A scanning electron microscope study of the pecten oculi of the black kite (*Milvus migrans*): possible involvement of melanosomes in protecting the pecten against damage by ultraviolet light

S. G. KIAMA[1], J. BHATTACHARJEE[2], J. N. MAINA[1] AND K. D. WEYRAUCH[3]

[1] *Department of Veterinary Anatomy, University of Nairobi, Kenya,* [2] *Department of Zoology, Egerton University, Njoro, Kenya, and* [3] *Institute of Veterinary Anatomy, Free University of Berlin, Germany*

(*Accepted 23 June 1994*)

ABSTRACT

The pecten oculi of the black kite (*Milvus migrans*), a diurnally active bird of prey, has been examined by scanning electron microscopy. In this species the pecten consists of 12 highly vascularised pleats, held together apically by a heavily pigmented 'bridge' and projects freely into the vitreous body in the ventral part of the eye cup. Ascending and descending blood vessels of varying calibre, together with a profuse network of capillaries, essentially constitute the vascular framework of the pecten. A distinct distribution of melanosomes is discernible on the pecten, the concentration being highest at its apical end, moderate at the crest of the pleats and least at the basal and lateral margins. Overlying and within the vascular network, a close association between blood vessels and melanocytes is evident. It is conjectured that such an association may have evolved to augment the structural reinforcement of this nutritive organ in order to keep it firmly erectile within the gel-like vitreous. Such erectility may be an essential prerequisite for its optimal functioning, as well as in its overt use as a protective shield against the effects of ultraviolet light, which otherwise might lead to damage of the pectineal vessels.

Key words: Avian eye.

INTRODUCTION

The pectin oculi is a unique vascular and pigmented intraocular structure characteristic of the avian eye. Since the appearance of the first correct description and interpretation made by Perrault in 1676 (cited by Sillman, 1973), this intraocular structure has been the subject of investigation from various aspects. Through experimental manipulation, analogy and hypothesis, researchers have endeavoured to assign to it plausible function(s). These include (1) intraocular pressure regulation (Seaman & Himelfarb, 1963), (2) intraocular pH regulation (Brach, 1975), (3) stabilisation of the vitreous (Tucker, 1975), (4) reduction of intraocular glare (Barlow & Ostwald, 1972), (5) serving as a blood and fluid barrier for the retina and vitreous body (Rodriguez-Peralta, 1975), and above all as a supplemental nutritive organ (Mann, 1924; Meyer, 1977), a function which has been equivocally accepted by contemporary researchers.

Grossly the pecten oculi have been grouped 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 the 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 disc in the form of accordion-pleats held together at the free apical border by a heavily pigmented bridge of tissue (Seaman & Storm, 1963; Raviola & Raviola, 1967). A considerable variation in the number of pleats, size and shape exists in the pleated form of pecten within the avian species (Thomson, 1929). The variations are

Correspondence to Dr S. G. Kiama, Department of Veterinary Anatomy, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

