Introduction

The pecten oculi is a unique vascular and pigmented intraocular structure characteristic of the avian eye. Since the first correct description and interpretation made by Perrrault in 1676 (Sillman, 1973), this intraocular structure has been the subject of investigation from various aspects. Through experimental manipulation, analogy and hypothesis, researchers have endeavored to assign plausible functions to this structure. These functions include: (1) intraocular pressure regulation (Seaman and Himelfarb, 1963), (2) intraocular pH regulation (Brach, 1975), (3) stabilization of the vitreous body (Tucker, 1975), (4) reduction of intraocular glare (Barlow and Ostwald, 1972), (5) a blood and fluid barrier for the retina and vitreous body (Rodriguez-Peralta, 1975), and, above all, (6) a supplemental nutritive organ (Mann, 1924; Meyer, 1977), a function which has been equivocally accepted by contemporary researchers. Grossly, pectin oculi can be categorized into three types: the conical type reported in the kiwi, the vaned type described in the ostrich, and the pleated type found in most birds (Meyer, 1977; Martin, 1985). The pleated form of pecten oculi has been most thoroughly investigated and is comprised primarily of an extensive network of blood vessels with intervening pigmented tissue. It arises from the optic disk in the form of accordion pleats held together at the free apical border by a heavily pigmented bridge (Seaman and Storm, 1963; Raviola and Raviola, 1967). Considerable variation in the number of pleats and in the size and shape of the pectin oculi exists in the pleated form of pecten within the avian species (Thomson, 1929). These variations are believed to be related to the behavior of birds in relation to their general activity and visual pattern (Braekevelt, 1988). Although the vascular framework of pecten has been studied by flat-mounting following its severance from the underlying optic disk and apical bridge (Tanaka, 1938), routine histology (Bawa and Roy, 1974) and transmission electron microscopy (TEM) (Seaman and Storm, 1963; Raviola and Raviola, 1967).
the true framework of pecten, vis-a-vis the tissue components comprising the intraocular structure in birds, has remained elusive.

Light and transmission electron microscopic studies have shown that the pecten oculi consists almost exclusively of blood vessels, extravascular pigmented cells and a superficial covering membrane (Meyer, 1977; Martin, 1985) that lacks both muscular and nervous tissue (Meyer, 1977). The pecteneal blood vessels consist of arterioles, venules and highly specialized capillaries (Raviola and Raviola, 1967) that freely anastomose with each other (Dieterich et al., 1973; Hossler and Olson, 1984; Matusnaga and Amemiya, 1990). The area between the vessels is partly filled with pleomorphic pigment cells described as melanocytes (Braekevelt, 1984) containing melanin granules (Raviola and Raviola, 1967). As part of an ongoing comparative study of the supplementary retinal circulation and the pecten oculi in particular, this report attempts to elucidate the organization of the pecten oculi of the Japanese jungle crow (Corvus macrorhynchos), and compares and contrasts these findings with observations from other avian species.

Materials and Methods

Collection of the sample

Japanese jungle crows (Corvus macrorhynchos) of either sex were collected by trapping in the city of Niiza, Saitama Prefecture. A total of nine normal jungle crows with an average weight of 840 g were anesthetized by injection of pentobarbitone sodium (30 mg/kg) and perfused through the heart with warm (35°C) physiological saline. This was followed by perfusion fixation with 1 L of Ringer solution (0.9% NaCl, 0.042% KCl, 0.025% CaCl₂ in distilled water, wt/vol) and 1 L of Zamboni fixative (0.1 M phosphate buffer [pH 7.4] containing 2.0% paraformaldehyde and 0.2% picric acid, wt/vol), before which the right auricle was cut open for drainage. After cessation of respiration and the heartbeat, the eyeballs were removed from the orbital cavities. The cornea and lens were excised from the eyeball. The posterior half of the eyeball was then removed, and the vitreous body was washed carefully and immersed briefly in the Zamboni solution. The pecten oculi was carefully dissected out and cut into smaller pieces for light and electron microscopy processing. The pecten was examined in both eyes, and as fixation was judged to be good and no differences were noted between the two eyes, the sample was regarded as valid.

