Fine Structure of the Choroidal Coat of the Avian Eye

Lymphatic Vessels

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Purpose. To clarify the fine structure of the avian choroid and thus help explain the mechanisms for normal and abnormal eye function and growth.

Methods. Eyes from normal chickens and from experimental chickens subjected to unilateral paracentesis were fixed either by perfusion or in situ, with or without post-fixation by microwave irradiation, and then processed for light and electron microscopic analysis.

Results. The avian choroid contains thin-walled lacunae, whose fine structure is identical to that of lymphatic vessels. The lacunae are much smaller toward the anterior chamber and the Schlemm's canal than posteriorly in the eye bulb. Large lacunae are situated primarily in the suprachoroidea, and their blind-ended capillary branches enter the choriocapillaris and the walls of large veins. The walls of the large veins contain villous structures that protrude into their lumina and are penetrated by thin lacunar branches and by side lines of the venous lumen. In normal chickens, the lacunae usually are devoid of blood cells. After paracentesis of the anterior eye chamber, the lacunae become filled with erythrocytes on the side that was operated on, but not on the contralateral side.

Conclusions. The authors propose that the lacunae of the avian choroid represent a system of posterior short lymphatic vessels, which drain intraocular fluids directly into the eye's venous system, and that the villous structures are sites of communication between lacunae and veins. The demonstration of a choroidal lymphatic system opens new insights into the processes of fluid removal, control of intraocular pressure, and regulation of choroidal thickness in the avian eye under normal and experimental conditions. Invest Ophthalmol Vis Sci. 1997;38: 1241–1260.

The eye's choroidal coat, which together with the ciliary body and the iris forms the uvea, is one of the most highly vascularized tissues in the body.[1–7] In addition to representing the major source of nourishment and oxygen for the retina,[1–5,8] the choroid also may work as a "cooling system" involved in the dissipation of heat produced from light absorption by the retinal photoreceptors.[3,9–12] Furthermore, the amount of plasma proteins in the tissue fluid of the mammalian choroid is high[13] and, by virtue of the ensuing oncotic pressure, fluids filter from the retina, through the pigment epithelium, to the choroid itself.[1–4] It has been suggested that this mechanism helps to keep the retina attached to the choroid.[1] The inner layer of the choroid, the choriocapillaris, is tied functionally to the retinal pigment epithelium in developmental, maintenance, and disease processes. Because of such complex relations, the choroid is an important model to study mesenchymal–epithelial interactions and the regulation of epithelial cell polarity.[14] The loose structure of the choroid plays a major role in the maintenance of intraocular pressure (IOP). Tissue fluids can be filtered from the capillary endothelium or reabsorbed into the capillaries themselves, depending on changes of the hydrostatic pressure gradient. In mammals, the choroid also is involved in the drainage of the aqueous humor from the anterior chamber of the eye. Part of the aqueous humor, which is secreted in the posterior chamber by the ciliary processes,[1,2,5,15,16]

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flows through the pupil into the anterior chamber and filters through the tissues of the anterior chamber angle and the interstitial spaces of the ciliary muscles into the supraciliary and suprachoroidal spaces. From the suprachoroidea, the fluids reach the sclera, and then the episcleral tissues, by simple diffusion in the scleral matrix or in the perivascular spaces.[1-3,5,17-21] This route of outflow has been termed uveoscleral. The rate of drainage through uveoscleral routes varies among species, from 3% in cats[22] to 35% in humans[23] to 30% to 65% in cynomolgus monkey.[24-26] The principal, or conventional, route for aqueous humor drainage, however, uses the Schlemm's canal, a circular vessel placed in the irido-corneal angle, that conveys the fluids from the anterior chamber directly into the episcleral veins.[1,2,5,7,20,21,27,28]

The choroid is provided with a rich autonomic innervation,[29-32] derived from various sources including the ciliary, pterygopalatine, and superior cervical ganglia, that regulates choroidal blood flow with the contribution of nitric oxide derived from retinal and choroidal cells[33-38] and endothelins of choroidal endothelia.[39,40]

The choroid has been the focus of renewed attention after the introduction of experimental defocus and its compensatory mechanisms in primates[41-54] and other mammals[55-58] to study the regulation of postnatal eye growth and, in particular, the process of emmetropization, that is the matching of optical power and axial eye length at neutral accommodation.[59-61] This complex vision-dependent process involves cornea, retina, choroid, and sclera. The avian eye, which had been used widely for studies of parasympathetic functions in development and aging,[62,63] has become a favored animal model for experimental ophthalmology because of its rapid growth, high visual qualities, and general tractability.[64-92] Yet, relatively little information was available on the structure and function of the avian choroid until recently.[29,93-99] Despite the presence of nonvascular smooth muscle cells in the stroma of the avian choroid[93-95] that are thought generally to be absent in the primate choroid,[7] the assumption seems to be that the avian and mammalian choroidal coats are largely similar.