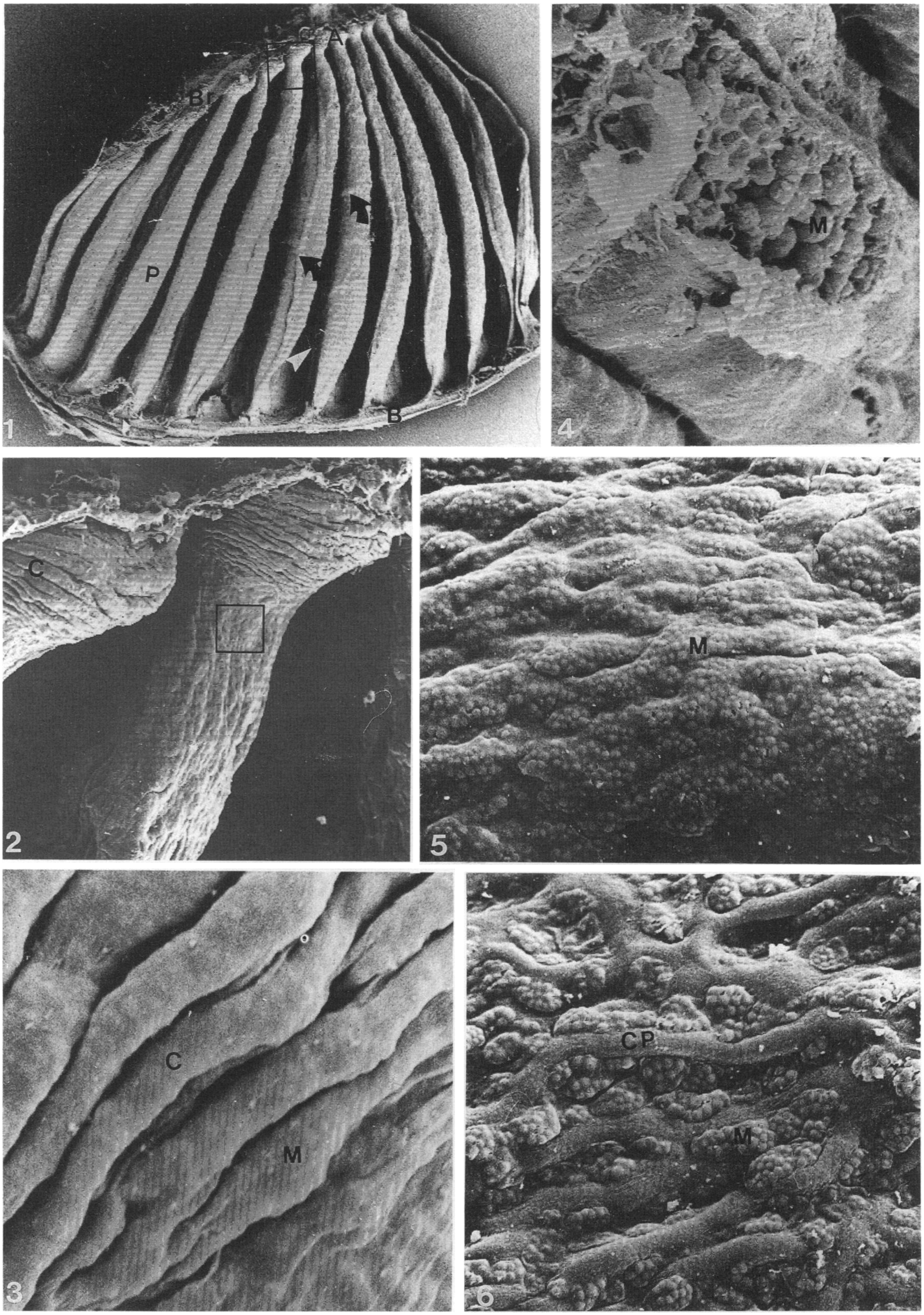


Fig. 1. The lateral view of the pecten oculi of the black kite (*Milvus migrans*) displaying 12 vertical pectineal pleats (P) separated by alternating depressions (arrow-head). The base (B) is wider than the apical end (A). Note the thickenings at the apical ends of each pectineal pleat and the blood vessels appearing in form of mid-rib-like prominences and ill defined prominences on the surface (arrows). Br, bridge. × 14.

Fig. 2. Enlarged view of the area shown in the square (Fig. 1). Several concentric cord-like structures (C) can be identified. These run transversely to the long axis of the pectineal pleat. × 100.

believed to be related to the behaviour of birds in relation to their general activity and visual pattern (Braekevelt, 1988).

