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Fine structure of the conus papillaris in the bobtail goanna (*Tiliqua rugosa)*

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Summary. The structure of the conus papillaris in an Australian lizard, the bobtail goanna *(Tiliqua rugosa)* was investigated by light and electron microscopy. In this strongly diurnal species, the conus papillaris consists of a heavily vascularized and pigmented, finger-like structure about 1 mm in diameter and 3-4 mm in length. It is situated over the optic nerve head and projects into the vitreous chamber. Within the conus are numerous capillaries and larger blood vessels, melanocytes and occasional mast cells. Many of the capillaries display prominent luminal and abluminal microfolds. Other capillaries show no microfolds while still others display an intermediate number of microfolds. The larger blood vessels are usually indistinguishable as to being either arterioles or venules. The endothelial cells of all blood vessels show a population of cytoplasmic granules. The melanocytes are large pleomorphic cells usually rich in microfilaments. Unmyelinated nerve processes are plentiful within the conus and the Schwann cells enclosing these nerve fibres are occasionally seen to be pigmented. The morphology of the conus papillaris indicates a heavy involvement in the transport of materials. It is considered to be homologous to the pecten oculi of the avian eye; to the falciform process of the teleost eye; to the supraretinal vessels of amphibians and to the intraretinal vessels of the mammalian eye.

Key words: Conus papillaris, Electron microscopy, Lizard, *Tiliqua rugosa*

Introduction

While the outer retina (which includes the photoreceptors and retinal pigment epithelium) is supplied by the large-caliber capillaries of the choriocapillaris, in most vertebrates a second vascular system is present to supply the requirements of the inner retina. This second vascular supply, termed a supplemental nutritive device or SND (Walls, 1942) or a supplementary retinal circulation (Rodieck, 1973) can take several forms, the most conspicuous of which is the pecten oculi of the avian eye and the conus papillaris of many reptilian eyes (Dunn, 1968; Nguyen, 1969, 1970; Jasinski, 1973; Brach, 1976; Braekevelt, 1984, 1986, 1988).

As part of a comparative morphological study of this supplementary retinal circulation in general and of the pecten oculi and conus papillaris in particular, the fine structure of the conus papillaris in a diurnal Australian lizard (the bobtail goanna *Tiliqua rugosa)* is described in this report.

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Materials and methods

For this study the eyes from ten healthy adult bobtail goannas *(Tiliqua rugosa)* were examined by light and electron microscopy. Specimens were 3-4 years old, of both sexes, weighed 420-550 gm and were light-adapted when sampled.

With the animals under deep anesthesia, the eyeballs were quickly removed, opened at the equator and fixed for 5 h in 5% glutaraldehyde buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer af 4°C. The eyeballs were then opened completely and the conus papillaris was removed, washed in 5% sucrose in 0.1 M Sorensen's buffer (pH 7.3) and either left intact or cut into smaller segments. The tissue was then post-fixed for 2h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reorientated to desired angles by means of a wax mount. Thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (60-70 nm) were then cut and collected on copper grids. These sections were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

Results

The conus papillaris in the bobtail goanna *(Tiliqua rugosa)* consists of a finger-like structure about 1 mm in diameter and 3-4 mm in length which tapers only slightly towards its tip. It is situated over the optic nerve head and projects out into the vitreous chamber.

Internally the conus consists of a large array of melanocytes, capillaries and larger blood vessels widely separated by a loose arrangement of collagenous fibrils and amorphous ground substance (Fig. 1).

The melanocytes are large pleomorphic cells which do not isolate the blood vessels completely (Figs. 1, 9, 10). The nucleus of the melanocyte is large, vesicular and often itself very pleomorphic (Figs. 9, 10). Melanocytes are extensively branched with the finest ramifications often less than 1.0 μm in width (Figs. 2, 7). The melanosomes within these cells are extremely electron dense and all appear to be spherical (Figs. 9-11). Melanosomes vary in diameter from 1.5 to 0.5 μm (Figs. 9-11). The majority of cell organelles are in a paranuclear location with the smaller processes only rich in microfilaments (Figs. 3, 7).

