses of glycogen granules (*g*)

different from those closer to the surface as they contain larger numbers of mitochondria and fewer vacuoles. They also contain glycogen granules and neurotubules.

A nerve plexus containing myelinated and unmyelinated nerves is located in the superficial dermis immediately beneath the dermal papilla. Unmyelinated nerves 0.2–1.0 µm in diameter arise from this plexus and pass through the dermal papilla to the epithelial part of the sensory organ.

No other epidermal sensory terminals were observed in this study.

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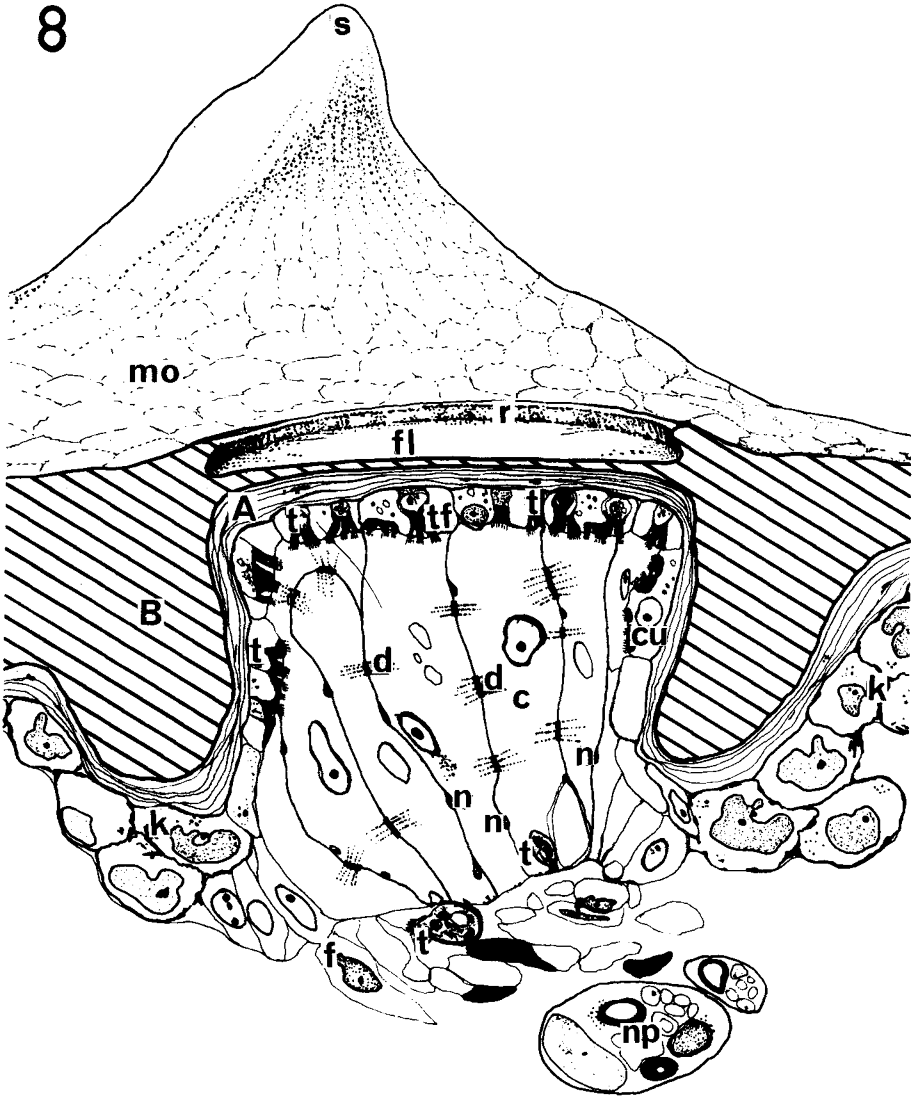

Fig. 8. Diagrammatic representation of the structure of the sensory organ (approx. ×1200). Surface features include the scale's spine (*s*), microornamentation (*mo*) and the crater, with its rim (*r*) and floor (*f*). *β* keratin (*B*) is thinned to form the floor of the crater and is thickened to form the rim and a collar around the organ. Superficial terminals (*t*) lie close to the *α* keratin layer (*A*) embedded in the cuboidal cells (*cu*) and are supported in this position by tonofilaments (*tf*). The cuboidal cell layer is continuous with the epidermal keratinocytes (*k*). Nerves (*n*) pass between the columnar cells (*c*) which are joined by desmosomes (*d*). Deep terminals (*t*) lie at the bases of the columnar cells often in the dermal papilla which contains loose connective tissue with fibrocytes (*f*). A nerve plexus (*np*) lies just beneath the dermal papilla in the superficial dermis

Discussion

The present results indicate that the intraepidermal receptors of *Amphibolurus barbatus* have similarities and differences to receptors described by other authors in other species as mechanoreceptors.