Light microscopy study

Hematoxylin and Eosin (H&E) and Azan staining

Intact fixed pectens were dehydrated, cleared using xylene, infiltrated in liquid paraffin and embedded in wax. The embedded tissues were mounted on wooden blocks and 7-μm-thick sections were cut using a microtome. The sections were stained using hematoxylin and eosin and viewed, and photographed under a light microscope. For Azan staining, the sections were deparaffinized and shaken in azocarmine G solution (40°C) for 30 minutes. The samples were treated in aniline blue orange G. After dehydration and clearing, the coverslip was mounted using Canada balsam (MGK-S; Matsunami Glass Ind., Kishiwada, Osaka, Japan).

Immunohistochemistry for determination of the type of collagen

The sections containing pecten were deparaffinized using xylene and then heated in an autoclave at 121 °C for 20 minutes in citrate buffer (pH 7.4) to retrieve antigenicity (Shi et al., 1993). The sections were then cooled to room temperature and rinsed with phosphate-buffered saline (PBS; pH 7.4, 0.01 mol/L). To reduce background staining, the sections were pre-incubated with 3% hydrogen peroxide (H₂O₂) in methanol and then in 10% normal goat serum in PBS, for 30 min each. Rabbit polyclonal anti-chicken collagen 1 antibody (AbD Serotec, Oxford, UK) was used at a concentration of 0.1 mg/mL. The specificity of this antibody for chick embryo was confirmed in a previous study (Mauger et al., 1982). The sections were incubated with anti-chicken collagen 1 antibody at 4°C for 18 h. After they were rinsed in PBS, all of the sections were incubated with a goat anti-rabbit antibody (ICN Pharmaceuticals, Costa Mesa, CA, USA) diluted 1:500. Immunoreactions were visualized by incubating the sections in Tris-HCL buffer containing 0.02% 3’, 3’-diaminobenzidine (Dojin Laboratories, Kumamoto, Japan), 0.3% ammonium nickel (II) sulfate hexahydrate and 0.015% hydrogen peroxidase for 10 min and then dehydrating them in an ascending alcohol series and clearing them with xylene.

Transmission electron microscopic study

For transmission electron microscopy, the pecten samples were sliced into 2 × 2 mm samples. The samples were fixed in 1% osmic acid solution for 1 hr, and then immersed in 2.5% glutaraldehyde for 1 hr. Then, the samples were dehydrated with ethanol and rinsed in 90% ethanol: 90% acetone (1:1 v/v), followed by 90%, 95% and 100% acetone. The samples were then immersed in Epon 812 with absolute acetone (1:1 v/v, overnight), followed by Epon 812 (overnight). The immersion in Epon 812 was repeated twice under vacuum in a desiccator for
3–4 hrs. The samples were placed into molds, and the blocks were cured in an oven at 60°C for 24 hr. Ultrathin sections (70-nm thick) were cut using an Ultractut S ultramicrotome (Reichert-Jung Optische Werke AG, Wien, Austria) equipped with a diamond knife (Diatome AG, Biel, Switzerland). Sections were placed on a 200-mesh copper grid, stained with 2% uranyl acetate in 50% ethanol, and post-stained with 0.2% lead citrate. The sections were observed using a transmission electron microscope (H-800; HITACHI, Tokyo, Japan) under an accelerating voltage of 100 kV at a magnification of ×3,000–6,000.

To calibrate the images, the resulting micrographs were scanned at 300 d.p.i. using an Epson GT X-700 flatbed scanner.