A second population of cells is noted within the connective tissue framework of the conus which also contains a large number of granules (Fig. 11). These granules are of approximately the same diameter as melanosomes but are much less electron dense (Fig. 11). These cells display the morphology of the mast cells of connective tissue.

The capillaries of the conus papillaris in this species are quite variable in their appearance as to the presence or absence of processes on their luminal and/or abluminal borders. Many capillaries present quite smooth borders with essentially no processes on either edge (Figs. 2, 3). Other capillaries show only abundant luminal processes (Fig. 4) while still others display extensive processes on both the luminal and abluminal borders (Figs. 5, 6). In those capillaries with processes on both aspects of the endothelial cell, the cell body is often reduced to a thin unfenestrated band of cytoplasm from which the numerous processes arise (Fig. 6). Presumed transport vesicles appear to be more numerous in capillaries without processes (Figs. 2-4, 6). These processes when present are always longer and more abundant on the luminal aspect of the capillaries (Figs. 4, 6). The processes are felt to be microfolds as they are quite long and can often be seen to branch (Figs. 4, 5).

The endothelial cells are joined by large and often elaborate cell junctions of the occludens type (Figs. 2, 3, 6). In all capillaries, the nuclear region is the thickest part of the endothelial cell with most cell organelles located in this paranuclear region (Figs. 2-4). Mitochondria, polysomes and abundant arrays of microfilaments are however located throughout the endothelial cells (Figs. 3, 5, 6). All capillaries also display an abundant population of small electron-dense granules (Figs. 2-5). These granules can be of differing electron densities but are always individually homogeneous (Figs. 2-4).

The basal lamina around the capillaries of the conus also displays a range of morphologies. In some cases the basal lamina appears as several indistinct fibrillar layers (Figs. 2, 5). In other locations, a single more prominent basal lamina has a varying amount of fibrillar material associated with its outer aspect (Figs. 3,4). In capillaries with extensive abluminal folds, the fibrillar material of the basal lamina is insinuated amongst these processes (Figs. 5, 6). No correlation was noted between the number of endothelial processes and the type of basal lamina present.

Apparent pericytes are associated with the conus capillaries and the extent to which they are enclosed within the basal lamina depends upon the thickness of the basal lamina present around that particular capillary (Figs. 2, 4). Pericytes did not show microfolds and indeed often display a paucity of cell organelles of any type. (Fig. 4).