Although the vascular framework of the pecten has been studied by flat-mounting following its severance from the underlying optic disc and apical bridge (Tanaka, 1938), routine histology (Bawa & Roy, 1974), as well as transmission electron microscopy (Seaman & Storm, 1963, Raviola & Raviola, 1967; Braekevelt 1988), the true framework of the pecten, vis-à-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) which lacks both muscular and nervous tissue (Meyer, 1977). The pectineal blood vessels consist of arterioles, venules and highly specialised capillaries (Raviola & Raviola, 1967) that freely anastomose with each other (Dietrich et al. 1973; Hossler & Olson, 1984; Matsunaga & Amemiya, 1990). The area between the vessels is partly filled with pleomorphic pigment cells described as melanocytes (Braekevelt, 1984) which contain melanin granules (Raviola & Raviola, 1967).

In the present study an attempt has been made to elucidate the organisation of the pecten in a variety of avian species selected on the basis of their adaptation to diurnal, nocturnal and crepuscular activity.

MATERIALS AND METHODS

Two black kites (*Milvus migrans*) weighing about 702.25 g each were anaesthetised using pentobarbitone sodium and perfused via the left and right internal carotid arteries with warm (35 °C) physiological saline. This was followed by perfusion fixation with 2.5%, 0.2 M, phosphate buffered (pH 7.4) glutaraldehyde solution, before which the right auricle was cut open for drainage. The eyes were enucleated, cut around the equator and pecten oculi carefully dissected out and immersed in the same fixative for 6 h. Intact pectens and small pieces from different parts of the pecten were postfixed in 1% phosphate buffered osmium tetroxide, dehydrated in a graded series of ethanol, critical point dried and then mounted on aluminium stubs with silver conductive points, after which they were sputter coated with gold palladium complex and examined with JEOL JSM 35SF or Zeiss DSM 950 scanning electron microscope at 17 kV.

RESULTS

The pecten oculi of the black kite is a fan-like intraocular structure situated over the optic disc and the contiguous stretch of the nerve fibre layer of the retina, and projects freely into the vitreous in the ventral half of the eye. A scanning electron micrograph (SEM) taken at low magnification, showed presence of 12 pleats (Fig. 1) which varied in length from 1.92 to 4.43 mm, and in width from 0.07 to 0.43 mm. The pecten measured about 7 mm at the long axis of its base and 3 and 5 mm in height at its dorsal and ventral margins respectively. The pectineal pleats were held together apically by a connecting bridge of tissue. Lying externally on the surface of some of the pleats were low midrib like prominences considered to be longitudinal blood vessels. Similarly, some ill defined prominences, disposed obliquely in a transverse plane, were also discerned on the surface of some of the pleats (Fig. 1).

A high-power photomicrograph taken at the tapered apical end of the pecten revealed presence of a globose thickening at the terminal end of each pleat (Fig. 2). Each of such terminal thickenings was characterised by the presence of several concentric cord-like prominences that ran transversely to the long axis of the pleats and gave the region a highly rugged appearance (Figs 2, 3). A surface tear of one such concentric prominence (Fig. 4) demonstrated presence of numerous closely packed spheroidal bodies, considered to be melanosomes, measuring between 1.5 and 2.2 μm in diameter.

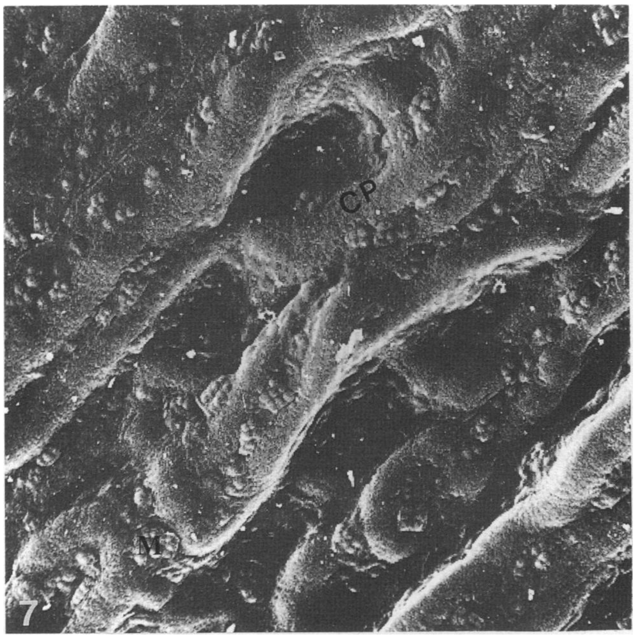
At higher magnification the surface of the pleats revealed an irregular array of branching and anastomosing ridges, considered to be pectineal capillaries, interspersed with clusters of spheroidal bodies similar to those evident at the thickened terminal ends of the pleats, and identified as melanosomes (Figs 5–8). The

