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Fine structure of the pecten oculi in the Australian Galah (*Eolophus roseicapillus*) (Aves)

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Summary. The pecten oculi of the Australian galah (*Eolophus roseicapillus*) has been examined by both light and electron microscopy. In this species the pecten is large relative to the size of the eye and is of the pleated type. It consists of 20-25 accordion folds that are joined apically by a bridge of tissue which holds the pecten in a fan-like shape widest at its base. Within each fold are many melanocytes, numerous capillaries as well as larger supply and drainage vessels. The capillaries are extremely specialized for transport functions and display extensive microfolds on both their luminal (inner) and abluminal (outer) borders. Except for the nuclear region which also contains most of the organelles, the endothelial cell bodies are extremely thin. These capillaries are surrounded by thick fibrillar basal laminae which are felt to be structurally important. Pericytes are a common feature within the basal lamina of capillaries. The numerous pleomorphic melanocytes which more or less surround the capillaries are also presumed to be important in structural support of the pecten. The pecten represents a supplementary retinal circulation and is comparable to the falciform process of some teleosts, the conus papillaris of reptiles, the supraretinal vessels of amphibians and some teleosts and the intraretinal vessels of mammals, all of which are felt to be alternative methods of bringing nutrients to the inner retina.

Key words: Pecten oculi, Fine structure, Aves, Galah, *Eolophus roseicapillus*

Introduction

In all vertebrates, the outer retina which includes the retinal pigment epithelium and photoreceptors is nourished by the large fenestrated capillaries of the choriocapillaris. In most vertebrates a second vascular system supplies the inner retina (nearest the vitreous chamber). This other vascular system which is termed a supplemental nutritive device (Walls, 1942) or supplementary retinal circulation (Rodieck, 1973) can take several forms and in avian species is seen as the pecten oculi.

The pecten oculi appears as a highly vascular and pigmented organ which projects from the optic nerve head (along the former course of the choroid or retinal fissure) out into the vitreous chamber (Michaelson, 1954; Prince, 1956). Histological studies have confirmed its vascularity and while several secondary (and often fanciful) functions have also been ascribed to it, the primary role is felt to be nutritive to the inner avascular avian retina (O'Rahilly and Meyer, 1961; Wingstrand and Munk, 1965). Fine structural studies have shown an extremely specialized capillary morphology and confirm its role as a nutritive organ (Raviola and Raviola, 1967; Fielding, 1972; Dieterich et al., 1973; Braekevelt, 1984, 1986, 1988, 1990, 1991a,b, 1993, 1994).

As part of an ongoing comparative study of the pecten of birds, this report deals with the fine structure of the pecten of an Australian cockatoo, the galah (*Eolophus roseicapillus*) and compares and contrasts these findings with observations from other birds.

Materials and methods

For this study the eyes from two-light adapted galahs (*Eolophus roseicapillus*) were examined by light and electron microscopy. The adult birds were captured in mist nets under the Western Australian Department of Conservation and Land Management Licence # SF 000503. With the animals under deep anesthesia, the eyes were quickly enucleated, slit open at the equator and immersion fixed for 5 h at 4 °C in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and the pecten and its underlying retinal tissue was carefully dissected out. For each specimen, one pecten was left intact while in the other it was further divided into pieces less than 1 mm^2. In all cases

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the tissue was then postfixed for 2 h in 1% OsO_4 in the same phosphate buffer, dehydrated up through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

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

Results

The pecten oculi of the Australian galah (*Eolophus roseicapillus*) is situated over the optic nerve head and projects out into the vitreous chamber. In this species the pecten measures about 5 mm along its base, tapers to about 3 mm at its apex or bridge and projects to about 3 mm in height. The pecten displays 20-25 pleats or accordion folds that are held together apically by a slightly more pigmented bridge of tissue.

Each pleat or fold measures 25-30 µm in width and contains plentiful melanocytes, numerous very specialized capillaries and two or more larger blood vessels which are difficult to differentiate as either arterioles or venules (Figs. 1, 2, 4). A fine basal lamina continuous with the inner limiting membrane of the retina encloses the entire pecten (Figs. 1, 2, 5, 6). Hyalocytes are occasionally noted adherent to the outer edge of this basal lamina (Fig. 1).