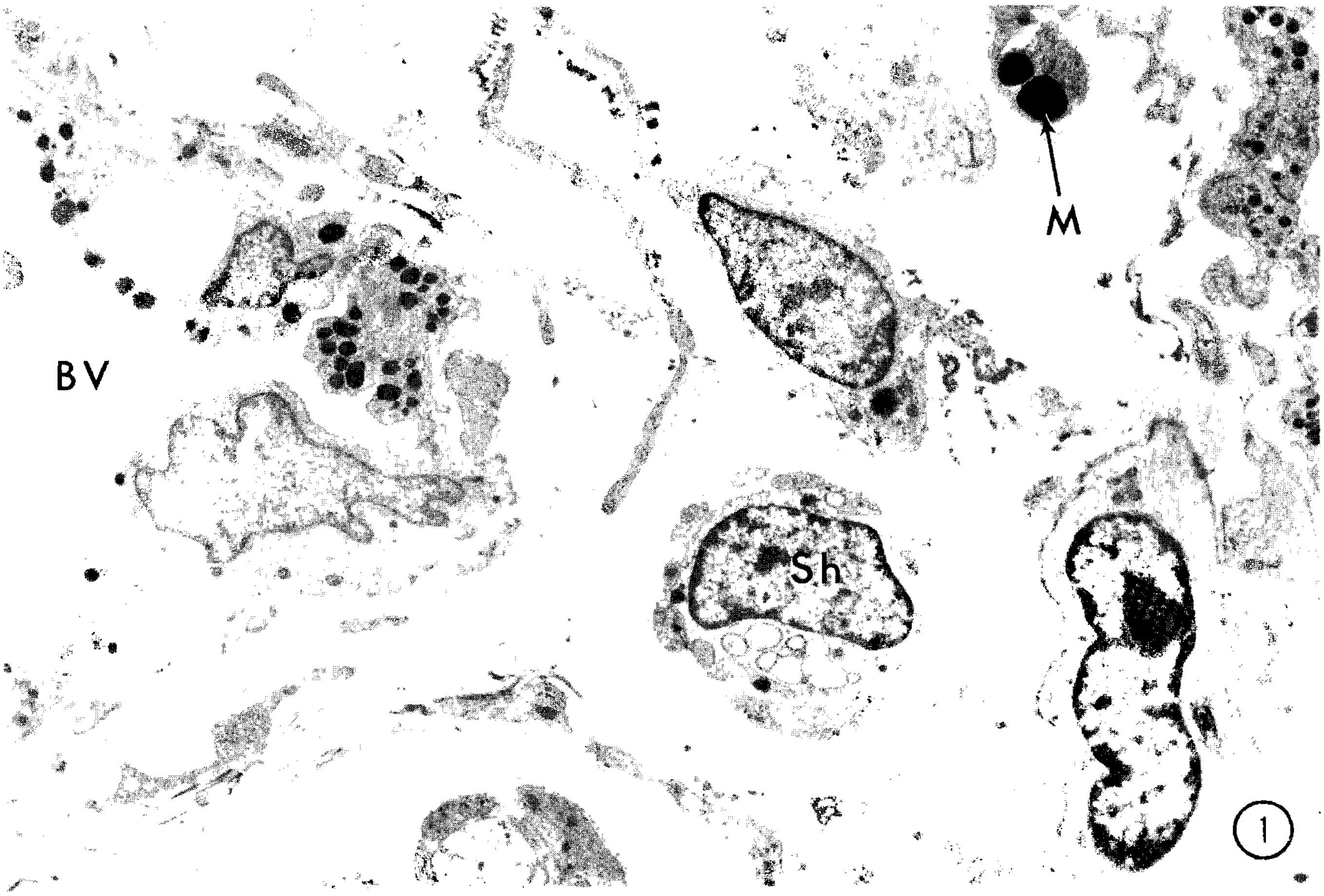
The larger blood vessels of the conus papillaris of this species are difficult if not impossible to distinguish as to either supply (arteriole) or drainage (venule) vessels (Fig. 7). These vessels show a non-fenestrated endothelium and do not possess microfolds on either border (Fig. 7). This endothelium shows a more compact basal lamina than is noted for the capillaries (Fig. 7). Forming part of the wall of these vessels is a layer or two of cells which display the morphology of smooth muscle cells including dense bodies, caveolae and abundant microfilament bundles (Fig. 7).

Another conspicuous feature of the conus papillaris is the presence of an extensive non-myelinated nerve supply (Figs. 1, 8, 9). These unmyelinated fibers are more or less enclosed within the cytoplasm of Schwann cells (Fig. 8). An unusual feature is that these presumed Schwann cells can also contain pigment granules (Fig. 9). These nerve fibres appear to be haphazardly arranged and can be observed to lie in close proximity to both capillaries and the larger blood vessels (Fig. 1).

Discussion

The conus papillaris of the reptilian eye is a heavily pigmented and richly vascular structure which projects from the optic nerve head. It is considered to be homologous to the pecten oculi of the avian eye, with both being employed as a supplementary nutritive device for the inner retina (Walls, 1942; Rodieck, 1973; Meyer, 1977).

