Histol Histopath (1991) 6: 345-351

Histology and Histopathology

Electron microscopic observations on the pecten of the great blue heron (*Ardea Herodias*)

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Summary. The pecten oculi of the great blue heron *(Ardea herodias)* has been examined by both light and electron microscopy. In this species the pecten is large and of the pleated type. It consists of 14-15 acordion folds that are joined apically by a more heavily pigmented bridge of tissue which holds the pecten in a fan-like shape widest at its base. As in other species it is situated over the optic nerve head and projects out into the vitreous. Within each fold are numerous capillaries, larger supply and drainage vessels and many melanocytes. The capillaries are extremely specialized vessels which display extensive microfolds on both their luminal and abluminal borders. The endothelial cell bodies are extremely thin with most organelles present in a paranuclear location. The capillaries are surrounded by thick fibrillar basal laminae which are felt to be structurally useful. Pericytes are a common feature of these capillaries. The numerous pleomorphic melanocytes which form an incomplete sheath around the capillaries and other blood vessels are also felt to be important in structural support of the pecten. The morphology of the pecten of the great blue heron is indicative of a heavy involvement in the transport of materials.

Key words: Pecten oculi, Electron microscopy, Bird, *(Ardea herodias)*

Introduction

In the vertebrate eye, the outer retina (which includes the photoreceptors and retinal pigment epithelium) is supplied by the large-caliber, fenestrated capillaries of the choriocapillaris. In most vertebrates, a second vascular system is present to supply the inner retina. This other vascular supply, termed a supplementary retinal circulation (Rodieck, 1978) can take several forms and in birds appears as the pecten oculi. It is a highly vascular and pigmented organ that projects from the optic nerve head out into the vitreus chamber (Michaelson, 1954; Prince, 1956).

Histological studies of the pecten have revealed its very vascular structure and while numerous secondary functions have also been ascribed to it, the primary role was felt to be nutritive to the inner region of the avascular avian retina (Slonaker, 1918; Mann, 1924; Tanaka, 1938; O'Rahilly and Meyer, 1961). Fine structural studies have shown an extremely specialized capillary morphology and substantiated its role as a nutritive organ (Seaman, 1966; Raviola and Raviola, 1967; Fielding, 1972; Dieterich et al., 1973; Braekevelt, 1984, 1986, 1988, 1990).

As part of a comparative study of the supplementary retinal circulation and the pecten oculi in particular, this report describes the fine structure of the pecten of the great blue heron *(Ardea herodias)* and compares these findings to observations from other avian species.

Materials and methods

For this study both eyes from an adult, light-adapted great blue heron *(Ardea herodias)* were examined by light and electron microscopy. With the bird under deep anesthesia, the eyeballs were quickly enucleated, opened at the equator and fixed for 5 h in 5% gluteraldehyde buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorenson's buffer (pH 7.3) and the pecten and its underlying retinal tissue was carefully dissected out. One pecten was left intact while the other was further divided into pieces less than 1 mm^2. The tissue was then further postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer (pH 7.3), dehydrated through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

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Pieces of plastic-embedded tissue were then reorientated to desired angles by means of a wax mount and both thick (0.5 μm) and thin (50-60 mm) sections were cut on an LKB ultramicrotome. Thick sections were stained with toluidine blue and examined by light microscopy. Thin sections of selected areas were stained in aqueous uranyl acetate and lead citrate and examined and photographed on a Philips EM201 transmission electron microscope.

Results

As in all avian species described to date, the pecten of the great blue heron is situated over the oval-shaped head of the optic nerve and projects free into the vitreous. In this species the pecten consists of 14-15 pleats or accordion-folds held together apically by a more heavily pigmented bridge of tissue. The pecten is 8-10 mm wide at its base, narrows to 4-5 mm at its apex or bridge and projects for about 5 mm out into the vitreus chamber.