During the past few years, the role of choroidal factors in the differentiation of ciliary ganglion neurons has become an important issue. Coulombe and Nishi[100] and Coulombe et al[101] have shown that a specific factor, the SSA (somatostatin stimulating activity), produced by cells located in the avian choroid, promotes somatostatin (Som) synthesis in avian ciliary ganglion neurons grown in dissociated cell cultures. Furthermore, Som and acetylcholinesterase-positive fibers have been localized in the vicinity of choroidal blood vessels in situ by immunofluorescence,[102-104] and Gray et al[103,104] have shown that acetylcholine (ACh) and Som are released from the same terminals through two different secretory pathways. Som acts as a neuromodulator and inhibits the Ca^{2+}-dependent, K^+-evoked ^{3}H-ACh release from the axon terminals of choroid neurons, and its action is mediated by a cascade mechanism involving nitric oxide and a cyclic guanosine monophosphate-dependent kinase.[103-105] In a previous article,[106] we have shown in adult birds that all of the neurons innervating the choroid ("choroid neurons"), but not the neurons innervating the iris and ciliary body ("ciliary neurons"), express Som. This peptide can be considered, therefore, as a cell class specific marker in the avian ciliary ganglion and can be used to identify, within the choroid, the axons originating from the choroid neurons. The choroidal coat contains several types of cells that may be involved in the induction of Som expression by choroid neurons, although it has been shown that the innervation of the choroid by the ciliary ganglion is directed, at least in part, to the vascular smooth muscle cells.[29,62,63,95,107]

Taken together, these considerations indicate that an extensive investigation of the avian choroid is highly warranted. We have performed, therefore, a detailed analysis of the fine structure of the choroid in the chicken to clarify the organization of the vascular system; the types, distribution, and intercellular relations of the different cell populations; and the innervation. The results are subdivided into two articles. The current article deals with the discovery of a lymphatic system, and the second article[108] is focused primarily on the contractile elements of the choroid and their innervation, including the immunoelectron microscopic demonstration of Som-positive axon terminals. The salient point of these studies is that the avian choroid, although resembling in certain general aspects the mammalian choroid, shows substantial morphologic peculiarities. This conclusion indicates the need for further functional studies of the avian eye and suggests that birds represent a special category as experimental model for human eye's diseases.

MATERIALS AND METHODS

Adult White Leghorn chickens (600 to 1400 g body weight) of either gender were used for these experiments. The animals were anesthetized deeply by injection of pentobarbital (65 mg/kg body weight) in the subalar vein and then perfused with an oxygenated calcium-free Ringer's variant, pH 7.3, followed by an aldehyde fixative. We applied different fixation protocols to preserve the choroid fine structure optimally under control and experimental conditions. Animals were housed in facilities at the University of Connecticut and handled according to guidelines proposed by the Society for Neuroscience and the ARVO Statement

for the Use of Animals in Ophthalmic and Vision Research.

Normal Animals

Perfusion Fixation. Two control chickens were perfused at a delivery pressure of 90 cm water with 4% polyvinylpyrrolidone-40 (PVP-40), 2% glutaraldehyde (Glu), 0.5% tannic acid (TA) in 0.1 M phosphate buffer (PB), whereas five other chickens were perfused, using the same fixative, at a lower pressure (70 cm water) to prevent swelling as much as possible of thin-walled blood vessels.