Aside from the so-called pseudo-conus papillaris observed in snakes (Nguyen, 1971) the conus papillaris assumes two basic shapes throughout the reptiles. The most common shape is a smooth, finger-like structure much like the conical pecten of the kiwi (Nguyen, 1969; Dieterich and Dieterich, 1975, 1977; Meyer, 1977). The other less common form consists of a central pillar from which vertical fins radiate, much like the vaned pecten of the ostrich (Nguyen, 1970; Meyer, 1977). While the conus papillaris never attains the complexity of the pleated form of pecten found in most birds, it is felt that the increase in surface area provided by the vaned conus represents a more pecten-like structure and is probably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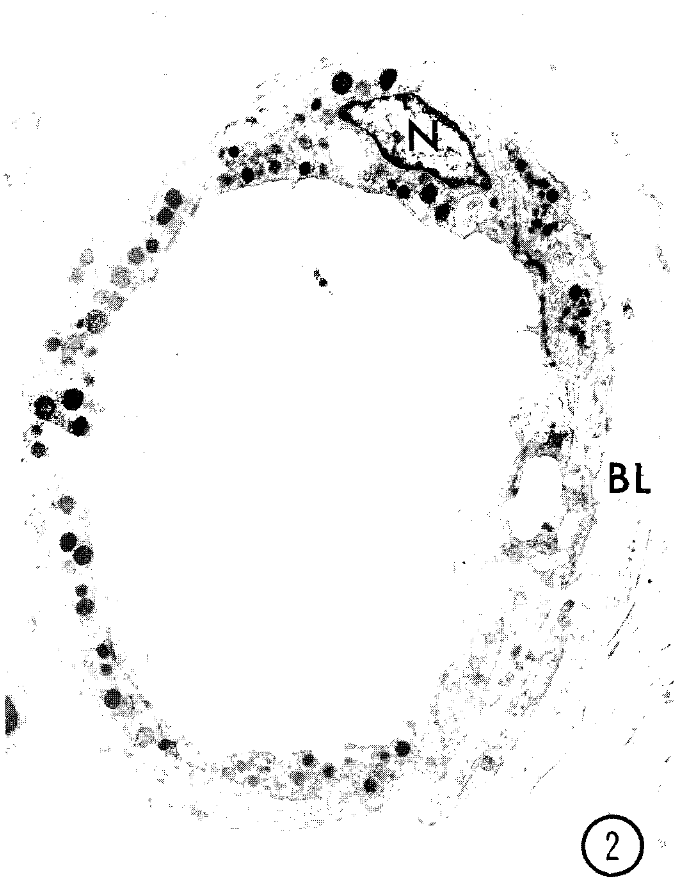
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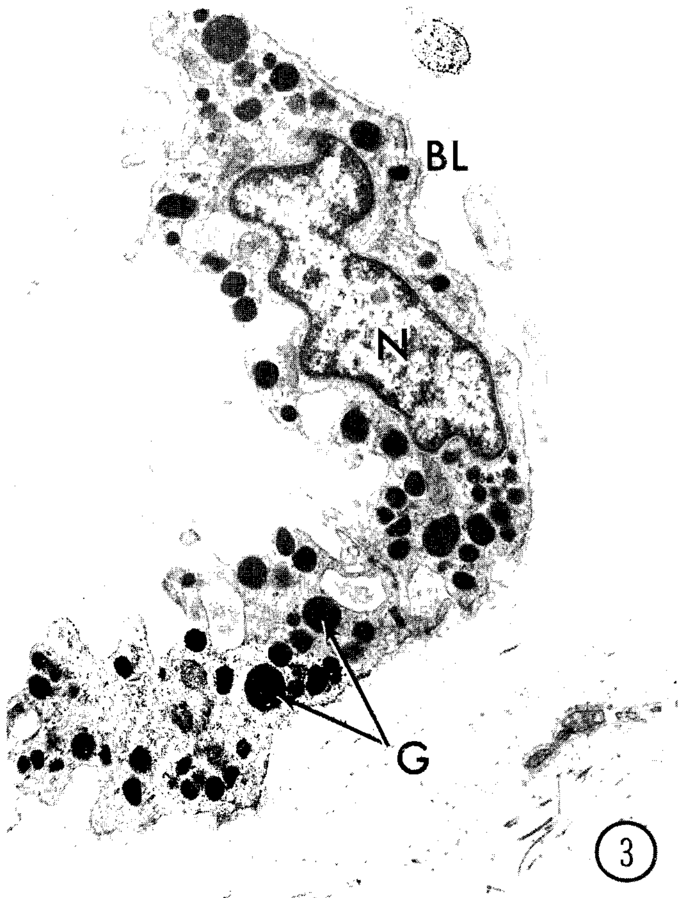
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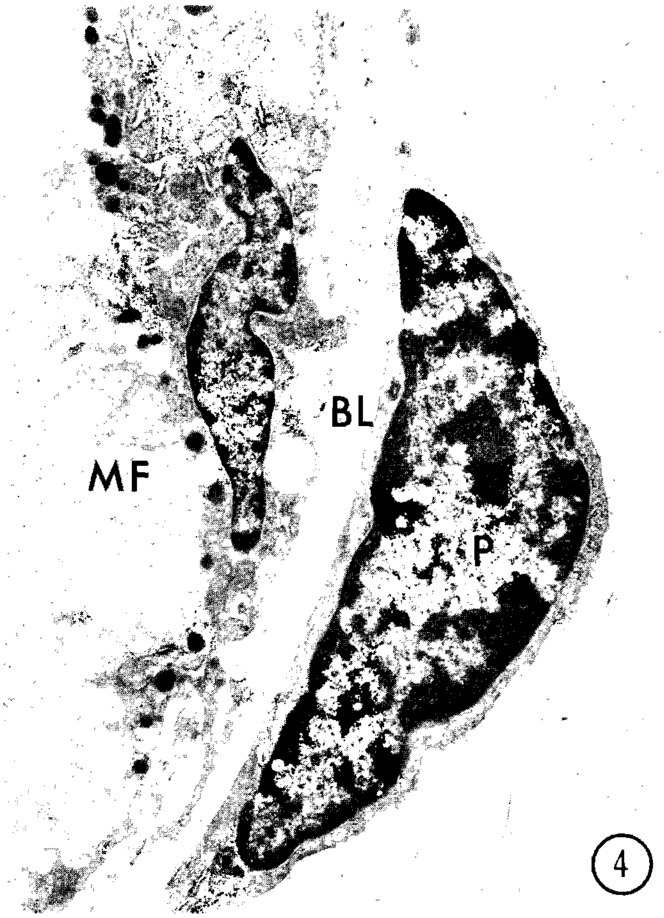
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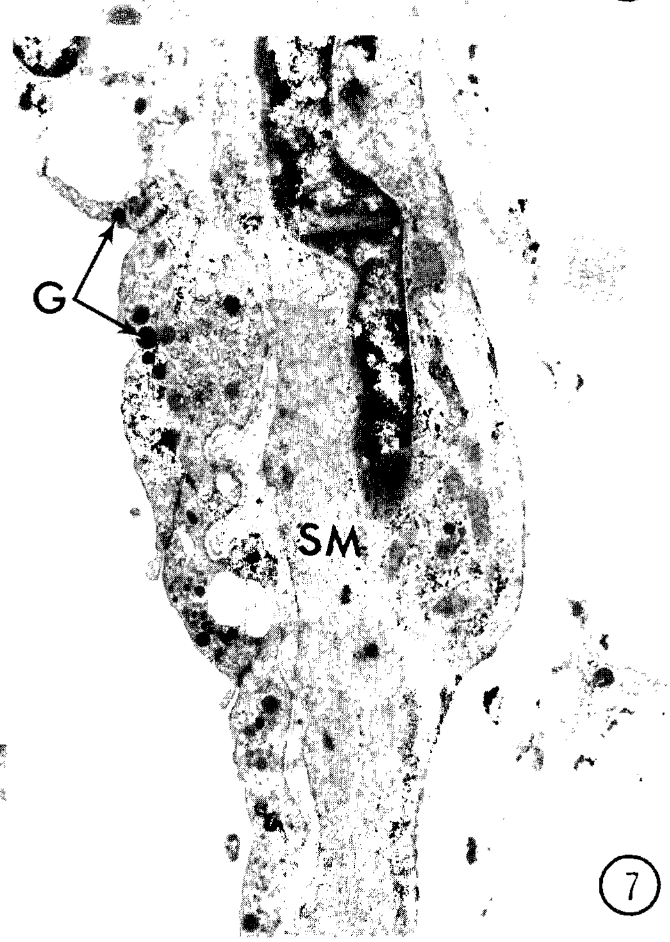