The melanocytes of the galah's pecten are large pleomorphic cells with long processes that form an incomplete covering around the capillaries but which more or less isolate the capillaries and larger blood vessels from one another (Figs. 1-3, 6). The nuclei of melanocytes are large and vesicular with isolated clumps of heterochromatin (Figs. 2, 3, 6). The round and extremely electron-dense melanosomes are mostly 1.5 to 2.0 µm in diameter but can be larger in the apex or bridge region of the pecten (Figs. 1-4). Melanosomes are scattered throughout the melanocyte cytoplasm however processes smaller than 1.0 µm in width seldom contained melanosomes (Figs. 1, 2, 6). Premelanosomes were not observed. While polysomes and small profiles of rough endoplasmic reticulum are scattered throughout the melanocyte's processes, most organelles including the mitochondria are predominantly in a perinuclear location (Figs. 2, 3, 6). In the galah, melanocytes are slightly more abundant in the bridge region than in other locations (compare Figs. 1 and 2).

The pecteneal capillaries are extremely specialized vessels measuring 8-12 µm in luminal diameter (Figs. 1, 2). These capillaries display a vast array of processes both on their luminal (apical or internal) and abluminal (basal or external) borders (Figs. 1-6). In many locations the actual cell body is a very thin central area measuring as little as 0.2 µm in width from which these numerous luminal and abluminal processes arise (Figs. 4-6). These processes are felt to be microfolds rather than microvilli as they exhibit a range of widths when cut in different planes (Figs. 4, 5) and they are also seen to branch (Figs. 3-6). Normally, the luminal microfolds are longer and straighter at 1.5 to 2.0 µm than the abluminal projections which are usually more compressed and tortuous but measure about 1.0 µm in length (Figs. 4-6).

The nuclear region of the capillary endothelial cells is always the widest portion of the cell body and luminal microfolds are numerically reduced in this area (Figs. 1, 2, 4). The nucleus is normally large, somewhat flattened and quite vesicular (Figs. 1, 2, 4). Most of the endothelial cell organelles are located in the perinuclear region although small mitochondria, polysomes, short profiles of rough endoplasmic reticulum and lysosome-like bodies are found throughout the cell (Figs. 3-6). At least two, and more usually three or four, endothelial cells surround a capillary lumen (Figs. 1, 2). These endothelial cells are joined by elaborate and extensive tight junctions (Figs. 3-5).