After perfusion fixation, the eyeballs were dissected carefully without compressing the bulb, the cornea was cut along the transition with the sclera, the lens and vitreous humor were removed with fine-tipped forceps, and the residues were cleaned gently with a cotton-tipped applicator. The eyeballs then were treated with a solution of 0.2 M ethylenediaminetetraacetic acid, 2.5% Glu in 0.1 M PB, pH 7.4 for 3 days, at 4°C, with a daily change of the solution to soften the sclera. In three of the chickens perfused at low delivery pressure, one of the eyeballs was placed in a jar surrounded by an ice bath in the same fixative used for the perfusion and then irradiated for 32 seconds (with steps of 4 seconds each) in a 800-W microwave oven to enhance the fixation.[109,110] All the specimens then were cut in large squares with sharp scissors without stretching the tissues, rinsed in 0.1 M PB, and osmicated with 2% OsO_4 in 0.1 M PB for 1 hour at 4°C. After several washes in distilled water, the specimens were treated with aqueous 2% uranyl acetate, rinsed again, dehydrated with a series of ethyl alcohol and propylene oxide, and embedded in Epon 812. Semithin sections (1- to 2-μm thick) and ultrathin sections (50- to 70-nm thick) were cut on a ultramicrotome and stained with 0.1% toluidine blue in 0.1% borax and with 2% uranyl acetate followed by 0.2% lead citrate, respectively.

En Bloc Fixation. To verify how much our perfusion fixation parameters affects the appearance of the vessels, one male chicken was decapitated while receiving anesthesia. The eyeballs were dissected rapidly and immersed in a fixative containing 4% freshly depolymerized paraformaldehyde, 2.5% Glu, 0.55% TA in 0.06 M PB (pH 7.4) for 2 hours at room temperature and then in the same fixative overnight at 4°C. One of the eyeballs was removed and exposed to microwave irradiation as specified, whereas the other eyeball was prepared in the standard way. The successive preparatory steps were as those described above.

Experimental Animals

In four chickens receiving anesthesia, the cornea of one eye was incised gently with a sharp razor blade, without compressing the eyeball, and the anterior chamber was emptied of the aqueous humor. Humor was withdrawn with a 27-gauge needle connected to a tuberculin syringe introduced into the anterior chamber, taking care to avoid damaging the anterior surface of the iris, and freshly secreted fluid was removed with a cotton-tipped applicator. After 10 minutes, two of the chickens were decapitated; both eyeballs from each bird were dissected out rapidly, cornea and lens were removed carefully, and the bulbs were immersed in a fixative containing 2% Glu, 0.5% TA in 0.1 M PB, pH 7.4. After microwave irradiation as specified above, the eyeballs were left in the same fixative for 4 hours at room temperature and then 2 hours at 4°C. For comparison, the other two chickens were perfused with the same type of fixative, preceded by a wash with oxygenated Ringer's solution, pH 7.3. The eyeballs were removed and exposed to microwave irradiation and then, together with the specimens from the previous animals, immersed in a solution of 0.2 M ethylenediaminetetraacetic acid, 2.5% Glu in 0.1 M PB, for 3 days with daily changes of the solution. Postfixation, dehydration, and embedding were performed as described above.

RESULTS

Light Microscopy

In accordance with the terminology adopted commonly for mammals,[7] we subdivide the choroidal coat of the avian eye into four layers: the Bruch's membrane; the choriocapillaris; the stroma, which consists of cells of various types surrounded by abundant intercellular substance and prominent, medium-sized vessels; and the suprachoroidea, which in birds consists largely of thin-walled vessels described previously as "lacunae"[95] and the membrana fusca (Figs. 1, 2, 3A, 4).

In the light microscope, the Bruch's membrane is recognized easily between the choriocapillaris and the retinal pigmented epithelium (Figs. 1, 5A, 5B). The capillaries are localized only in the area above the retina and are organized in a monolayer apposed closely to the Bruch's membrane (Figs. 1, 2, 4, 5A, 5B).

The stromal vascular bed consists primarily of numerous arterioles and venules, which communicate with arteries and veins in the cartilaginous sclera and the fibrous episcleral tissue and with the capillaries of the choriocapillaris layer (Figs. 1A, 2A, 3A, 4, 5A). The episcleral and scleral vessels are the largest and often are surrounded by pigmented cells (not shown). The episcleral arteries, which include the cerebral ophthalmic, internal carotid ophthalmic, posterior cerebral, ethmoidal, and stapedial arteries, are anastomosed and the short ciliary arteries, which supply the choroid, are derived primarily from the ophthalmic branch of the stapedial artery.[111] The scleral veins fed