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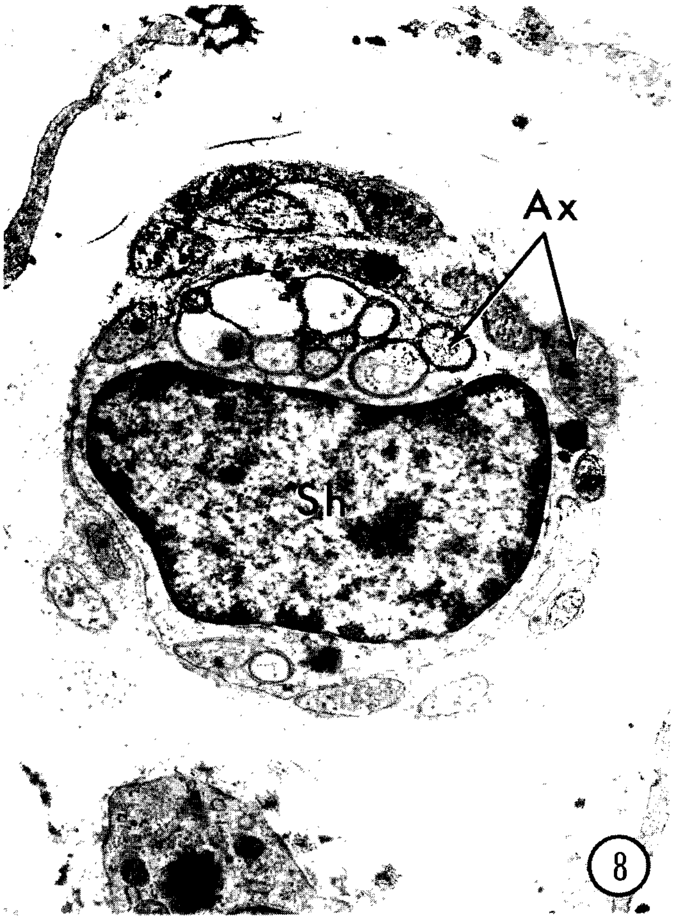
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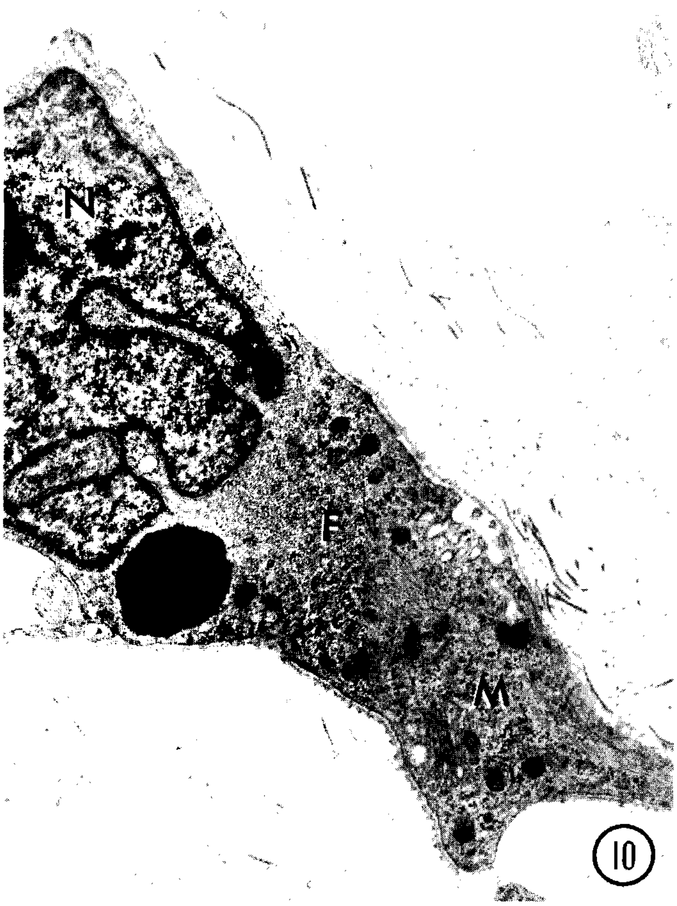
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Fig. 1. Low power electron micrograph of the conus papillaris of the bobtail goanna to illustrate the loose arrangement of connective tissue elements and ground substance. A blood vessel (BV), a melanocyte (M) and a Schwann cell (Sh) are indicated. × 8,600