Results

The pecten of the crow was a trapezoid fan-like intraocular structure and was black or brown in color (Fig. 1). The base of the trapezoid inserted upon the head and the cauda of the optic nerve, and the rest projected into the vitreous body. It consisted of a pleated lamina, 120 to 348 µm in thickness, whose sinuous course was fixed distally by its attachment to a compact cord of pigmented tissue, “the bridge,” running like a handrail along the free margin of the organ. The pectineal pleats were separated by alternating depressions, and the base was wider than the apical end (Fig. 1). The number of pleats varied from 24 to 25, one or two of which did not reach the distal edge and merge with the dorso-temporal side of the trapezoid (Fig. 1). The pecten looked like a thin, pleated lamina in a transverse section. The pleats of the pecten appeared in the form of an accordion pattern. Abundant blood capillaries and a few afferent and efferent blood vessels were observed (Fig. 2). The capillaries were generally arranged in two layers and surrounded the larger vessels (Fig. 2). The larger vessels and capillaries were provided with a thick endothelium and a prominent adventitial coat. The lumen of the vessels was occupied by numerous erythrocytes (Figs. 3, 4). Cells containing granules of melanin occupied the grooves between the vessels, and thus smoothed out the surface of the lamina (Figs. 2, 3, 4). The bridge was crescent-shaped in vertical sections of the pecten. Its surface was often provided with narrow depressions, occasionally deep and tortuous, that afforded attachment for fibers of the vitreous body (Fig. 2). The bridge consisted mainly of a network of anastomosing cords of pigment cells. The meshes of the network were occupied by capillaries, some of which had a thick or thin endothelial lining (Fig. 4).

Serial sections through the base of the pecten and the underlying cauda of the optic nerve demonstrated that these vessels arose from the basal artery of the pecten (Fig. 5). They must therefore be regarded as afferent vessels. The second type of vessel ranged from 5 to 80 µm in diameter and was provided with an endothelium 2 to 3 µm thick (Fig. 5). Capillaries 20 µm or less in diameter were preponderant, while large vessels with a thick endothelium numbered only 2 or 3 in each fold and were usually located at the edges. Serial sections through the base of the pecten and the underlying ocular tunics

Fig. 1. Lateral view of the pecten oculi of the jungle crow (Corvus macrorhynchos) illustrating the pectineal pleats (P) separated by alternating depressions (arrowhead). The base (B) is wider than the apical end. The pleats arise from the base and attach to the bridge (Br). Note the thickenings at the apical ends of each pectineal pleat, the blood vessels appearing in the form of mid-rib-like prominences, and the ill-defined prominences on the surface (arrows). Bar: 5 mm.

Fig. 2. Transverse section of the pecten appearing as a thin, pleated lamina. The pleats appear in the form of an accordion pattern. Numerous blood capillaries (C) and a few afferent (arrow) and efferent (arrowhead) blood vessels are observed. The capillaries are generally arranged in two layers and surround the larger vessels. H&E stain. Bar: 100 µm.
showed that the largest vessels of the pecten, which had a thick endothelium in the lamina, acquired a thin endothelial lining at the base of the pecten, then passed through the bundles of the optic nerve fibers, and finally joined a large vein situated in the choroid, immediately beneath and parallel to the cauda of the optic nerve (Fig. 6). Therefore, the large vessels with thick endothelium were efferent. Under a light microscope, the walls of the capillaries and the efferent vessels appeared similar. The vessels occasionally anastomosed to form the ampulla (Fig. 7). The ampulla was more frequently located towards the apical part of the pleats. Within the ampulla, the intercapillary spaces were occupied by pigment cells. The diameter of an ampulla was 100 to 200 µm (Fig. 7).

Fig. 3. Light micrograph of a large blood vessel in the pecten oculi of the crow (Corvus macrorhynchos), illustrating the attenuated endothelium (arrow) and the thick basement membrane (M). Erythrocytes (arrowheads). H&E stain. Bar: 50 µm.

Fig. 4. Light micrograph illustrating a portion of the heavily pigmented bridge (Br) and the vascularized pleated lamina. Note the tightly packed network of pigment cells (P) and the limited number of capillaries (C) within the bridges. H&E stain. Bar: 50 µm.

Fig. 5. Oblique section through the insertion of the pecten upon the optic nerve cauda (ONC). A basal artery (Ar) is lodged in a groove on the vitreal surface of the mass of optic nerve fibers. An artery (A) is present with the regular distance of a fold in the pecten. Re: retina. Azan stain. Bar: 100 µm.

Fig. 6. Serial section of the same specimen shown in Fig. 5. A venule (Ve) is present at the edge of a fold in the pecten. The vein (V) has now reached the base of the pecten and merged toward the deep choroidal vein. ONC: Optic nerve cauda; Re: retina. Azan stain. Bar: 100 µm.
The wall of the vessels in the pecten was composed of unspecialized endothelial cells, a single sheath of smooth muscle cells and a thick adventitia. The inner region of the adventitia mainly consisted of layers of basement lamina-like materials, while the outer region was rich in collagen fibrils type I (Fig. 8).