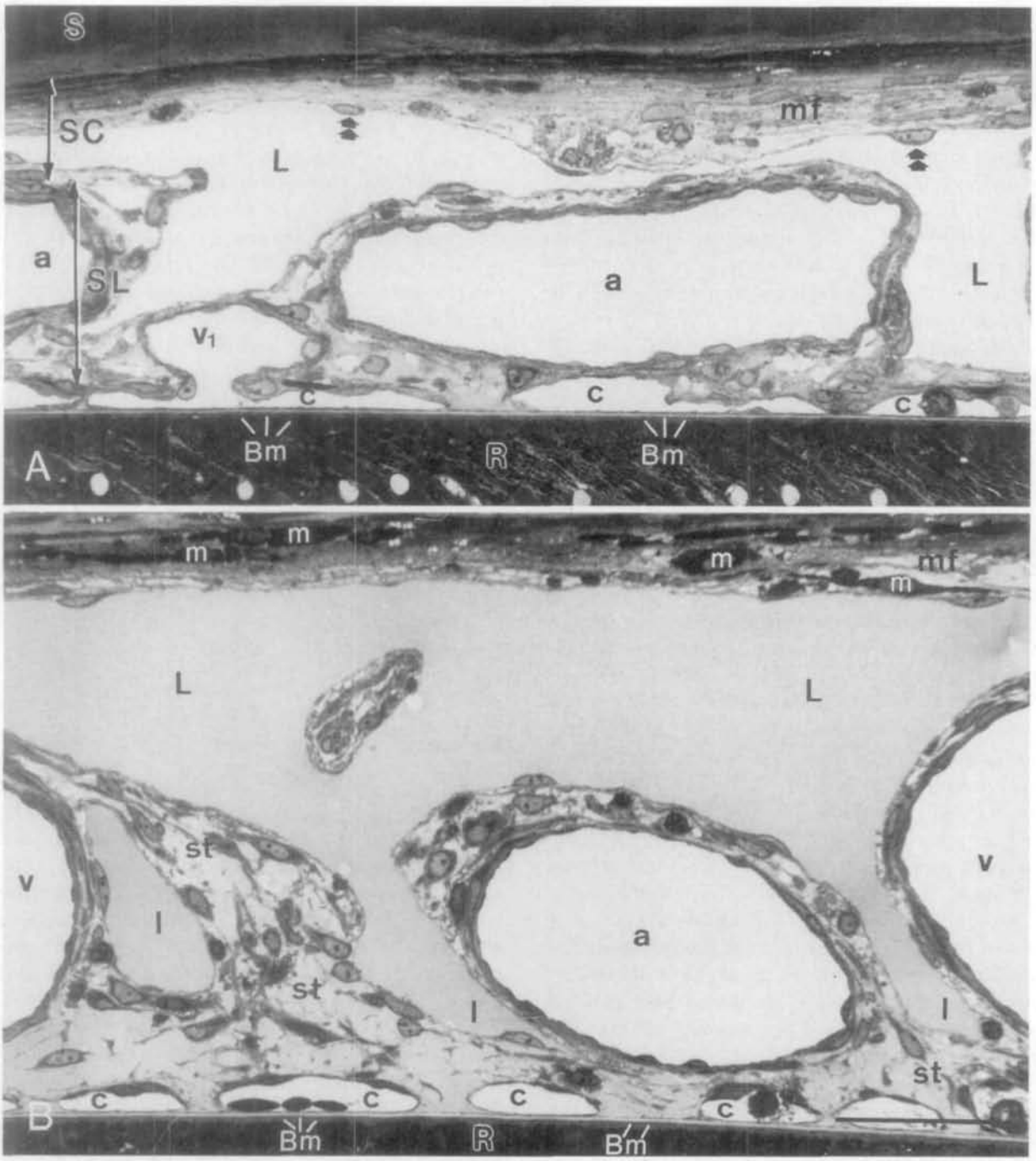

FIGURE 1. Light micrographs of semithin sections of the avian choroid, after perfusion fixation at normal delivery pressure (**A**), and after perfusion fixation at low delivery pressure followed by microwave irradiation (**B**). S = sclera; SC = suprachoroidea (formed by the membrana fusca [mf] and the large lacunae [L]); SL = stromal layer; c = choriocapillaris; Bm = Bruch's membrane; and R = retina. (**A**) A large lacuna in the inner part of the suprachoroidea forms blind-ended branches situated between the blood vessels of the stroma and adjacent to the choriocapillaris. Two arterioles are indicated by a. The blood capillaries form a single layer above the Bruch's membrane, and one of them opens into a small venule (v_1). (*double block arrows*) Endothelial cell nuclei bulging into the lumen of the lacuna. (**B**) A homogeneous precipitate completely fills the lumen of both the large and small (l) lacunae. Small lacunar branches insinuate themselves between the blood vessels (a = arteriole; v = venules) in the stroma. The blood vessels and the lacunae are sustained by trabeculae of supporting tissue (st). Several melanocytes (m) are observed in the membrana fusca. Scale bar = 50 μm.