Fig. 2. Electron micrograph of a capillary with only very few luminal and abluminal microfolds. The thickened basal lamina (BL) and an endothelial nucleus (N) are indicated. Note the granules within the endothelial cells. × 8,900

Fig. 3. Electron micrograph of a capillary with an abundance of granules (G) within the endothelial cells. An endothelial nucleus (N) is indicated. The basal lamina (BL) is relatively thin. × 12,900

Fig. 4. Electron micrograph of a capillary with abundant luminal microfolds (MF) and only a few abluminal processes. A pericyte (P) and the basal lamina (BL) are indicated. × 12,900

Fig. 5. Electron micrograph of a capillary with abundant luminal and abluminal microfolds. Microfilaments (F) are also abundant and granules (G) are obvious. × 18,200

Fig. 6. Electron micrograph of a capillary with an abundance of both luminal and abluminal processes. A cell junction (J) is indicated. The endothelial cell body (E) is quite thin. × 25,000

Fig. 7. Electron micrograph of a blood vessel with a single layer of smooth muscle cells (SM) in the wall. Granules (G) are abundant within the endothelial cells but the cells show no processes. × 13,200

Fig. 8. Electron micrograph of a Schwann cell (Sh) enclosing several axons (Ax). × 18,700

Fig. 9. Electron micrograph of a pigmented cell (P) which is enclosing several axons (Ax). × 13,200

Fig. 10. Electron micrograph of a melanocyte (M) to indicate the nucleus (N) and the abundance of microfilaments (F). × 13,200

Fig. 11. Electron micrograph of melanocytes (M) and a mast cell (Mc). Note the difference in electron density of the inclusions in the two cell types. 13,200

more efficient (Nguyen, 1970). Intermediate forms between the finger-like and vaned conus papillaris are also reported (Nguyen, 1974).

Perhaps more important to the efficiency of the conus papillaris however than its gross morphology is the presence of numerous capillaries within the structure that are highly specialized for enhanced transport (Nguyen, 1970, 1971, 1974; Brach, 1976; Jasinski, 1977). Dieterich et al. (1976) have correlated the presence of the most elaborately specialized capillaries with numerous deep and slender microfolds to diurnal species with thicker inner retinas than was the case in the nocturnal species.

In the bobtail goanna *(Tiliqua rugosa)* which is diurnally active, the conus papillaris is the finger-like structure noted for most reptiles. Grossly the conus appeared heavily pigmented in this species but upon histologic examination was found not to be excessively pigmented internally but with most of the melanocytes arranged in a peripheral location. This peripheral accumulation of melanocytes has previously been noted in the pecten and has been conjectured to be useful in raising the temperature and probably the metabolic rate of the entire pecten (Bawa and Yash Roy, 1974; Braekevelt, 1986, 1988). Variations in the amount of pigmentation within the conus papillaris of various species of gecko have also been reported (Dieterich et al., 1976).

The melanocytes in the bobtail goanna are highly branched and often rich in bundles of microfilaments. This would seem to indicate that they fulfil a structural role in the conus. In addition the Schwann cells which enclosed unmyelinated nerve fibres are often seen to be pigmented. It is not known if these are true melanocytes fulfilling a secondary function or whether these are truly Schwann cells which have become pigmented.