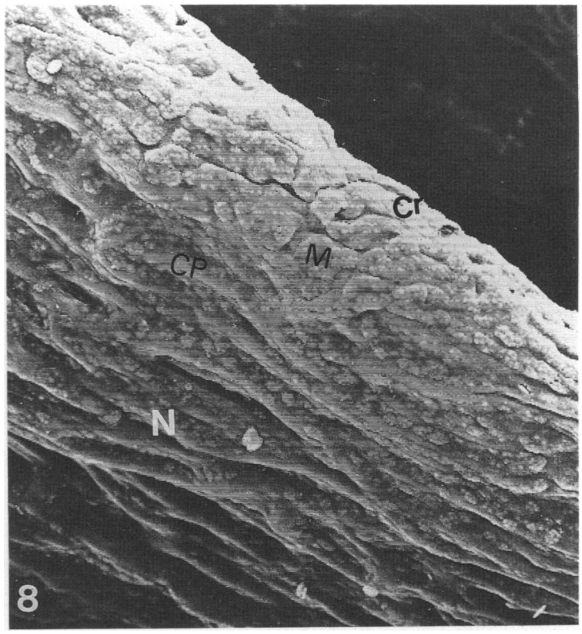
Fig. 3. High magnification of the cord-like structures shown in Figure 2. These appear to be expanded by the spheroidal bodies (M) contained in them. × 1000.

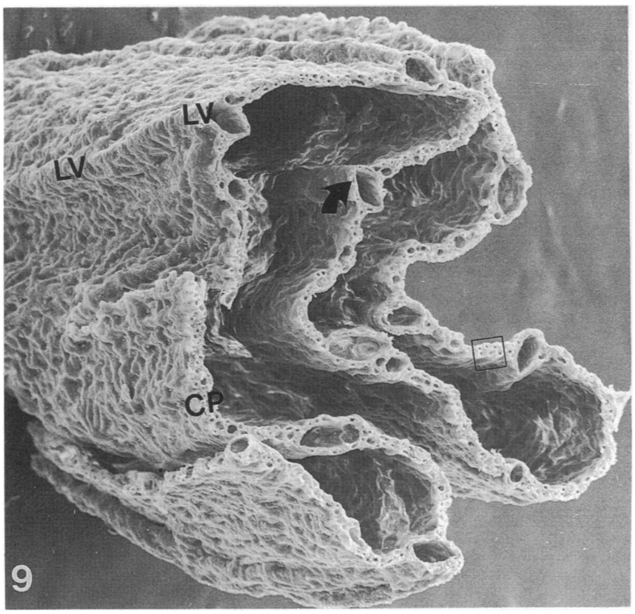
Fig. 4. High-magnification photomicrograph of the cords shown in Figure 3; a surface tear on one of the cords exposes the contained melanosomes (M). × 2000.

Fig. 5. High magnification of the area shown in the square in Figure 2. Note the thick blanket of melanosomes (M). Blood vessels are not discernible. × 500.

Fig. 6. Surface view of a portion of a pectineal pleat at about the middle of the pecten. The anastomosing capillaries (CP) are easily discerned and are interspersed with mulberry-like clusters of melanosomes (M). × 500.