Each pleat or fold consists of numerous specialized capillaries, two or more larger blood vessels which are difficult to differentiate as to either arterioles or venules and numerous intervening melanocytes (Fig. 1). A fine basal lamina continuous with the inner limiting membrane of the retina encloses the entire pecten (Fig. 1).

The melanocytes of the pecten are large pleomorphic cells with long processes which form an incomplete covering around the capillaries but which more or less isolate the capillaries from one another (Figs. 1, 2, 3). The nuclei of the melanocytes are large and vesicular with isolated clumps of heterochromatin (Figs. 1, 2). The round and extremely electron-dense melanosomes are mostly located in the perikaryon region (Figs. 1, 2). Most melanosomes are about 1.0 to 1.5 μm in diameter and melanocyte processes smaller than this seldom show melanosomes (Fig. 2). Premelanosomes were not observed. While polysomes and small profiles of rough endoplasmic reticulum are scattered throughout the melanocyte processes, most organelles are also located in a perinuclear location (Figs. 2, 3). The melanocyte processes are rich in both microtubules and microfilaments (Figs. 2, 3, 8). Melanocytes are most numerous in the bridge region but are found throughout the pecten (Fig. 1).

The capillaries of the pecten are extremely specialized vessels which show an abundance of processes on both their luminal (apical or internal) and abluminal (basal or external) borders (Figs. 3, 4, 5, 7). In many locations the actual cell body is only a thin central area (0.30 to 0.50 μm in width) from which these numerous processes arise (Figs. 4, 6, 7). 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, 7) and they are also seen to branch (Figs. 4, 7). The luminal microfolds are usually somewhat deeper and straighter at 1.0 to 1.5 μm than the abluminal projections which range from 0.75 to 1.0 μm and are usually more compressed and tortuous (Figs. 3-5).

The nuclear region of the capillary endothelial cells is always the widest portion of the cell body (Figs. 2, 3, 5). The nucleus is usually large, quite vesicular and somewhat flattened (Figs. 2-4). Most of the endothelial cell organelles are located in the perinuclear region although small mitochondria, microbodies, polysomes and bundles of microfilaments are found throughout the cell (Figs. 4, 5, 7). A pair of centrioles is often found near the nucleus (Fig. 5). At least two and often three or more endothelial cells encircle a capillary lumen (Figs. 1, 2). The endothelial cells are joined by elaborate and often extensive cell junctions of the occludens types (Figs. 4, 6, 7).

The basal lamina of these capillaries is very thick, averaging about 1.0 μm in thickness (Figs. 2-5). This basal lamina consists of concentric layers of fine fibrillar material separated by an amorphous material (Figs. 2, 4, 6). The outermost layer of this thickened basal lamina has the appearance of a «regular» basal lamina and is separated from the endothelial cell body by several layers of fibrous material (Figs. 4, 6, 7).

Pericytes are often enclosed within the thickened basal lamina of these capillaries (Figs. 1, 2, 4, 6). These cells appear quite undifferentiated with a minimum of cell organelles and no microfolds (Figs. 2, 4). Occasionally these pericytes will display melanosomes within their cytoplasm (Fig. 6). The pericytes may either be separated from the endothelial cells by basal lamina material (Figs. 2, 6) or be in apparent contact with the abluminal folds of the endothelial cells (Fig. 4).

All of the larger blood vessels (i.e. above capillary size) of the pecten have essentially the same morphology and cannot be definitely categorized as either arterioles or venules (Figs. 1, 6, 8). The endothelium of these vessels is non-fenestrated and shows no microfolds on either border (Figs. 6, 8). The endothelial cells are however rich in microfilaments, polysomes and microbodies (Figs. 6, 8). As with the capillaries the nuclear region is the thickest portion of the endothelial cell (Figs. 1, 8). These vessels are surrounded by a thickened, fibrous basal lamina similar to that around the capillaries (Figs. 1, 6, 8). Within this thickened basal lamina are normally found flattened cells which have the morphology of smooth muscle fibers (Figs. 6, 8).