Electron microscopy revealed that the entire pecten was delineated by a fine basal lamina (Figs. 9, 10). The basal lamina of the pecten capillaries was unusually thick, averaging 0.5 µm with areas of up to 1.5 µm in thickness (Figs. 9, 10, 11). The basal lamina consisted of several fibrillar layers, each of which had the appearance of a thin basal lamina. The melanocytes of the pecten were large pleomorphic cells with long projections that

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**Fig. 7.** Light micrograph illustrating a capillary ampulla (Am) (arrow). Note the tightly packed network of pigment cells within the capillaries. Azan stain. Bar: 100 µm.

**Fig. 8.** Immunohistochemical staining revealing collagen type I (arrow) fibers in the basement lamina of the capillaries. Bar: 100 µm.

**Fig. 9.** Electron micrograph indicating the multilayered basal lamina (Bl) of the capillaries, the lumen of the capillaries (Lu), and nuclear regions (N) of melanocytes. ×3200.

**Fig. 10.** Electron micrograph along the capillary and its peripheral areas, showing the nuclear regions (N) of melanocytes (M). The multilayered basal lamina (Bl) of the capillaries is indicated. Lu: lumen of the capillaries. ×3200.
more or less isolated the capillaries from one another. The nucleus of the melanocytes was large and vesicular (Figs. 9, 10). Melanocytes were most abundantly located peripherally in the pecten immediately below the basal lamina (Figs. 9, 10), but they could also be observed more centrally within the pecten. The capillaries of the crow pecten were extremely infolded on both the luminal and abluminal borders (Figs. 11, 12). In many cases, the actual cell body was only a thin central area from which numerous projections arose (Figs. 11, 12). These processes were thought to be microfolds, rather than microvilli, as they exhibited a range of widths when cut in different planes (Figs. 11, 12). The microfolds projecting into the lumen were longer (1.0 to 2.0 µm) than those formed on the abluminal surface, which ranged from 0.6 to 0.8 µm in length (Figs. 11, 12). It is difficult, however, to obtain true measurements of the lengths of these folds, as they are often extremely tortuous in shape. On both the luminal and abluminal borders, these microfolds often appeared to branch and anastomose (Fig. 11). In the capillary endothelial cells, the nuclear region was the widest portion of the cell body (Fig. 11). Pericytes were often enclosed within the thick compound basal lamina of the capillaries (Fig. 12). The pericytes did not display microfolds (Fig. 12). The pericytes might be separated from the endothelial cells by basal lamina materials (Fig. 12), or they might be in apparent contact with the abluminal folds of the endothelial cells, with no intervening basal lamina layers (Fig. 12). The wall of the blood vessels consisted of a thin nonfenestrated endothelium surrounded by a basal lamina of much the same thickness and appearance as that described for the pecten capillaries (Figs. 11, 12).