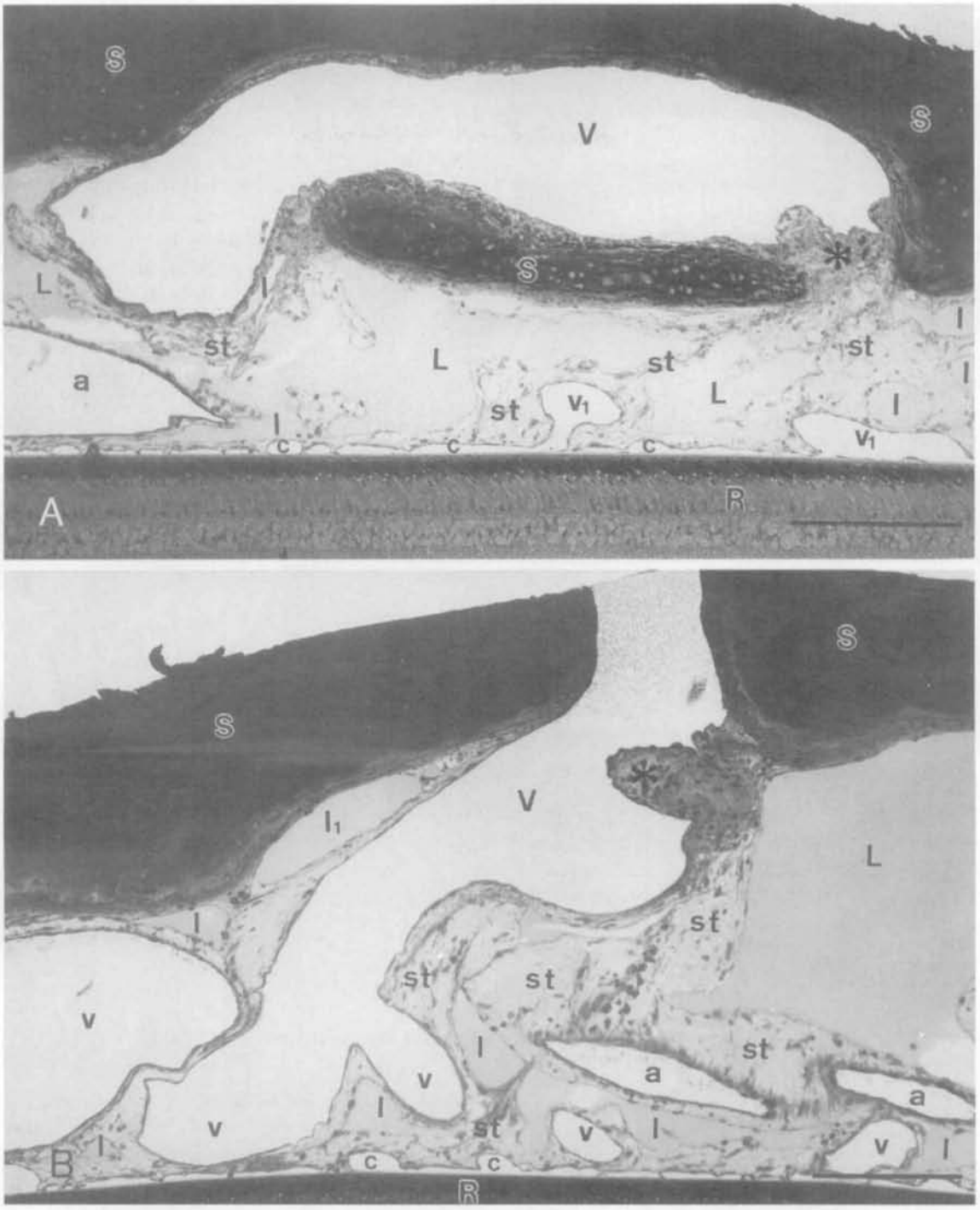

FIGURE 2. Light micrographs of semithin sections of the choroid after perfusion fixation at low delivery pressure. S = sclera; R = retina. (**A**) A large vein (V) runs in the sclera. One side branch has pierced the sclera on the left side of the micrograph. On the opposite side, a villous structure (asterisk) arising from the venous wall bulges into the lumen. Immediately below the large vein, an extensive system of large lacunae (L), bordered by bridges of supporting tissue (st), occupies most of the suprachoroidea and intrudes into the stromal layer. Profiles of the capillary net merging into venules (v_1) are indicated by c. l = small lacunae; a = arteriole. (**B**) A vein (V) crosses the entire choroid and the sclera. A large lacuna (L) and smaller lacunar branches (l) are present in the surrounding area; a small branch (l_1) lies along the vein wall, next to its exit through the sclera. On the right side, the wall of the vein enlarges into a cell plug (*asterisk*), which protrudes into the vessel lumen. Some of the capillaries are indicated by c. a = arterioles; v = venules; st = supporting tissue. Scale bar = 200 μm.

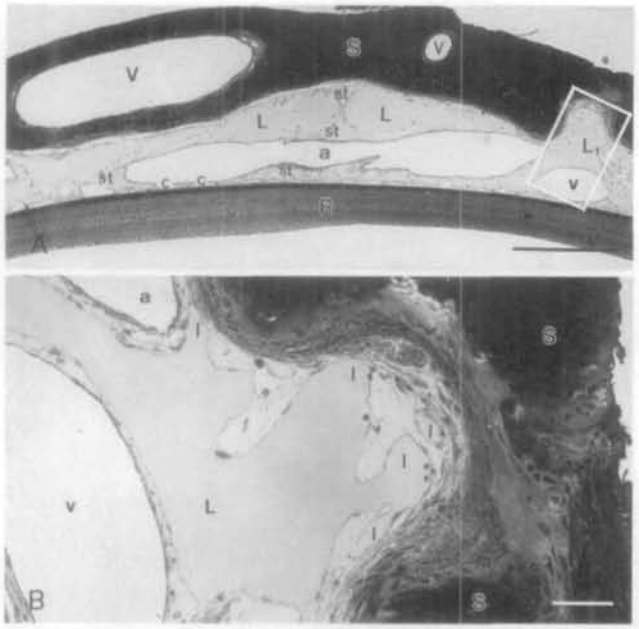

FIGURE 3. Light micrographs of semithin sections of the choroid after perfusion fixation at low delivery pressure. (**A**) In the boxed area, a lacuna (L_1) branches and pierces the sclera (S) near two veins (V). In the choroidal stroma, an arteriole (a), merging into the capillary (c) net, is separated from the extensive system of lacunae (L) by bridges of supporting tissue (st). v = venule; R = retina. Scale bar = 400 μm. (**B**) Higher magnification of the boxed area in **A**, turned approximately 70°. The large lacuna (L) sends smaller branches (l) inside the sclera (S). a = arterioles; v = venule. Scale bar = 50 μm.