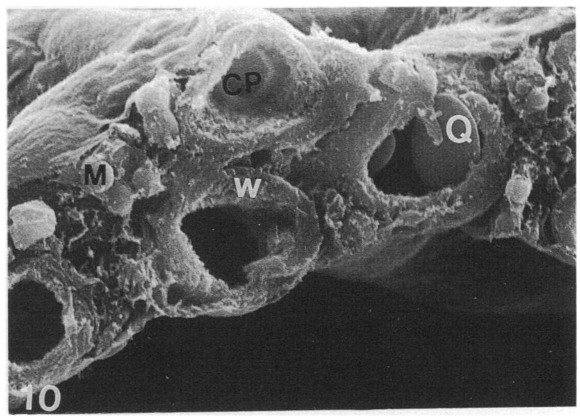


Fig. 7. Surface of the pectineal pleat near the base. The melanosomes (M) are scanty and the capillaries (CP) lie exposed over most of the surface. × 500.

Fig. 8. Lateral aspect (N) and crest (Cr) of a pectineal pleat as viewed from the mid-section of the pecten. Note the predominance of the capillaries (CP) on the lateral aspect as compared with the crest of the pectineal pleat, where the melanosomes (M) dominate. × 500.

Fig. 9. Transversely cut surface of the pecten oculi displaying the afferent and efferent vessels (LV). Note that these are situated at the crest and in the middle of the pectineal pleat while the capillaries (CP) lie between and around the large blood vessels (arrow). × 60.

Fig. 10. An enlarged view of the rectangle marked in Figure 9. Note thick-walled capillaries (CP) and the melanosomes (M) appearing in form of spheroidal bodies between them. Red blood cell (Q). Capillary wall (W). × 1600.

melanosomes at the pectineal surface, however, were clustered in the form of a mulberry over the basal and middle thirds of the pectineal surface where most of the capillaries lay exposed (Figs 6,7). In contrast, the capillaries at the apical end of the pleats remained completely hidden under a thick blanket of melanosomes (Fig. 5) where their branching and intersecting pattern could not be discerned. Similarly the distribution of the melanosomes showed a decreasing pattern from the crests of the pleats to their lateral aspects (Fig. 8).

The vascular framework of a portion of the pleats showed large ascending and descending blood vessels with a vast anastomosing network of capillaries lying between them. These appeared as surface prominences on the pleats and were evident as a collection of translucent canals on the transected surface of the pleats (Fig. 9). The large blood vessels showed a characteristic disposition. The vessels of relatively larger dimension mostly ran along the crest and the middle of the pleats. These are considered to be efferent and afferent in function. Conversely, the capillaries were relatively smaller in size and were most often disposed between and around these vessels, their walls appearing relatively thicker than those of their larger counterpart (Figs 9, 10).

DISCUSSION

The black kite is a largely diurnal bird of prey exhibiting a cosmopolitan distribution (Brown, 1976). Although no substantive record is available regarding the visual capacity of this avian species, birds of prey are reputed to have keen vision (Shlaer, 1976) and the pecten oculi has been considered to play a major role in the acquisition of the efficient visual mechanism (Mann, 1924).

The location of the pecten in the black kite conforms to that evident in other diurnal species of birds possessing a pleated type of pecten. It overlies the optic disc and the adjoining strip of the nerve fibre layer of the retina as shown in chick (Seaman & Storm, 1963; Bhattacharjee, 1993). The present study demonstrates how the large conglomerate of pectineal blood vessels of varying calibre 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 from TEM studies (Meyer, 1977; Braekevelt, 1991), the functional relationship between the melanocytes and the pectineal vessels has remained unresolved. Scrutiny of the figures presented in this study raises the possibility of a twofold causal relationship between the two components of the pectineal 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 pectineal 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 in them. 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 possibly accounting for the differential distribution of melanosomes in the pecten (compare Figs 2, 3, 5–8).

