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Fine structure of the pecten oculi in the great horned owl *(Bubo virginianus)*

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Summary. The pecten oculi of the great horned owl *(Bubo virginianus)* has been examined by light and electron microscopy. The pecten in this species is of the pleated type and is small in comparison to the size of the eyeball. It consists of 7-8 accordion folds which are joined apically by a pigmented bridge of tissue. Within each fold are numerous capillaries, larger supply and drainage vessels and plentiful pleomorphic melanocytes. The capillaries are extremely specialized vessels, most of which display plentiful microfolds on both their luminal and abluminal surfaces although some capillaries show but a few microfolds. The endothelial cell bodies are extremely thin with most organelles located near the nucleus. All capillaries are surrounded by a thick fibrillar basal lamina which is felt to be structurally important. Pericytes are a common feature within these thickened basal laminae. The numerous melanocytes form an incomplete sheath around the capillaries and are also presumed to be fulfilling a structural role. While the morphology of the pecten in the great horned owl is certainly indicative of a heavy involvement in transport, when compared to the pecten in species that are more visually oriented it is smaller, displays fewer folds and a reduced number of microfolds within the capillaries.

Key words: Pecten oculi, Electron microscopy, Great horned owl, *Bubo virginianus*

Introduction

In all vertebrates, while the choriocapillaris supplies the outer retina (photoreceptors and retinal pigment epithelium) there is always a second vascular system to supply the inner retina. This other vascular supply termed a supplemental nutritive device or SND (Walls, 1942) or a supplementary retinal circulation (Rodieck, 1973) can take several forms, appearing in the avian eye as the pecten oculi. The pecten forms a highly vascular and pigmented organ that projects as a fan-like structure out into the vitreous chamber from the head of the optic nerve (Walls, 1942; Michaelson, 1954; Prince, 1956).

The structure and function of the pecten has intrigued investigators for years and many often quite fanciful activities have been ascribed to this structure but the only function well based in fact is its role as a nutritive source for the inner region of the avascular avian retina (Walls, 1942; Wingstrand and Munk, 1965). Light and electron microscopic studies have revealed extremely specialized capillaries heavily involved in transport and substantiated its role as a nutritive organ (O'Rahilly and Meyer, 1961; Raviola and Raviola, 1967; Fielding, 1972; Dieterich et al., 1973; Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992).

As part of an ongoing comparative morphological study of the pecten oculi, this report describes the fine structure of the pecten in the great horned owl *(Bubo virginianus)* and compares and contrasts these findings with observations in other avian species.

Materials and methods

For this study both eyes from an adult, light-adapted great horned owl *(Bubo virginianus)* were examined by light and electron microscopy. With the bird under deep anaesthesia, the eyeballs were quickly enucleated, sliced open at the equator and 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 and washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and then pecten and the underlying retinal tissue was carefully dissected out. One pecten was left intact to allow for gross measurements while the other was further divided into pieces less than 1 mm^2. The tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer (pH 7.3) dehydrated up through graded ethanols to methanol and

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then propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently reoriented using a wax mount and both thick (0.5 μm) and thin (50-60 nm) sections were cut on an LKB ultramicrotome. Thick sections were stained with toluidine blue and examined by light microscopy. Thin sections were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM 201 transmission electron microscope.

Results

The pecten oculi in the great horned owl *(Bubo virginianus)* is as in all avian species situated over the oval-shaped head of the optic nerve. In this species the pecten is 5-6 mm long at its base, tapers to a length of about 3 mm at its apex or bridge and projects out into the vitreous chamber for 5-6 mm. The pecten itself consists of 7 or 8 pleats or accordion folds held together apically by a pigmented bridge of tissue. Each pleat or fold measures 25-30 μm in width and is composed of numerous melanocytes, many highly specialized capillaries and two or more larger blood vessels which are difficult to categorize as either arterioles or venules (Figs. 1, 8). A fine basal lamina continuous with the inner limiting membrane of the retina, encloses the entire pecten (Fig. 1).