Discussion

In birds where the retina is avascular, the pecten, a highly vascularized intraocular organ with sparse pigmented tissue, is strongly suspected of playing a nutritive role (Duke-Elder, 1958; Meyer, 1977). A tropic role calls for an expansive surface area of the diffusion surfaces and a high blood supply. This must, however, be achieved after meeting certain constraints: the pecten must not be too large to interfere with the optical function of the eye and must also be stable enough to resist lateral swings. Our present observation of the pecten of the jungle crow, *Corvus macrorhynchos*, and findings from a study of pectens from birds with diverse habitats and differing visual acuity (Kiama *et al.*, 2001) suggest that the pecten has been designed to overcome the above constraints. The jungle crow is a largely diurnal bird of prey, exhibiting a cosmopolitan distribution. Shlaer (1976) reported that birds of prey have keen vision, and our previous observations regarding the visual capacity of the jungle crow (Rahman *et al.*, 2006) also support this notion. The pecten oculi has been considered to play a major role in the acquisition of an efficient visual mechanism (Mann, 1924). The location of the pecten in the jungle crow conforms to that evident in other diurnal species of birds possessing a pleated type of pecten. It lies over the optic disc, and the free end projects into the vitreous, as shown in the chick (Bhattacharjee, 1993). Since the optic disc constitutes a blind spot on the retina, it is of great advantage for the pecten to also be located in a position that optimizes the retinal area available for the capture of images. Although the location of the pecten is the same in all birds so far studied, the specific characteristics of the organ, such as its size, form and the
number of pleats, vary greatly (Thomson, 1929; Meyer, 1977). Other than the pleated type, there are two other morphological types of pecten (Meyer, 1977), e.g., the conical type and the vaned type. The conical pecten is a finger-like structure resembling the conus papillaries of reptiles and is found only in the kiwi bird (Apteryx mantelli) (Meyer, 1977). The vaned pecten consists of a central flattened pillar from which vertical vanes arise. This type of pecten is found in the ostrich and the rheas (Walls, 1942; Meyer, 1977). The pleated pecten is by far the most common type of pecten, with variations found in all other birds. These variations are believed to depend on the behavior of birds in relation to their general activity and visual pattern (Kiama et al., 2001). A basal lamina or vitreo-pecteneal limiting membrane continuous with the inner limiting membranes of the retina covers the entire pecten (Braekevelt, 1986, 1988, 1990, 1991). In some species, hyalocytes are a regular feature adherent to the outer surface of this membrane (Braekevelt, 1984, 1990). Within the folds of the pecten were located many specialized capillaries, supply (afferent) and drainage (efferent) vessels and numerous large, branching melanocytes. Unlike the chicken (Dieterich et al., 1973) and pigeon (Raviola and Raviola, 1967), where the authors could differentiate arterioles and venules in the pecten, in the crow, as in all previous studies of other species by these authors, it is very difficult if not impossible to adequately categorize these larger vessels in the pecten as to being either arterioles or venules (Braekevelt, 1984, 1986, 1988, 1990, 1991). 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 capillaries within the avian pecten are extremely specialized vessels; one of the striking features of these capillaries is the presence of a huge array of long processes on both their luminal (apical or internal) and abluminal (basal or external) borders. While the authors of some ultrastructural studies have referred to these as microvilli (Nguyen et al., 1967), most other investigators have referred to them as microfolds (Dieterich et al., 1973; Meyer, 1977). In the present study in Japanese jungle crows, these processes were thought to be microfolds, rather than the finger-like structure implied by the term microvilli (Braekevelt, 1984, 1986, 1988, 1990, 1991). The microfolds on the luminal surface always appeared to be longer, straighter and more numerous than those on the abluminal edge, perhaps indicating enhanced transport out of the capillaries. While some variation in the height of both the luminal and abluminal folds has been reported across species, these variations do not seem to be correlated with the overall size of the pecten, as even the relatively small pecten of the night hawk shows microfolds similar in height to those reported in species with larger pectens, such as those found in the great blue heron (Braekevelt, 1984, 1991). Since the range of heights of the luminal microfolds is very small (normally between 1.0 and 1.5 µm), this may indicate the optimum size of microfolds or may perhaps represent the tallest microfolds that can be supported adequately by the endothelial cells. Another striking feature of pecten capillaries is the unusually thick basal lamina described in all species to date (Meyer, 1977, Braekevelt, 1984, 1986, 1988, 1990, 1991). This thickened basal lamina might appear incongruous associated with capillaries so obviously involved in transport functions. However, despite its overall thickness, the fibrillar layers of the basal lamina are not closely packed, and the entire structure would not appear to offer a serious barrier to the movement of materials. It is speculated that this thickened basal lamina may actually serve an important structural function, as it supports fragile endothelial cells that have very thin cell bodies and numerous processes. The pecten of the Japanese jungle crow was relatively large and the basal laminae were relatively thick. Similarly thick basal laminae have been reported to be present in the larger pectens of the great blue heron, red tailed hawk, loon and pigeon (between 1.0 and 2.0 µm), compared with the thinner basal lamina in the smaller pecten of the nighthawk (0.5 µm) and the basal lamina with intermediate thickness in the intermediate-sized pecten of the mallard (0.75 µm), possibly reflecting this structural role (Braekevelt, 1984, 1988, 1990, 1991). Pericytes, which are a common feature of the wall of both retinal and hyaloid capillaries, are also present in the walls of pectinal capillaries (Ashton and De Oliviera, 1966; Braekevelt and Hollenberg, 1970; Jack, 1972). Similar to the function of these cells in other locations, the function of the pericytes in pecten capillaries is uncertain, and they may be supportive or contractile in nature or may perhaps represent reserve cells that could become endothelial cells, as required.