Von Düring (1974) has described the dermal papilla of the touch corpuscle in *Boa constrictor* as containing layered receptor axons and fibrocytes. The only nerve terminals found within the dermal papilla in *A. barbatus* are associated with the columnar epidermal cells. Extremely complex touch corpuscles have also been described in *Natrix* and *Caiman crocodilus* (von Düring 1973a, b). These consist of a fluid filled dermal papilla containing Merkel cell neurite complexes and lamellated receptors. Von Düring and Miller (1979) have mentioned several other species in which Merkel cell neurite complexes occur, but these are considered to be rare in reptiles. No Merkel cell neurite complexes could be found in *A. barbatus,* while lamellated receptors occur only in the deep dermis. Organised columns of cells in the dermal papilla of lizard cutaneous sensory organs have been described by Landmann (1975) and von Düring and Miller (1979), but in *A. barbatus* no such orderly arrangement is apparent. The papilla is completely absent in the early resting stage of the sloughing cycle (Maderson 1965), and at later stages it is distinct from the surrounding superficial dermis because of its lack of iridophores rather than the presence of the fibrocytes and loose connective tissue it contains.

The modification of the epidermis in the region of the intra-epidermal receptors usually takes the form of an increase in the height of the cellular part and a reduction in the thickness of the cornified part (Schmidt 1917, 1920; Cohn 1914; Miller and Kasakara 1967; Hiller 1976a, b, 1977; von Düring and Miller 1979), although exceptions have been noted in *Typhlops braminus* (Aota 1940), varanid lizards (Miller and Kasahara 1967), the Texas rat snake (Jackson and Doetsch 1977) and other snakes (Jackson 1971, 1976).

Jackson (1971, 1976) and Jackson and Doetsch (1977) have observed a touch corpuscle consisting of columnar cells of dermal origin, which intrude into the thin epidermis above the corpuscle. The columnar cells found in the intraepidermal mechanoreceptors of *A. barbatus* skin are underlaid by a basal lamina which suggests that they are of epidermal origin. Dermal melanophore processes do, however, penetrate between these cells on the more heavily pigmented skin of the dorsal body surface.

The nerve terminals within the intraepidermal mechanoreceptor have been variously described as orbiculate bodies (Aota 1940), end buttons (Proscke 1969a), bubble receptors (von Düring 1974), expanded tip receptors (Miller 1967) and discoid terminals (Landmann and Villiger 1975). Von Düring and Miller (1979) have suggested that the term "discoid terminals" be adopted since, with improved fixation techniques for electronmicroscopy, many of the previously described terminals will probably be found to be of this type. The intraepidermal nerve terminals in *A. barbatus* are round rather than discoid, but the presence of glycogen, mitochondria and vacuoles within the terminals is typical of the receptor matrix described by von Düring and Miller (1979) and Landmann and Villiger (1977). The intraepidermal terminals have previously been described as being located in the superficial layers of keratinocytes (von Düring, 1973a, 1974; Aota 1940; Hiller

1976a, b, 1977). In *A. barbatus* these terminals are pressed against the α keratin layer. This association with the α keratin has not previously been reported. The arrangement of the tonofilaments has previously been described as a tonofibrillar basket (von Düring 1973a, 1974; von Düring and Miller 1979). The condensed tonofilaments in the cuboidal cells of the epidermal receptors in *A. barbatus* skin may be more accurately described as pedestals, whose bases originate at the desmosomes connecting cuboidal cells with columnar cells, and whose distal cups hold the terminals against the α keratin. This arrangement has not been previously described although Hiller (1976a) has noted that the mass of tonofibrils surrounding the terminals are denser at the bases of the terminals.

Although no other epidermal nerve terminals have been found in *A. barbatus*, methylene blue staining, currently being employed, may reveal other free nerve terminals.

The more complex touch corpuscles found on the heads of snakes and *Caiman* appear on the skin surface as small elevations (Jackson 1977; von Düring 1973a, b, 1974). The specialised projections of the Oberhäutchen over the intraepidermal mechanoreceptors of lizards and the Texas rat snake have been described as pegs (Jackson and Doetsch 1977), bristles (Landmann 1975; Schmidt 1920), hairs (Cohn 1914; Miller and Kasahara 1967; Grandison 1968) and setae (Hiller 1976, 1977). The intraepidermal mechanoreceptors in the skin of *A. barbatus,* lack any surface projections, but Landmann (1975) has illustrated diagramatically the sensory organ described in the present study, calling it a crater, and mentioning that it is found only in association with a bristle.

The function of these receptors has been suggested to be mechanoreception (Schmidt 1920), but difficulties have been experienced in showing this experimentally. Proske (1969a) has observed several kinds of cutaneous receptors in the skin of the Australian black snake but has been unable to attribute physiological properties to the specific receptors he describes (Proske 1969b, c). Jackson and Doetsch (1977) have shown that deflection of the central peg of the epidermal mechanoreceptors on the scales of the head of the Texas rat snake causes nervous stimulation. In *A. barbatus* the surface structure gives no indication of function, but the close association of the receptors with the spine and reinforced lip of the scale, as well as the similarity in histological structure, does suggest that the function is the same. Nerve impulses are possibly initiated when deflection of the β keratin of the spine or scale edge causes corresponding deformation of the extremely thin β keratin covering the organ. The thickened β keratin surrounding the organ may amplify this effect and give directional information.

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