On the free apical half of the pectineal surface melanosomes form a thick investment over the anastomosing network of blood vessels, giving the latter a bloated appearance (Fig. 2). The melanosomes at this part of the pecten appear 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 (Fig. 5) tends to implicate similar melanosomal function. Such an inference seems plausible in view of the increase of melanosomes in the keratinocytes of man following exposure to ultraviolet light (Ghadially, 1982). Furthermore, melanin is an efficient absorber of light: this is nonspecific 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, the reported conditions including cataract (Van-Kuijk, 1991; Xiao-Lan & Marjorie, 1993) and retinal degeneration (Van-Kuijk, 1991). The vertebrate retina is believed to be particularly susceptible to this kind of damage because (1) it has a high oxygen tension (Weiter, 1987), (2) it is exposed to ultraviolet light (van-Kuijk, 1991), and ultraviolet light 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 day-time exposure to ultraviolet light. Although no data are available with respect to the absorption spectra of the ocular media in birds of prey, birds in general have been reported to utilise ultraviolet light for visual detection (Jacobs, 1992), suggesting that the ocular media of birds allows ultraviolet light transmission, although this may vary among species. Moreover, in view of the existing notion that melanin reacts with excited molecules and radicals (Foote, 1982; Larsson, 1993), close association between melanin bearing melanocytes and the pectineal blood vessels in the black kite may be looked upon as a strategy to protect the integrity of the capillary endothelium. High pigmentation of the pectineal bridge has been reported in the chick (Seaman & Storm, 1963; Bhattacharjee, 1993), pigeons (Raviola & Raviola, 1967) and vultures (Bawa & Yash Roy, 1974). Bawa & Yash Roy (1974) suggested that this would favour light absorption and a consequent increase in pectineal temperature leading to increased 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 fully explained by the provision of a rise of 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 (Figs 6–8).

ACKNOWLEDGEMENTS

This work was carried out at the Institute of Veterinary Anatomy, Free University of Berlin, Germany and Department of Veterinary Anatomy, University of Nairobi, Kenya. We wish to thank the German exchange programme (DAAD) for funding this project. We also wish to thank Eckart-Funken, Hindorf and Schiel for their excellent technical assistance.

REFERENCES

Barlow HB, Ostwald TJ (1972) Pecten of the pigeon's eye as an inter-ocular eye shade. *Nature* **236**, 88–90.

Bawa SR, Yashroy RC (1974) Structure and function of vulture pecten. *Acta Anatomica* **89**, 473–480.

Bhattacharjee J (1993) A presumably astroglial cell in the retino-pectineal junction in *Gallus domesticus* demonstrated by non-specific esterase staining. *European Journal of Morphology* **31**, 169–173.

Brach V (1975) The effect of intraocular ablation of the pecten oculi of the chicken. *Investigative Ophthalmology* **14**, 166–168.

Braekevelt CR (1984) Electron microscopic observations on the pecten of the Nighthawk (*Chordeiles minor*). *Ophthalmologica* **189**, 211–220.

Braekevelt CR (1988) Fine structure of the pecten of the pigeon (*Columba livia*). *Ophthalmologica* **196**, 151–159.

Braekevelt CR (1991) Electron microscopic observations on the pecten of the Great blue heron (*Ardea herodias*). *Histology and Histopathology* **6**, 345–351.

Brown L (1976) Classification and distribution. In *The African Birds of Prey, their Biology and Ecology*, pp. 6–17. London: Hamlyn.

Delmelle M (1979) Possible implication of photo-oxidation reactions in retinal photo-damage. *Photochemistry Photobiology* **29**, 713–716.

Dieterich CE, Dieterich HJ, Spycher MA, Pfautsch M (1973) Fine structural observations of the pecten oculi capillaries of the chicken. *Zeitschrift für Zellforschung* **146**, 473–489.