The capillaries of the pecten are extremely specialized vessels measuring in most cases 10-12 μm in luminal diameter (Fig. 1) although larger capillaries measuring 15-20 μm are also common (Fig. 2). These capillaries show a vast array of processes on both their luminal (apical or internal) border as well as on their abluminal (basal or external) border (Figs. 1-4). Except for the nuclear region the endothelial cell body is a thin central area from which these numerous processes arise (Figs. 1, 2, 5, 7). These processes are microfolds rather than microvilli as they exhibit a range of widths when cut in different planes (Figs. 4, 7) and they are also seen to branch (Figs. 2, 3, 7). The luminal microfolds are typically longer and straighter at about 1.0 μm than the abluminal projections which are usually more compressed and tortuous and average about 0.7 μm in length (Figs. 2-5). While most capillaries show a wealth of these microfolds, particularly on the luminal surface, a number of capillaries show only very few projections (Figs. 4, 6).

The nuclear region of these endothelial cells is always the widest part of the cell body (Figs. 1, 2, 4) with the nucleus being typically large, vesicular and somewhat flattened (Figs. 1, 2). Most of the endothelial cell organelles are congregated in the perinuclear region although small mitochondria, polysomes and short profiles of rough endoplasmic reticulum (RER) are distributed throughout the cell body (Figs. 4-6). Microbodies are also widely distributed in these endothelial cells as are arrays of microfilaments although the later are often more abundant in the nuclear region (Figs. 2, 4, 6, 7). At least two and more usually three or four endothelial cells encircle the capillary lumen (Figs. 1, 2). The endothelial cells are joined by elaborate cell junctions of the occludens type (Figs. 4, 5, 7).

The basal lamina of these specialized pecteneal capillaries is very thick and averages about 1.0 μm in width (Figs. 1, 2, 5). This basal lamina consists of concentric incomplete layers of fine fibrillar material separated by an amorphous substance. The outermost layer of this thickened basal lamina has the appearance of a «regular» basal lamina but is separated from the endothelial cell body by several additional layers of fibrillar material (Figs. 2, 4, 6).

Pericytes are a common feature of these capillaries and are enclosed within the thickened basal lamina (Figs. 1, 3). These cells appear quite undifferentiated with a minimum of cell organelles and no microfolds on either surface (Figs. 1, 3). In this species the pericytes are normally separated from the endothelial cell abluminal folds by intervening basal lamina fibrillar material (Figs. 1, 3).

All blood vessels within the pecten above capillary size display essentially the same morphology and cannot be definitely categorized as either arterioles or venules (Fig. 8). The endothelium of these vessels is non-fenestrated and shows no microfolds on either border (Fig. 8). The endothelial cells measure at least 0.5 μm in thickness (in non-nuclear regions) and contain polysomes, microbodies and short profiles of RER (Fig. 8). As with the capillaries, the nuclear region is the thickest portion of the endothelial cell and these cells are joined by tight junctions. These vessels are surrounded by a thickened basal lamina within which are found flattened cells which have the morphology of smooth muscle cells (Fig. 8).