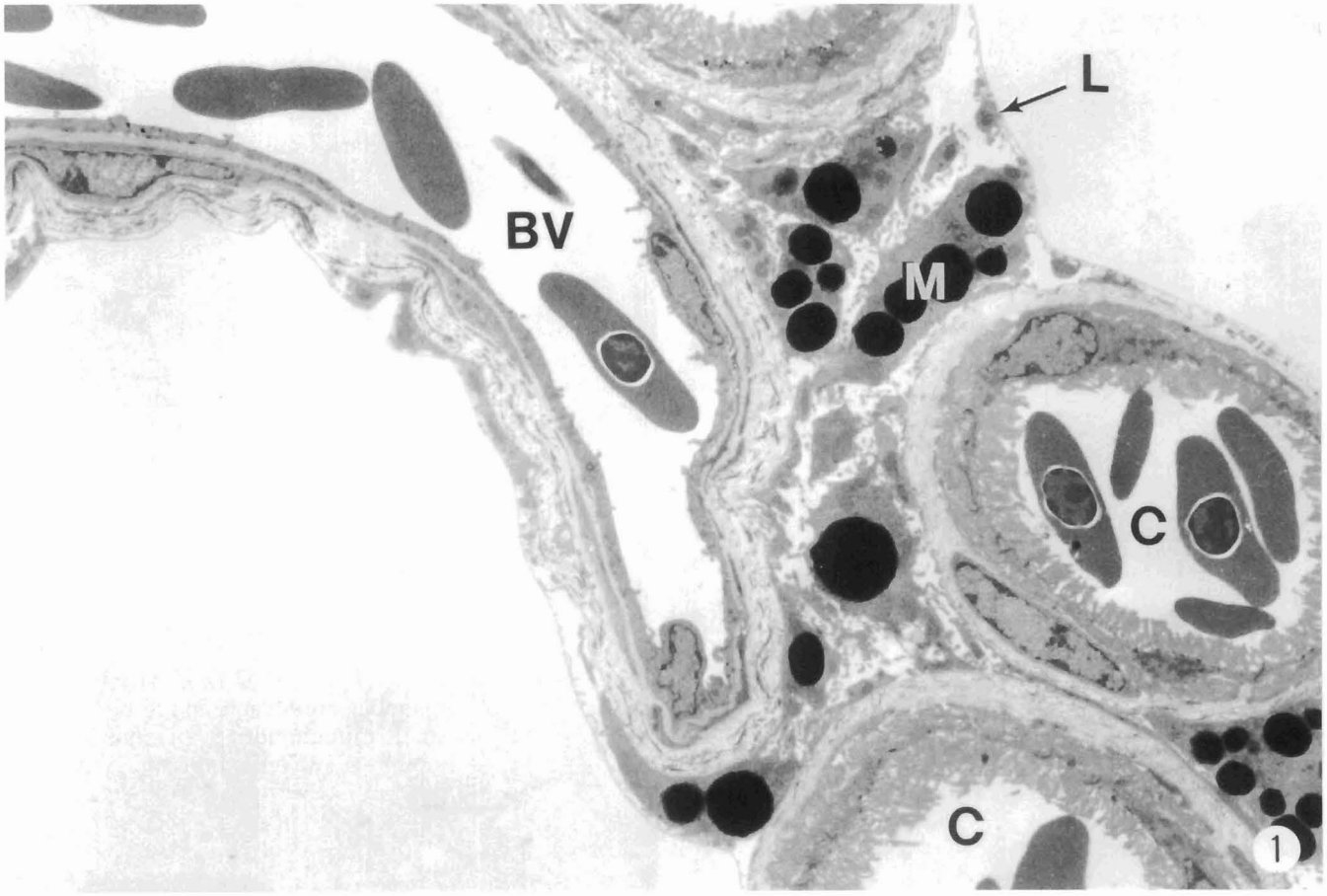
The basal lamina of these capillaries consists of several concentric layers of fine fibrillar material, each separated by amorphous ground substance (Figs. 3-6). The outermost layer of this thickened basal lamina has the appearance of a «regular» basal lamina and it is separated from the endothelial cell body by several additional layers of fibrillar material (Figs. 3-6). This multilayered basal lamina measures about 1.0 µm in thickness (Figs. 3, 4).

Pericytes are often enclosed within this thickened basal lamina of pecteneal capillaries (Figs. 1, 2, 5). These cells appear quite undifferentiated with a minimum of cell organelles and no microfolds on any surface (Fig. 5). The pericyte nucleus is normally flattened and vesicular and the cytoplasm contains polysomes, profiles of RER and small mitochondria (Fig. 5). Pericytes may touch the abluminal microfolds of the endothelial cells or there may be intervening basal lamina material between the two cell types (Figs. 1, 2, 5).

All blood vessels above capillary size within the pecten folds display essentially the same morphology and cannot be categorized definitely as either arterioles or venules (Figs. 1, 4). The endothelium of these vessels is non-fenestrated and shows no microfolds on either

Fig. 1. Low power electron micrograph of one fold of the pecten taken from the basal region of the pecten. The limiting membrane (L), two capillaries (C) are indicated as is a melanocyte (M) and an afferent or efferent vessel (BV). x 4,000