Discussion

The pecten oculi of the avian eye has been classified into three morphologically different types by 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 of reptiles and to date has only been reported in the kiwi *Apterynx mantelli* (Meyer, 1977). The vaned type consists of a central flattened pillar from which vertically-oriented vanes

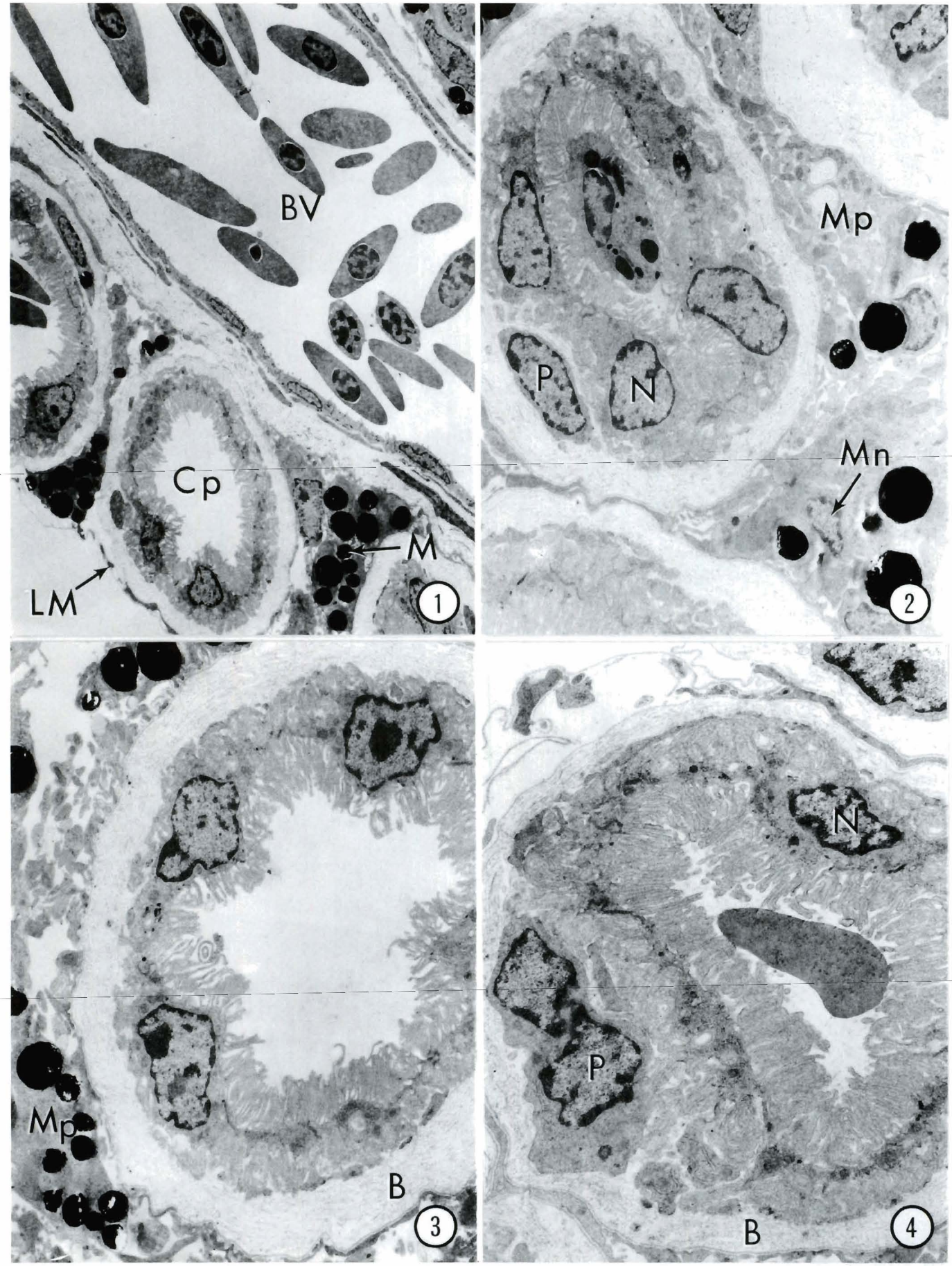

Fig. 1. Low power electron micrograph of one fold of the pecten of the great blue heron. A capillary (Cp), a larger blood vessel (BV), a melanocyte (M) and the limiting membrane (LM) are all indicated. × 4,300