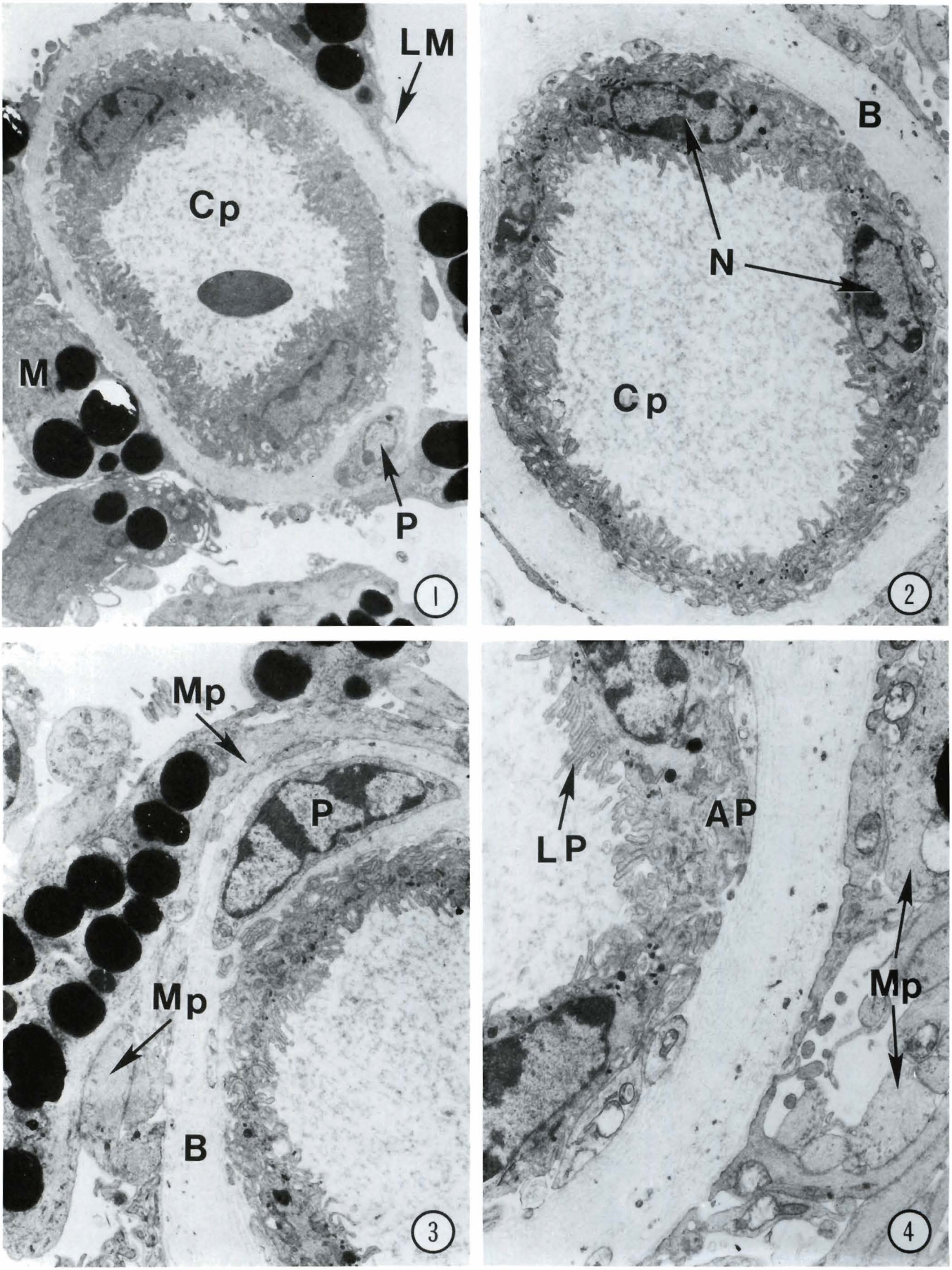
The melanocytes within the pecten are large pleomorphic cells with numerous long processes which form an incomplete covering around the capillaries and larger blood vessels and which at least semi-isolate the

Fig. 1. Electron micrograph of one capillary (CP) from the pecten. The limiting membrane (LM), melanocytes (M) and a pericyte (P) are all indicated. x 4,300

Fig. 2. Electron micrograph of a pecteneal capillary (Cp). Endothelial cell nuclei (N) and the thickened basal lamina (B) are indicated. x 5,800

Fig. 3. Electron micrograph to illustrate melanocyte processes (Mp), a pericyte (P) and the thickened basal lamina (B). x 6,600