Fig. 2. Low power electron micrograph of one fold of the pecten taken near the bridge or apical region. The limiting membrane (L) and two capillaries (C) are indicated as is a hyalocyte (H). Melanocytes (M) are slightly more abundant in this location. x 4,000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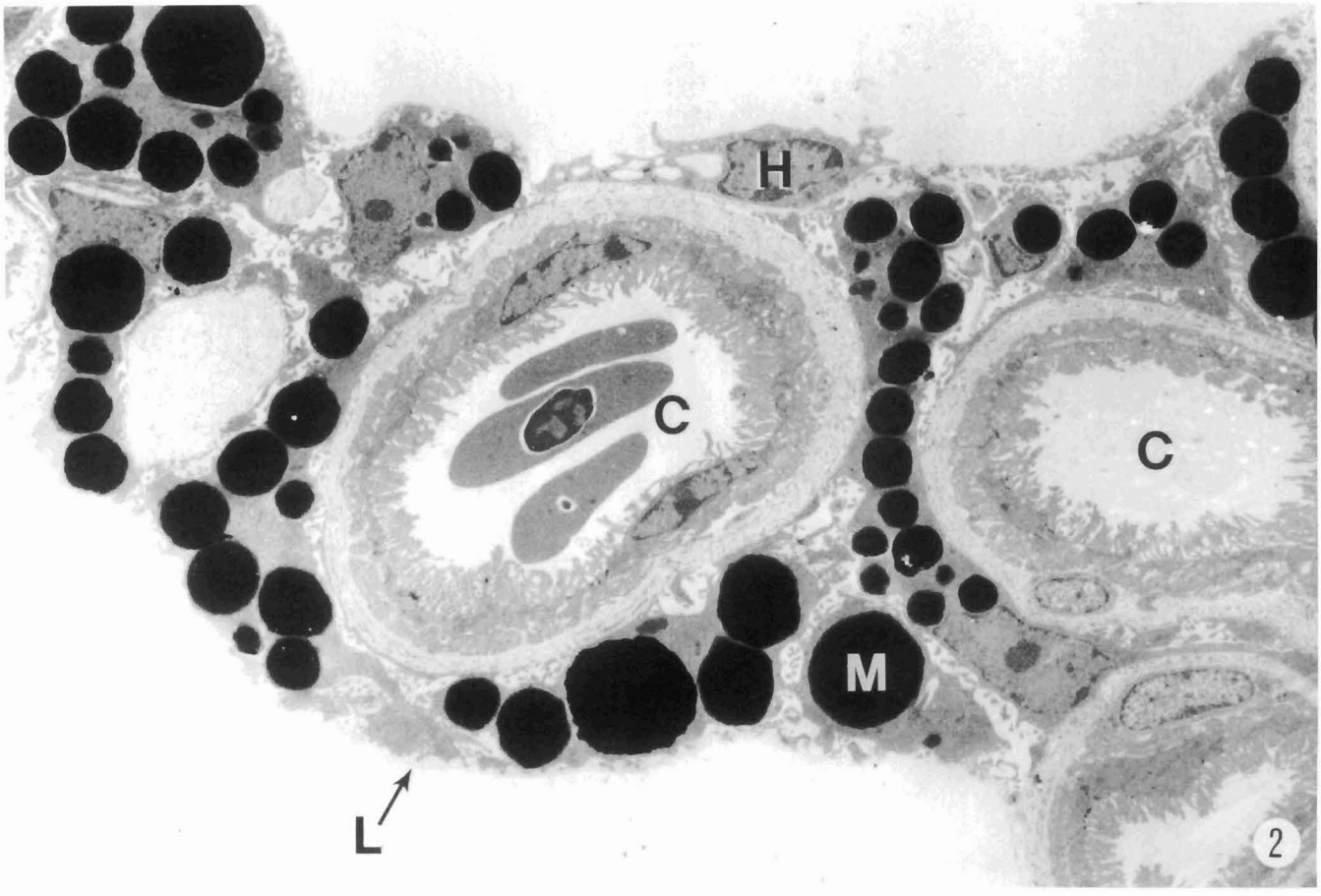
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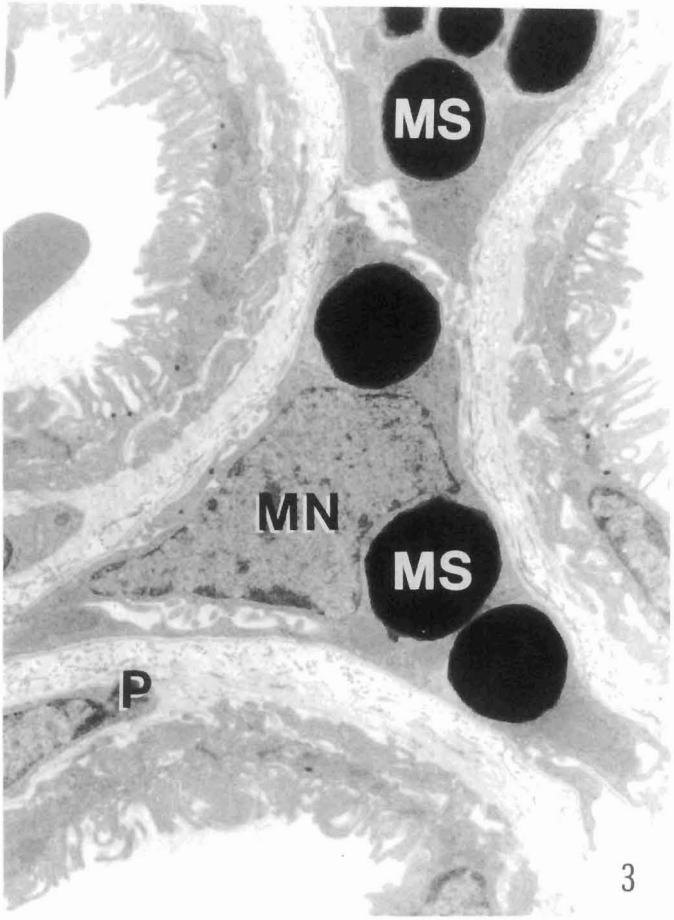