Fig. 2. Electron micrograph to illustrate numerous melanocyte processes (Mp), a melanocyte nucleus (Mn), an endothelial nucleus (N) and a pericyte (P). × 6,000

Fig. 3. Electron micrograph to indicate the thickened basal lamina (B) and melanocyte process (Mp) × 6,000

Fig. 4. Electron micrograph to indicate the basal lamina (B), a pericyte (P) and an endothelial cell nucleus (N). × 8,900

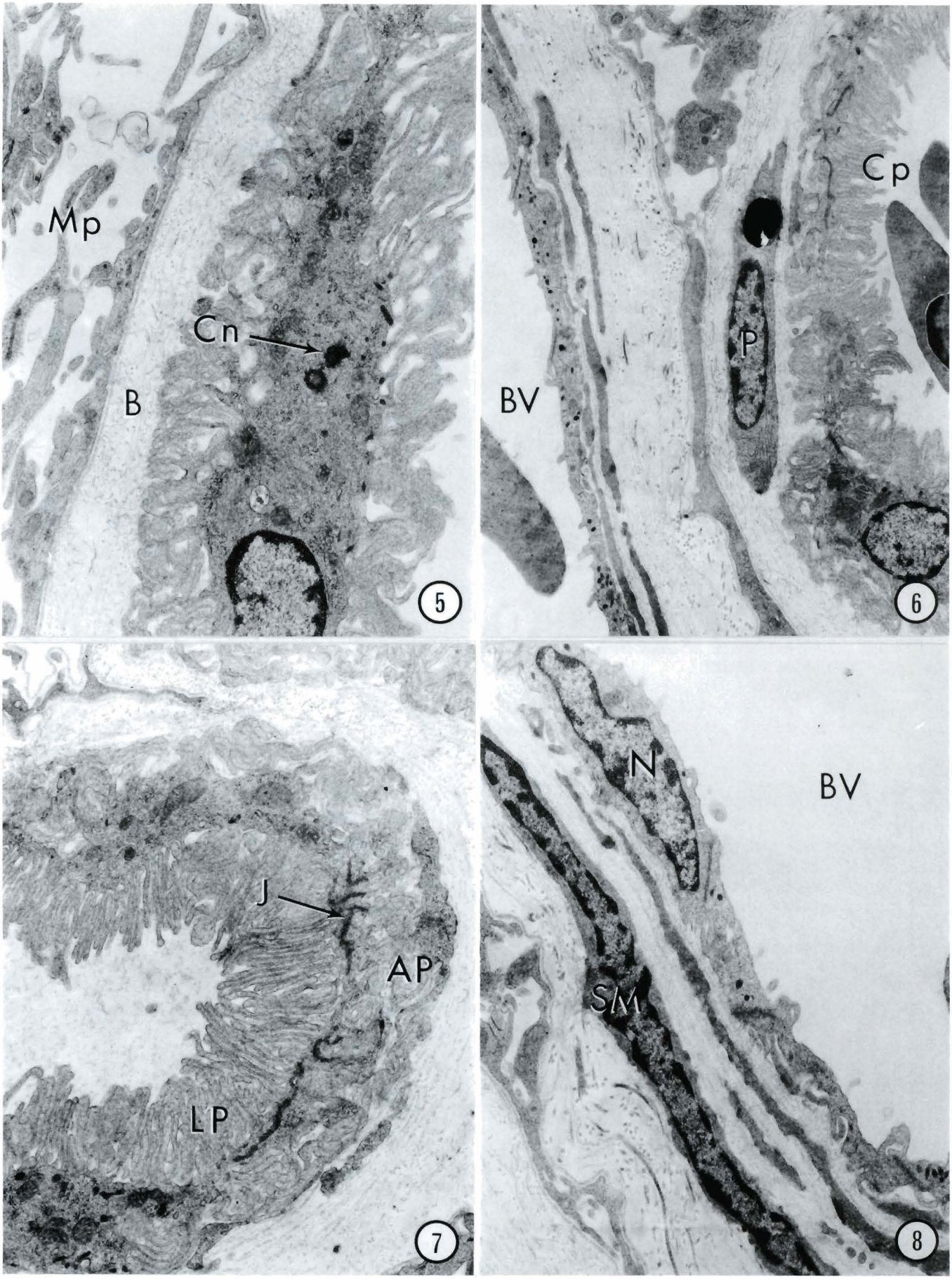

Fig. 5. Electron micrograph to illustrate the paranuclear region of an endothelial cell. Centrioles (Cn), the basal lamina (B) and melanocyte processes (Mp) are well indicated × 8,900

Fig. 6. Electron micrograph to indicate a capillary (Cp) and an adjacent larger blood vessel (BV). A pigmented pericyte (P) is also indicated × 12,900

Fig. 7. Electron micrograph of a pecten capillary to illustrate the extensively luminal (LP) and abluminal processes (AP). A cell junction (J) is also indicated. × 12,900

Fig. 8. Electron micrograph of the wall of a larger blood vessel (BV). An endothelial cell nucleus (N) and a presumed smooth muscle cell (SM) are indicated. × 12,900

arise. This type of pecten is found in ostriches and rheas (Walls, 1942; Meyer, 1977). The pleated pecten is by far the most common type and with variations is apparently found in all other birds (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990). The great blue heron (*Ardea herodias*) displays a pleated type of pecten situated over the optic nerve head.