Fig. 4. Electron micrograph to illustrate luminal (LP) and abluminal processes (AP). Note the microtubules and microfilaments in the melanocyte processes (Mp). x 9,900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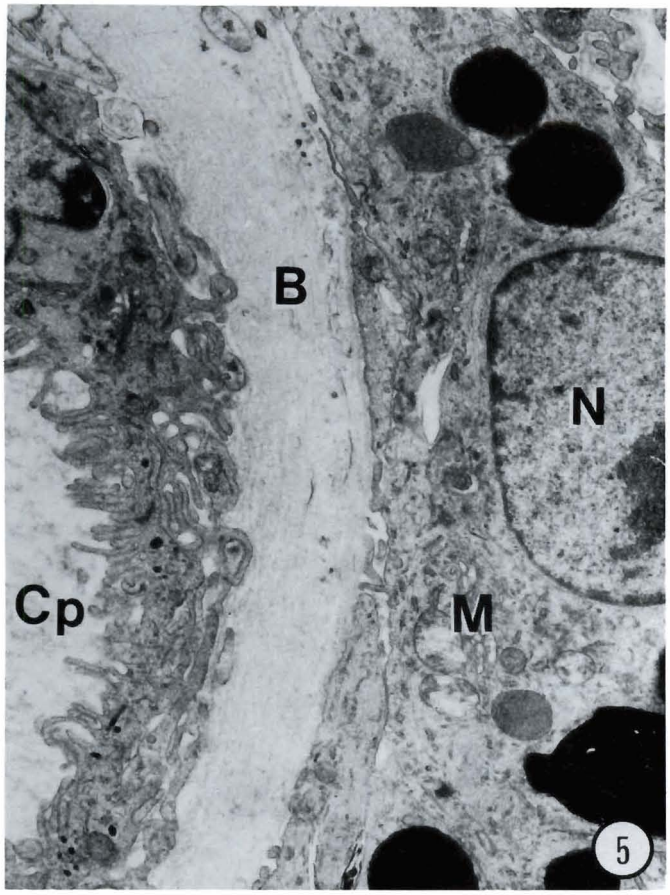
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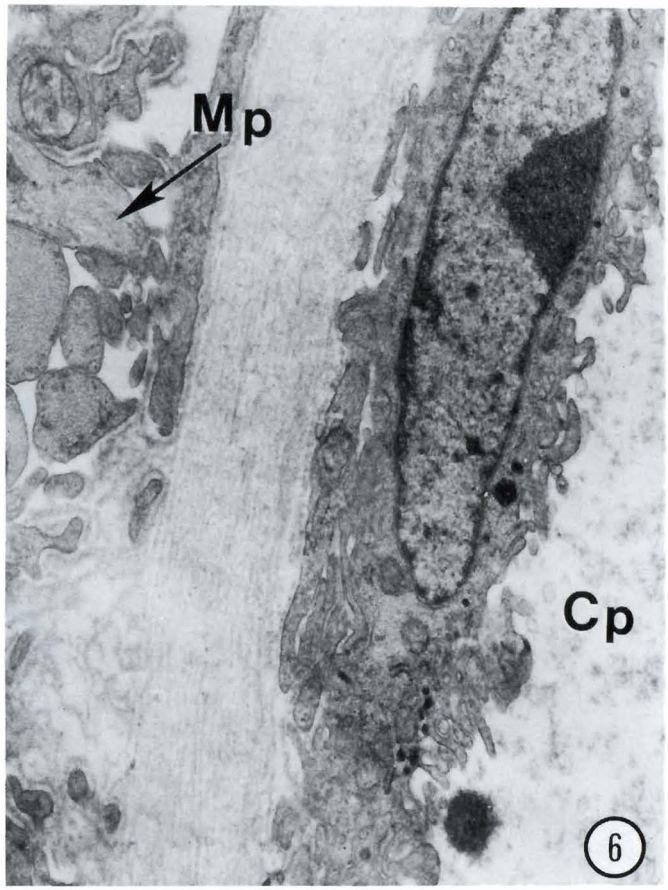
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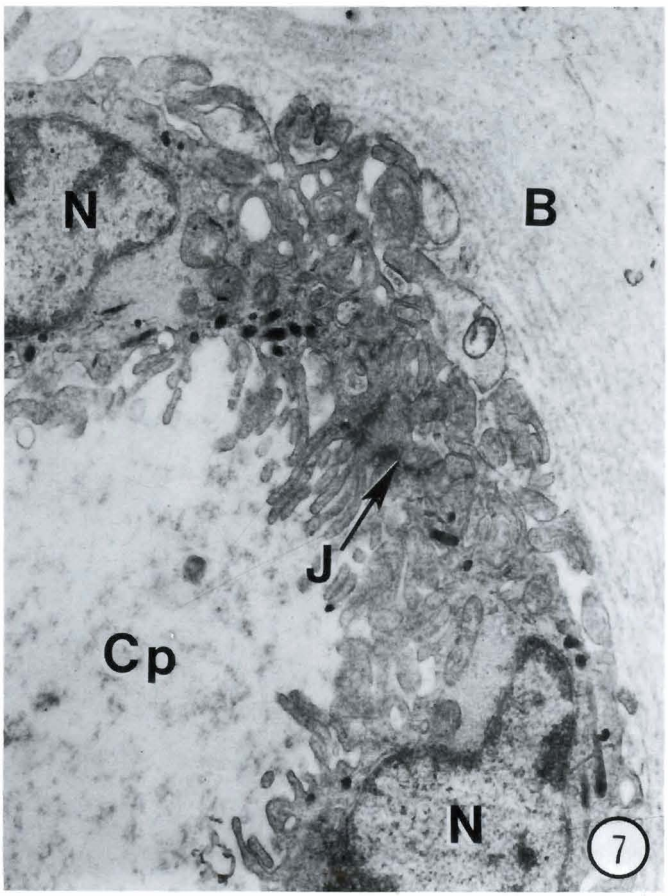
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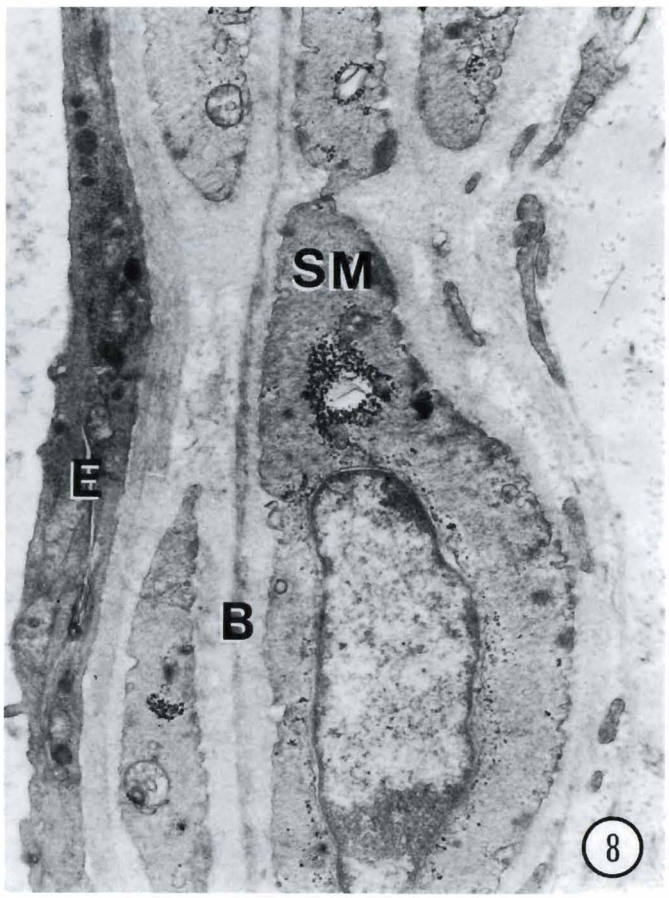
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Fig. 5. Electron micrograph to illustrate the nuclear region of a melanocyte (M). The melanocyte nucleus (N), the thickened basal lamina (B) and a capillary (Cp) are all indicated. x 10,300