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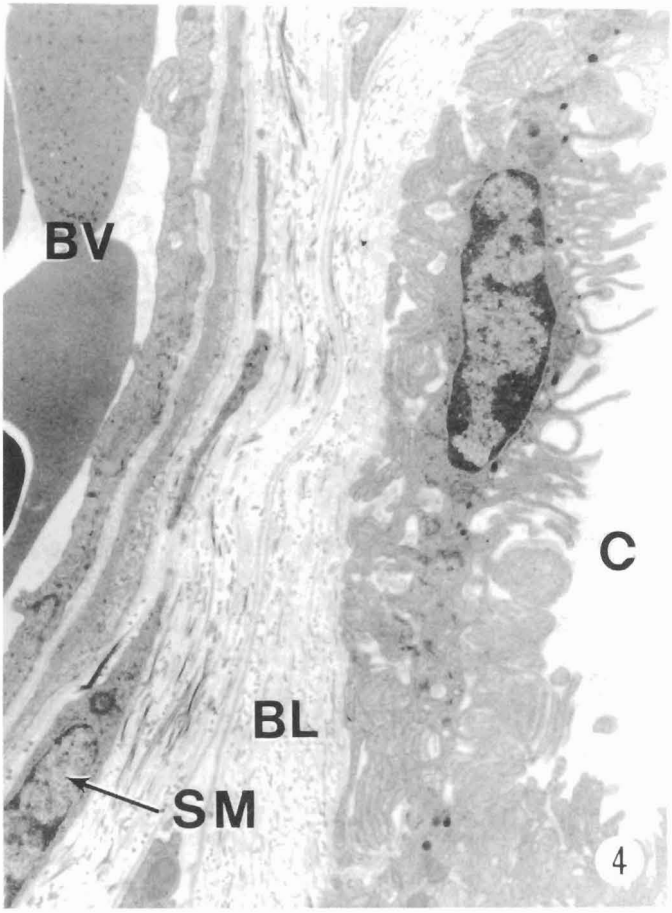
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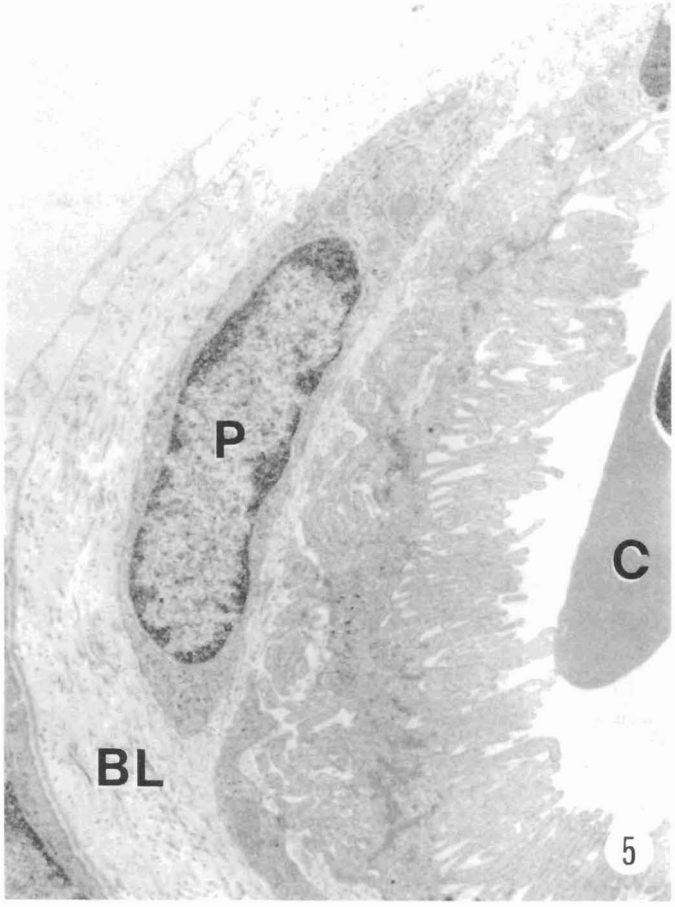
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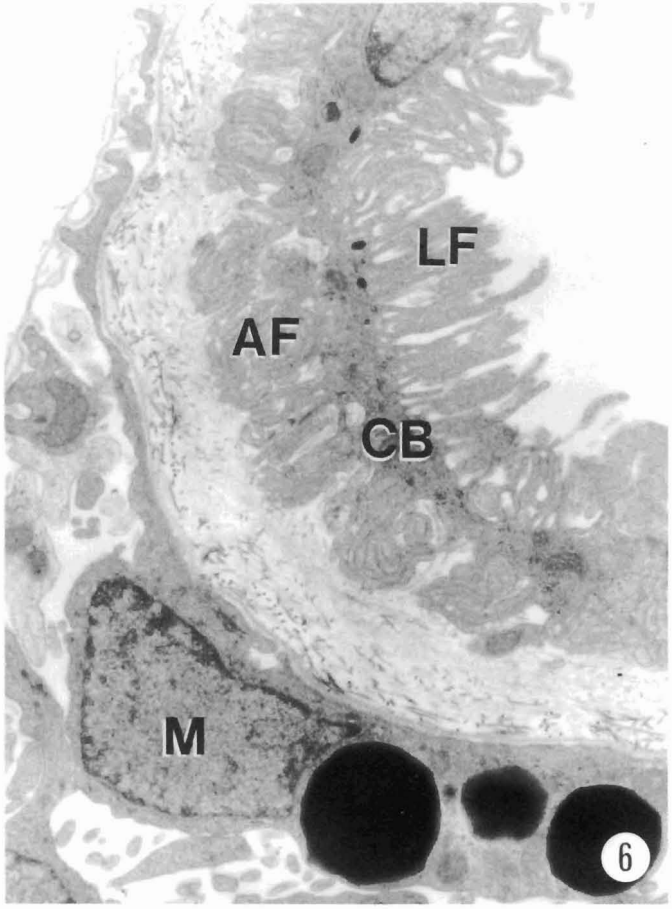
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border (Figs. 1, 4). The endothelial cells contain polysomes, short profiles of rough endoplasmic reticulum, measure about 0.7 µm in width and are joined by tight junctions (Fig. 4). As with the capillaries the nuclear region is the widest portion of these cells (Fig. 1). These vessels are surrounded by a thickened basal lamina (like the capillaries) which contain flattened cells which show the morphology of smooth muscle cells (Fig. 5).