A simple basal lamina or vitreo-pectineal limiting membrane continuous with the inner limiting membrane of the retina covers the entire pecten (Dieterich et al., 1973; Braekevelt, 1986, 1988, 1990). In some species, hyalocytes are regularly noted adherent to the outer surface of this membrane (Semba, 1962; Braekevelt, 1990). In most species including the great blue heron however these hyalocytes have not been reported (Braekevelt, 1984, 1986, 1988). While the function of these hyalocytes (when present) is unknown, they display a morphology indicative of phagocytes and appear to be ameboid in nature (Braekevelt, 1990).

Within the folds of the pecten are found numerous specialized capillaries, supply (afferent) and drainage (efferent) vessels and many large branching melanocytes. 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 describe arterioles and venules, in the great blue heron as in all previous studies of other species by this author, it is very difficult if not impossible to adequately categorize these larger vessels of the pecten as to being either arterioles or venules (Braekevelt, 1984, 1986, 1988, 1990). This apparent lack of structural difference between most of these supply and drainage vessels within the body of the pecten may indicate a lowered blood pressure within the pecten.

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, 1984, 1986, 1988, 1990). 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 role. The presence of numerous microtubules and microfilaments within the processes of these cells would tend to support this view (Braekevelt, 1986, 1988, 1990). In addition the absorption of light by the pigment of these cells probably raises the temperature of the pecten and hence the rate of metabolic reactions within it (Bawa and Yash Roy, 1974). The raised number of melanocytes in the bridge region and periphery of the pecten might be so placed as to subserve this function.