Fig. 6. Electron micrograph of a capillary (Cp) which has a reduced number of luminal and abluminal processes. Note the microtubules and microfilaments in the melanocyte processes (Mp). x 14,375

Fig. 7. Electron micrograph of a pecteneal capillary (Cp) to indicate a cell junction (J), endothelial nuclei (N) and the thickened basal lamina (B). Note the microfilaments near each nucleus. x 15,000

Fig. 8. Electron micrograph of a supply or drainage vessel in the pecten. The endothelium (E), presumed smooth muscle cells (SM) and the basal lamina (B) are all indicated. x 15,000

capillaries from one another (Figs. 1, 3, 4, 6). The nuclei of melanocytes are large and vesicular and the majority of the cell organelles are located in the perinuclear region (Fig. 5). The melanosomes within these cells are about 2.0 μm in diameter and are normally round and very electron-dense (Figs. 1, 3, 5). Premelanosomes were not observed. Melanocyte processes smaller than 2.0 μm do not normally contain melanosomes but are rich in both microtubules and bundles of microfilaments (Figs. 3-6). While melanocytes are found throughout the pecten they are more plentiful at the periphery and at the apex or bridge of the pecten.

Discussion

The pecten oculi of the avian eye is normally classified into three morphologically distinct groups (Meyer, 1977). These are the 1) conical 2) vaned and 3) pleated pecten.