The present study demonstrated how a large conglomerate of pectinal blood vessels of varying caliber is effectively arranged and reinforced by an extensive contingent of melanosomes. Although investigators have attributed a supportive role to the pigmented intervascular tissue of the pecten in TEM studies (Braekevelt, 1991), the functional relationship between the melanocytes and the pectinal vessels has remained unresolved. Scrutiny of the figures presented in this study raises the possibility of a two-fold causal relationship between the two components of pectinal tissue: (1) structural reinforcement of the vascular lattice, and (2) shielding of the blood vessels from exposure to ultraviolet light. The structural reinforcement of the pectinal blood vessels is important for maintaining the erectile function of the pecten and protecting the visual efficiency of the eye. This aspect of melanocytic function is provided by the perikarya of the melanocytes as well as by the melanin granules contained within. Whereas the perikarya of the melanocytes lie wedged between adjoining blood vessels, the melanosomes, which occur in sizeable numbers, plug...
into the interstices of the vascular network in different conformational patterns to reinforce the pecten at different locations. This may account for the differential distribution of melanosomes in the pecten. On the free apical half of the pectineal surface, the melanosomes formed a thick investment over the anastomosing network of blood vessels, giving the latter a bloated appearance. The melanosomes at this part of the pecten appeared to provide protection for the ensheathed blood vessels against exposure to incident ultraviolet light. The great preponderance of melanosomes at the apical end of the pecten tends to implicate a similar melanosomal function. Such an inference seems plausible in view of the increase in the number of melanosomes in the keratinocytes of man following exposure to ultraviolet light (Ghadially, 1982). Furthermore, melanin is an efficient absorber of light: this is a non-specific phenomenon and extends through the ultraviolet region into the visible range, but it is most pronounced toward the shorter end of the spectrum (Jimbow et al., 1991; Kollias et al., 1991).

When tissues are exposed to reactive oxygen radicals, a variety of pathological changes may occur such as protein denaturation, lipid peroxidation and cell degeneration (Fridovich, 1978). The eye is no exception, with reported conditions including cataract (Van-Kuuk, 1991; Xiao-Lan and Marjorie, 1993) and retinal degeneration. The vertebrate retina is believed to be particularly susceptible to this kind of damage because (1) it has a high oxygen tension (Weiter, 1987), and (2) it is exposed to ultraviolet light, which has been shown to initiate oxygen radical formation (Delmelle, 1979). This lends further support to the envisaged function of melanosomes as a protective shield against sustained daytime exposure to ultraviolet light. Moreover, in view of the existing notion that melanin reacts with excited molecules and radicals (Foote, 1982; Larsson, 1993), a close association between melanin-bearing melanocytes and the pectineal blood vessels in the jungle crow may be looked upon as a strategy to protect the integrity of the capillary endothelium.

A high degree of pigmentation of the pectineal bridge has also been reported in chicks (Seaman and Storm, 1963; Bhattacharjee, 1993), pigeons (Raviola and Raviola, 1967) and vultures (Bawa and Roy, 1974). Bawa and Roy suggested that this would favor light absorption and a consequent increase in pectineal temperature, leading to an increase in the physiological activity of this intraocular structure in the transport of nutrients to the retina. However, it should be pointed out that the close association between the melanosomes and the capillaries, and the differential distribution of the melanosomes on the pectineal surface, as demonstrated in this study, cannot be completely explained by the provision of a rise in temperature in the pecten alone, although such a possibility cannot be excluded. The nutritive role of the pecten may possibly be mediated more efficiently along the basal half of the pecten and the lateral aspect of the pectineal pleats, where melanocytes are much fewer and the capillary network of blood vessels is partially exposed.

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