The capillaries within the avian pecten are extremely specialized vessels with a morphology unparalleled in vertebrate vascularization (Tanaka, 1960; Seaman and Storm, 1963; Fielding, 1972; Welsch, 1972; Hanzley et al., 1979; Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990). The most obvious feature of the endothelial cells 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 them as microvilli (Nguyen et al., 1967) most others have referred to them as microfolds (Dieterich et al., 1973; Meyer, 1977). In the great blue heron as in other species studied by this author these processes are felt to be microfolds rather than the finger-like shape implied by the term microvilli (Braekevelt, 1984, 1986, 1988, 1990). The microfolds on the luminal surface also always appear to be straighter and more numerous than on the abluminal edge, perhaps indicating enhanced transport out of the capillaries.

While some of the earlier fine structural studies of these capillaries stated that the endothelium was a syncytium, (Seaman and Storm, 1963; Seaman, 1966; Fielding, 1972) better fixation has shown that the endothelial cells are indeed distinct and joined by elaborate occludens type (tight) junctions (Dieterich et al., 1973; Meyer, 1977; Braekevelt, 1988, 1990).

With most of the area of the endothelial cell 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 (Raviola and Raviola, 1967; Meyer, 1977). In addition the work of Welsch (1972) has shown a highly alkaline phosphatase level in the pecten. Alkaline phosphatase is required in the active transport of materials across cell membranes and the extensive microfolds of the endothelial cells may also be present to subserve the alkaline phosphatase system. The pecten therefore appears to be heavily implicated in both the passive diffusion of materials as well as the active transport of substances (Wingstrand and Munk, 1964; Welsch, 1972; Bawa and Yash Roy, 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). Such a thickened basal lamina might appear to be out of place with capillaries so obviously involved in transport functions. However despite its overall thickness, the fibrillar material of the basal lamina is not closely packed and the entire structure would not appear to offer a serious barrier to the movement of materials. This thickened basal lamina may actually serve an important structural function as they support the «fragile» endothelial cells which have very thin cell bodies and numerous processes. The thicker basal laminae found in the larger pecten of the great blue heron, red-tailed hawk, loon and pigeon (1.0 - 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 size, may reflect this structural role (Braekevelt, 1984, 1986, 1988, 1990).

Pericytes which are a common and constant feature of the wall of both retinal and hyaloid capillaries are also present in the walls 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.

Studies to date would seem to indicate that while the pleated pecten is widespread and is essentially similar in most species, there is a variation in such parameters as shape, size, number of folds and the thickness of capillary basal laminae (Meyer, 1977; Braekevelt, 1984, 1986, 1988, 1990). 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 larger and elaborate pecten while more crepuscular or less visually oriented species would have a smaller, simpler pecten. The smaller size and fewer folds of the crepuscular nighthawk (4-5 folds) and the larger pecten 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) would tend to support this generalization (Meyer, 1977). Further comparative studies are however necessary to establish the validity of this hypothesis.

A large number of sometimes quite fanciful functions have been ascribed to the avian pecten in addition to its role as a supplemental nutritive device (SND) (Walls, 1942; Wingstrand and Munk, 1965; Meyer, 1977). While the elaborate folding and heavy pigmentation have usually been the bases for most of these theories, the only proven function of the pecten is still that of a nutritional source. As such the pecten appears to be comparable to the falciform process of some teleosts, the conus papillaris of reptiles, the supraretinal or vitreal vessels of amphibians and teleosts and the intraretinal vessels of mammals which are all felt to be alternative methods of bringing nutrients to an avascular inner retina (Walls, 1942; Duke-Elder, 1958; Nguyen, 1974; Braekevelt, 1988, 1990). From an architectural and optical standpoint, the placing of a supplemental nutritive device (SND) over the blind spot of the retina (optic nerve head) may be preferable as it probably interferes less with vision than say the intraretinal vessels of mammals.

Acknowledgements. Thanks are extended to Renatta Scriven for supplying the great blue heron used in this study. The excellent technical assistance of D.M. Love is also gratefully acknowledged. This work was supported in part by funds from the Natural Sciences and Engineering Research Council (NSERC) and the Medical Research Council (MRC) of Canada.

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Accepted February 2, 1991