The conical pecten is a finger-like structure resembling the conus papillaris (an SND found in reptiles) and has only been reported to date in the kiwi *(Apternyx mantelli)* (Meyer, 1977). The vaned type of pecten consists of a central flattened pillar from which vertically-oriented vanes project. This type of pecten is reported in the large flightless ostrich and rhea (Walls, 1942; Meyer, 1977). The vast majority of birds however display the pleated type of pecten which consists of a varying number of accordion-like folds arranged in a fan-like configuration (Dieterich et al., 1973; Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992). The great horned owl *(Bubo virginianus)* also shows the pleated type of pecten with a smaller number of pleats or folds (7-8) than might be expected in such a relatively large species.

The pecten in the great horned owl is situated over the optic nerve head and a simple basal lamina or vitreo-pecteneal limiting membrane continuous with the inner limiting membrane of the retina covers the entire structure (Dieterich et al., 1973; Braekevelt, 1986, 1990). In some species, hyalocytes which are presumed to be phagocytic are noted adherent to the outer surface of this limiting membrane but they were not observed in the great horned owl (Semba, 1962; Braekevelt, 1988).

The pecteneal capillaries are extremely specialized vessels with a morphology unique in vertebrate vascularization (Fielding, 1972; Welsch, 1972; Hanzely et al., 1975; Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991,1992). The most pronounced feature of these capillaries is the presence of numerous long processes on both the luminal (apical or internal) and abluminal (basal or external) borders. While some ultrastructural studies have referred to these processes as microvilli (Nguyen et al., 1967) most have described them as microfolds (Dieterich et al., 1973; Meyer, 1977). In the great horned owl as in all other species studied by this author, these processes are felt to be microfolds rather than the finger-like shape implied by the term microvillus (Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992). The microfolds on the luminal surface always appear to be straighter, longer and more numerous than those on the abluminal surface, perhaps indicating enhanced transport out of these capillaries.

While the pecteneal capillaries of the great horned owl are in absolute terms rich in both luminal and abluminal folds they are comparatively less well endowed in microfolds than a number of other species (Braekevelt, 1986, 1988, 1990, 1991, 1992). Indeed some of the pecteneal capillaries in this species are almost devoid of microfolds and resemble the capillaries of the conus papillaris of reptiles (Nguyen, 1970, 1974; Braekevelt, 1989). The luminal and abluminal microfolds in this species are however comparable in length to that observed in other avian species (Braekevelt, 1988, 1990, 1991, 1992). Also as in other species the luminal microfolds are longer and less tortuous in shape than the abluminal microfolds.

Some of the earlier fine structural studies of pecteneal capillaries reported that the endothelium was a syncytium (Seaman and Storm, 1963; Seaman, 1966; Fielding, 1972). Better fixation has however shown that the endothelial cells are indeed distinct cells joined by often very elaborate occludens (tight) junctions (Raviola and Raviola, 1967; Dieterich et al., 1973; Meyer, 1977; Braekevelt, 1988, 1990, 1991, 1992).

With most of the area of the endothelial cell taken up by microfolds, reducing the cell body area to a thin central region and with the majority of cell organelles clustered in a paranuclear location, the entire morphology of these capillaries is indicative of vessels heavily involved in transport (Raviola and Raviola, 1967; Meyer, 1977). In addition the work of Welsch (1972) has shown a high level of alkaline phosphatase which is required in the active tranport of materials

across cell membranes and the extensive microfolds may also be present to subserve the alkaline phosphatase system. The pecten therefore appears to be heavily implicated in both the passive diffusion and the active transport of materials (Wingstrand and Munk, 1965; Bawa and Yash Roy, 1972; Welsch, 1972; Meyer, 1977).

The basal lamina of the pecten capillaries is unusually thick in all species described to date (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992). Such a thickened basal lamina might appear to be out of place in capillaries so obviously involved in transport functions. Despite its overall thickness however the fibrillar material of the basal lamina is not closely packed and the entire structure would not appear to represent a serious barrier to the movement of materials. The thickened basal lamina of these capillaries may however be required to serve an important structural function as they support the «fragile» endothelial cells with their thin cell bodies and numerous processes. The thicker basal laminae found in the larger pectens of the great blue heron and loon for instance (1.0-2.0 μm in thickness) as compared to the thinner basal laminae in the smaller pecten of the nighthawk (0.5 μm) and the intermediate thickness of the basal laminae in the mallard pecten (0.75 μm) which is also intermediate in overall size may reflect this structural role (Braekevelt, 1984, 1986, 1990, 1991). The thickness of the basal laminae in the great horned owl is reported at about 1.0 μm. From a comparative standpoint the pecten in this species is small when compared to the huge eye in which it is situated. In absolute terms however this is a fairly large pecten and probably requires this thicker basal lamina for support.