Discussion

The pecten oculi of the avian eye is normally classified into three morphologically distinct types (Meyer, 1977). These are the conical, vaned and pleated pecten.

The conical pecten is a finger-like structure resembling the conus papillaris of reptiles and to date has only been reported in the brown kiwi (*Apteryx australis mantelli*) (Meyer, 1977). The vaned type of pecten consists of a central flattened pillar from which ventrally-oriented vanes arise. This type of pecten is reported in ostriches and rheas (Walls, 1942; Meyer, 1977). The pleated pecten is however by far the most common type and with minor variations is apparently found in all other birds (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991a,b, 1993, 1994). The galah (*Eolophus roseicapillus*) displays the pleated type of pecten.

A basal lamina or vitreo-pecteneal limiting membrane continuous with the inner limiting membrane of the retina covers the entire pecten (Dieterich et al., 1973; Braekevelt, 1986, 1990, 1993). Hyalocytes are occasionally noted adherent to the outer surface of this membrane but they do not appear to be a regular feature in all species (Braekevelt, 1990, 1991a,b, 1994).

The pecten is always located over the normally oval optic nerve head and projects out into the vitreous chamber (Walls, 1942). In large eyes the pecten will be large in absolute terms but may be small in relation to the size of the globe. The size of the pecten seems to be dictated by the visual activity of the bird so that highly active diurnal species will have a large pecten with many folds while less visually reliant species will have a relatively smaller pecten (Walls, 1942; Meyer, 1977). In relation to eye size the galah has a large pecten with many folds (20-25) emphasizing the importance of vision in this cone dominant cockatoo (Braekevelt and Richardson, 1996).

The presence of melanocytes is a constant feature of all pectens described to date (Walls, 1942; Fischlsweiger and O'Rahilly, 1966; Fielding, 1972; Meyer, 1977; Braekevelt, 1984, 1988, 1990). As no other cell types are present within the pecten to act as supportive elements, it is felt that these cells at least in part fulfil a structural role. The normal presence of numerous microtubules and microfilaments within the processes of these cells would support this view (Braekevelt, 1990, 1991a,b, 1993). In addition the absorption of light by the melanin of these cells probably raises the temperature within the pecten and hence the rate of metabolism within it (Bawa and YashRoy, 1974). In most species described to date there is a marked increase of melanocytes in the apex or bridge region of the pecten (Braekevelt, 1984, 1986, 1990). This was not noted in the galah or American crow (Braekevelt, 1994) however both of which showed but a small increase in melanocytes in the bridge region.

Within the folds of the pecten are located many specialized capillaries as well as supply (afferent) and drainage (efferent) vessels all of which are more or less surrounded by melanocytes. The width of a pecteneal fold is quite constant at 25-30 µm across avian species and may represent some optimal thickness based on structural and/or diffusion criteria (Braekevelt, 1988, 1990, 1991a, 1993, 1994). Unlike the condition reported in the chicken (Dieterich et al., 1973) and a previous study on the pigeon (Raviola and Raviola, 1967) where the authors differentiate arterioles and venules, in the galah as in all previous studies of other species by this author, it is very difficult if not impossible to adequately differentiate these larger vessels of the pecten as to being either arterioles or venules (Braekevelt, 1984, 1986, 1988, 1990, 1991a,b, 1993, 1994). This apparent lack of structural differences between most of these afferent and efferent vessels within the body of the pecten may indicate a lowered blood pressure within the pecten.

The capillaries within the avian pecten are extremely specialized vessels with a morphology unparalleled in other vertebrates (Tanaka, 1960; Seaman and Storm, 1963; Fielding, 1972; Hanzely et al., 1975; Meyer, 1977; Braekevelt, 1988, 1990, 1993). One of the most striking features of these capillaries is the presence of numerous long processes on both their luminal and abluminal borders. While some ultrastructural studies have referred to these as microvilli (Nguyen et al., 1967), other investigators refer to them as microfolds (Meyer, 1977). In the galah as in all other species studied by this author,

Fig. 3. Electron micrograph to illustrate the nuclear region of a melanocyte. The melanocyte nucleus (MN) and melanosomes (MS) are indicated as is a pericyte (P) from an adjacent capillary. x 9,000

Fig. 4. Electron micrograph to indicate the differences between the wall of a capillary (C) and an afferent or efferent vessel (BV). Note the thickened basal lamina (BL) around each vessel and the flattened presumed smooth muscle cells (SM) in the wall of the large vessel. x 13,000

Fig. 5. Electron micrograph of a typical pecteneal capillary (C). Note the thickened basal lamina (BL) enclosing a pericyte (P). x 13,000

Fig. 6. Electron micrograph of a capillary to indicate the cell body (CB) and luminal (LF) and abluminal microfolds (AF). A melanocyte (M) is also indicated. x 13,000

these processes are felt to be microfolds rather than the finger-like structure implied by the term microvillus (Braekevelt, 1990, 1991a,b, 1993, 1994).