Foote CS (1982) Light, oxygen and toxicity. In *Pathology of Oxygen* (ed. P. Autor), pp. 21–44. New York: Academic Press.

Fridovich I (1978) The biology of oxygen radicals. *Science* **201**, 875–880.

Ghadially FN (1982) Melanosomes. In *Ultrastructural Pathology of the Cell and Matrix*, 2nd edn, pp. 599–623. London: Butterworths.

Hossler FE, Olson KR (1984) Microvasculature of the avian eye: studies on the duckling with microcorrosion casting, scanning electron microscopy, and stereology. *American Journal of Anatomy* **170**, 205–221.

Jacobs GH (1992) Ultraviolet vision in vertebrates. *American Zoologist* **32**, 544–554.

Jimbow K, Fitzpatrick TB, Wick MM (1991) Biochemistry and physiology of melanin pigmentation. In *The Physiology, Biochemistry, and Molecular Biology of the Skin*, 2nd edn (ed. L. A. Goldsmith), pp. 873–909. New York: Oxford University Press.

Kollias N, Sayre RM, Zeise L, Chedekel MR (1991) Photoprotection by melanin. *Journal of Photochemistry Photobiology B* **9**, 135–160.

Larsson BS (1993) Interaction between chemicals and melanin. *Pigment Cell Research* **6**, 127–133.

Mann IC (1924) The function of the pecten. *British Journal of Ophthalmology* **8**, 209–226.

Martin RG (1985) In *Form and Function in Birds*, vol. 3 (ed. A. S. Kings & J. McLeland), pp. 311–373. London: Academic Press.

Matsunga N, Amemiya T (1990) Pecten of the chick eye demonstrated by vascular casts. *Okajimas-Folia-Anatomica-Japonica* **67**, 263–270.

Meyer DB (1977) The avian eye and its adaptations. In *Handbook of Sensory Physiology*, V11/5, *The Visual System in Vertebrates* (ed. F. Crescittelli), pp. 549–612. Berlin: Springer.

Raviola E, Raviola G (1967) A light and electron microscopic study of the pecten of the pigeon eye. *American Journal of Anatomy* **120**, 427–462.

Rodriguez-Peralta LA (1975) Hematic and fluid barriers of the retina and vitreous body. *Journal of Comparative Neurology* **132**, 109–124.

Seaman AR, Himelfarb TM (1963) Correlated ultrafine structural changes of the avian pecten oculi and ciliary body of *Gallus domesticus*. *American Journal of Ophthalmology* **56**, 278–296.

Seaman AR, Storm H (1963) A correlated light and electron microscopic study on the pecten oculi of the Domestic fowl (*Gallus domesticus*). *Experimental Eye Research* **2**, 163–172.

Sillman AJ (1973) Avian vision. In *Avian Biology*, vol. III (ed. S. Farner, J. R. King & K. C. Parkes), pp. 349–387. New York: Academic Press.

Shlaer R (1976) An eagle's eye: quality of the retinal image. *Science* **176**, 920–922.

Tanaka H (1938) The blood-vessel distribution and pigmentation in pecten of Japanese birds. *Japanese Journal of Medical Sciences* **7**, 133–151.

Thomson A (1929) The pecten, considered from an environmental point of view. *Ibis* **12**, 608–639.

Tucker R (1975) The surface of the pectin oculi in the pigeon. *Cell and Tissue Research* **157**, 457–465.

van-Kuijk FJGM (1991) Effect of ultraviolet light on the eye: role of protective glasses. *Environmental Health Perspectives* **96**, 177–184.

Weiter JJ (1987) Phototoxic changes in the retina. In *Clinical Light Damage to the Eye* (ed. D. Miller) pp. 77–125. New York: Springer.

Xiao-Lan C, Marjorie FL (1993) The effect of recovery of long-term H_2O_2 exposure on lens morphology and biochemistry. *Experimental Eye Research* **57**, 157–167.