Pericytes which are a common and constant feature of the wall of both retinal and hyaloid capillaries are also present in the wall of pecteneal capillaries (Ashton and de Oliveira, 1966; Braekevelt and Hollenberg, 1970; Jack, 1972). The function of these cells is uncertain and they may be supportive or contractile in nature or perhaps reserve cells that could become endothelial cells as required.

In addition to the numerous specialized capillaries, within each fold of the pecten are situated larger supply (afferent) and drainage (efferent) vessels. Unlike the condition reported in the chicken (Dieterich et al., 1973) and the pigeon (Raviola and Raviola, 1967) where the authors described arterioles and venules, in the great horned owl as in all previous studies of other species by this author it is very difficult if not impossible to adequately differentiate the larger vessels of the pecten as to being either arterioles or venules (Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992). This apparent lack of structural differences between most of these supply and drainage vessels of the pecten may indicate a lowered blood pressure within the pecten again conducive to transport functions.

The presence of pigmented cells is a constant feature of all pectens described (Walls, 1942; Fischlschweiger and O'Rahilly, 1966; Fielding, 1972; Meyer, 1977; Braekevelt, 1988, 1990, 1991, 1992). As no other cell types are present within the pecten to act as supportive elements, it is felt that these melanocytes at least in part fulfil a structural function. The presence of numerous microtubules and microfilaments within the processes of these cells would tend to support this view (Braekevelt, 1986, 1988, 1990, 1991, 1992). The bundles of microfilaments typically found in the endothelial cells of the capillaries are likewise felt to be structurally important. While no statistical studies have as yet been attempted it does appear that the quantity of microfilaments and microtubules increases as pecten size increases. The absorption of light by the melanosomes of these cells also probably raises the temperature of the pecten and hence the rate of metabolic processes such as transport (Bawa and Yash Roy, 1974). The enhanced number of melanocytes in the bridge region and periphery of the pecten might be there to subserve this function.

Studies to date would seem to indicate that while the pleated pecten is widespread and essentially similar in most species, there are variations in such parameters as shape, size, number of folds, number of microfolds on the capillary endothelial cells and the thickness of capillary basal laminal (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992). Further these variations seem to correlate with the diurnal activity and/or visual requirement of the species. Active visually oriented birds would thus have a relatively larger and more elaborate (enhanced number of folds and microfolds) pecten while more crepuscular or less visually oriented species would have a smaller, simpler pecten (Walls, 1942; Meyer, 1977). The smaller size and fewer folds of the crepuscular nighthawk (4-5 folds) and nocturnal great horned owl (7-8 folds) as compared to the intermediate number of folds in the dabbling mallard duck (12-14 folds) and the larger pectens of the great blue heron (14-15 folds) and loon (14-15 folds), the strongly diurnal pigeon (15-17 folds) and the highly visually oriented red-tailed hawk (17-18 folds) and American crow (22-25 folds - unpublished observations) would tend to support this generalization (Braekevelt, 1984, 1986, 1988, 1990, 1991, 1992).

In conclusion while the elaborate folding, heavy pigmentation and rich vascularization of the pecten has lead to a number of theoretical functions, the only proven role of the pecten is that of a supplemental nutritive device or SND (Walls, 1942) or supplementary retinal circulation (Wingstrand and Munk, 1965; Rodieck, 1973). As such the pecten oculi can be considered to be comparable to the falciform process of some teleosts, the conus papillaris of reptiles, the supraretinal or vitreal vessels of amphibians and some teleosts and the intraretinal vessels of mammals (Walls, 1942; Michaelson, 1954; Duke-Elder, 1958; Nguyen, 1974; Braekevelt, 1988, 1989, 1990, 1991, 1992). From an optical standpoint the placing of a SND over the blind spot of the retina (optic nerve head) probably interferes the least with vision and may explain why avian species have developed the pecten in that location.

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