The microfolds on the luminal surface always appear to be longer, straighter and more numerous than on the abluminal aspect, perhaps indicating enhanced transport out of these capillaries. While some variation in the height of both luminal and abluminal microfolds is reported between species, it does not appear to correlate with overall pecten size as even the relatively small pecten of the nighthawk shows microfolds similar in height to those reported in relatively larger pectens such as the great blue heron, crow and galah (Braekevelt, 1984, 1991a, 1994). Also as the range of luminal microfold heights is very small (normally between 1.0 and 2.0 µm) this may represent the optimal height of microfolds or indicate the tallest microfolds that can adequately be supported by these endothelial cells.

While some of the earlier fine structural studies of the capillaries of the pecten stated that the endothelium was a syncytium (Seaman and Storm, 1963; Seaman, 1966) better fixation and resolution have shown that the endothelial cells are indeed distinct and are joined by large and often elaborate occludens type (tight) junctions (Dieterich et al., 1973; Meyer, 1977; Braekevelt, 1990, 1991a, 1993, 1994).

With most of the luminal surface area and cytoplasmic volume of the endothelial cells taken up by microfolds and the majority of cell organelles clustered in a paranuclear location, the entire morphology of these capillaries is indicative of vessels heavily involved in transport (Meyer, 1977). In addition the work of Welsch (1972) has shown a high alkaline phosphatase level within the pecten. Alkaline phosphatase is required for the active transport of materials across cell membranes and the extensive microfolding of the endothelial cells may also be present to subserve this function. Consequently, the pecten is probably heavily involved in both the passive diffusion of materials as well as the active transport of substances (Wingstrand and Munk, 1965; Bawa and Yash Roy, 1972; Welsch, 1972; Meyer, 1977).

Another striking feature of pecten capillaries is their unusually thick basal lamina as described in all species to date (Meyer, 1977; Braekevelt, 1988, 1990, 1991a,b, 1993, 1994). Such a thickened basal lamina may at first appear to be out of place associated with capillaries so obviously involved in transport functions. However, despite its overall thickness, the fibrillar layers of the basal lamina are not closely opposed and the entire structure probably does not offer a serious barrier to the movement of materials. It is felt that these thickened basal laminae may actually serve an important structural function as they support the «fragile» endothelial cells which have very thin cell bodies and numerous microfolds. The thicker basal laminae reported in the large pectens of the crow, heron, loon and pigeon (between 1.0 and 2.0 µm in thickness) as compared to the thinner basal lamina in the smaller pecten of the nighthawk (0.5 µm) and the intermediate thickness of the basal lamina in the mallard pecten (0.75 µm) which is also intermediate in overall relative size, may reflect this structural role (Braekevelt, 1984, 1986, 1988, 1990, 1991a, 1994).

Pericytes which are a common feature of the wall of both retinal and hyaloid capillaries are also present within the basal lamina of pecteneal capillaries (Ashton and de Oliveira, 1966; Braekevelt and Hollenberg, 1970; Jack, 1972). As is the case in other locations, the function of pericytes in the pecteneal capillaries is uncertain. They may be supportive or contractile in nature or perhaps represent reserve cells that could become endothelial cells as required.

Fine structural studies to date would seem to indicate that while the pleated pecten is widespread and essentially similar in most species, there is a variation in such parameters as shape, size, number of folds and the thickness of the capillary basal lamina (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991a, 1994). Further these variations seem to correlate with the diurnal activity and/or visual requirements of the species. Active visually oriented birds would thus have a relatively large and elaborate pecten while crepuscular and/or less visually active species would have a smaller less pleated pecten. The smaller size and fewer folds of the nighthawk (4-5 folds, Braekevelt, 1984) and great horned owl (7-8 folds, Braekevelt, 1993), the intermediate pecten of the dabbling mallard duck (12-14 folds, Braekevelt, 1990) as compared with the larger pecten of the diurnal and visually oriented great blue heron (14-15 folds, Braekevelt, 1991a), loon (14-15 folds; Braekevelt, 1986), pigeon (15-17 folds; Braekevelt, 1988), red-tailed hawk (17-18 folds, Braekevelt, 1991b) and crow (22-25 folds, Braekevelt, 1994) would further support this generalization. The large pecten with 20-25 folds reported here for the galah would indicate that the metabolic activity of the retina in this species is even higher than in the highly visually oriented redtailed hawk which only shows 17-18 folds within its pecten (Braekevelt, 1991b) and is comparable to that reported in the crow (Braekevelt, 1994). Further comparative studies are planned to establish the validity of these morphological observations.

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