The presence of relatively numerous mast cells within the stroma of the conus papillaris has been reported in other species (Nguyen, 1970; Dieterich and Dieterich, 1977). Mast cells are a common feature within the conus of the bobtail goanna but the significance of this observation is unknown.

The highly specialized capillaries observed in the avian pecten with their extensive luminal and only slightly less elaborate abluminal microfolds are felt to be the epitome of transport oriented vessels (Meyer, 1977; Braekevelt, 1984, 1986, 1988). In the bobtail goanna, capillaries with extensive luminal and abluminal processes are common but so were capillaries with essentially no surface specializations. The presence of these two types of capillary has also been noted in other species and is felt to reflect a lowered efficiency of the conus as a whole (Nguyen, 1969, 1970, 1971; Dieterich and Dieterich, 1975). The least efficient conus is thus felt to be in the slow worm *(Anguis fragilis)* which shows essentially no endothelial microfolds (Nguyen, 1969). In the day-active geckos, iguanids and chameleons on the other hand, the capillary endothelium shows the most extensive formation of both luminal and abluminal microfolds (Nguyen, 1970; Dieterich and Dieterich, 1975; Dieterich et al., 1976). The presence of some capillaries with highly specialized and others with relatively smooth endothelium may indicate an intermediate position for the bobtail goanna.

The specialized capillaries noted in the pecten oculi of various avian species always display an elaborate multilayered basal lamina (Meyer, 1977; Braekevelt, 1984, 1986, 1988). The purpose of this thickened basal lamina has been conjectured to be supportive around the heavily infolded and presumably relatively fragile endothelial cells (Braekevelt, 1988). In the bobtail goanna a range of basal lamina thicknesses is noted. This may reflect a lessened need for structural support around these conus capillaries as they are relatively less infolded than those of the pecten. In addition as the conus papillaris never attains the highly pleated morphology of the pecten, this may also lessen the need for structural support of the capillaries.

The presence of a prominent population of granules within the endothelial cells of the conus papillaris has also been noted in other species (Nguyen, 1970; Dieterich and Dieterich, 1975; Jasinski, 1977). The purpose of these granules is unknown but may indicate a secretory function for these cells. While other authors have readily distinguished the larger blood vessels of the conus to be either arterioles or venules (Nguyen, 1970; Dieterich and Dieterich, 1975) in the bobtail goanna such a categorization was very difficult if not impossible.

The presence of unmyelinated axons within the conus papillaris is a common feature of all examined species (Nguyen, 1970, 1974; Jasinski, 1977; Nguyen-Legros, 1978). Variations in their abundance and distribution are however noted with the most elaborate innervation being seen in the finger-like conus (Nguyen, 1970; Nguyen-Legros, 1978). In *Chameleon lateralis* which has a more vaned conus papillaris these nerve fibers are reported to be confined to the base of the conus only (Nguyen, 1970). As unmyelinated nerve fibres are never encountered within the body of the pecten oculi, their absence within a conus papillaris is considered to indicate a more pecten-like pattern and hence is considered to indicate a more efficient conus (Meyer, 1977).

The purpose of these unmyelinated nerve fibres is as yet unestablished. They may be useful in varying luminal size and hence the blood supply within the conus or perhaps they may influence the extent of the pigment dispersion within the conus. While ectotherms may have direct innervation to their chromatophores, it seems more likely that this nerve supply is important in determining the state of vasoconstriction and/or vasodilation of the blood vessels of the conus papillaris.

While the elaborate pleated structure of the typical pecten oculi has lead to the conjecture of several other functions for the pecten in addition to its established role as a nutritive organ, the finger-like conus papillaris has not been implicated in any other functions (Walls, 1942). It, like the pecten functions as a supplementary vascular supply to the inner retina and can be considered to be homologous to the pecten of the avian eye; to the falciform process of some teleosts; to the supraretinal vessels of amphibian, some teleost and a few mammalian species and to the intraretinal vessels found in the majority of mammals (Walls, 1942; Rodieck, 1973).

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