by choroidal veins form the vortex system, in which several veins converge into a single large vessel. Within the choroid, the larger arteries and veins (Figs. 2A, 2B, 3A) are clearly recognizable from each other. The arteries, which in cross section show a circular outline, are usually smaller than are the veins, their muscular wall is thicker, and the endothelial cell nuclei protrude distinctly into the lumen. Compared with those of the arteries, the veins usually have a wider caliber and their endothelial cell nuclei bulge less inside the lumen (Figs. 1, 2, 3, 4, 5A). Moreover, the veins often are surrounded by a more conspicuous tunica adventitia. Similar distinguishing features generally characterize the arterioles and venules in the stroma, although it is not always possible to classify each vessel in the light microscope. The walls of medium-sized and large veins exiting from the eye bulb show peculiar villous structures, reminiscent of arachnoidal villi (Figs. 2, 6). These appear as large cellular plugs penetrated by diverticula of the venous lumen and by thin-walled vessels interpreted as small branches of the lacunae (see below).

The system of large lacunae in the suprachoroidea initiates from small, blind-ended lacunar branches, or capillaries, situated near the choriocapillaris that enlarge as they enter the choroidal stroma between arterioles and venules and then merge to form the lacunae of the suprachoroidea (Figs. 1, 2, 3A, 4, 5A, 5B). The choroidal lacunae are easily distinguishable from arteries and veins because they have an extremely thin endothelial wall. Breaks of the endothelial lining occur only in specimens with obvious mechanical damage of the supporting tissue. Such artifacts are common despite the care taken to minimize push–pull movements during dissection of the tissue blocks. Moreover, the lacunae contain in their lumina a light precipitate that, after perfusion fixation, may appear

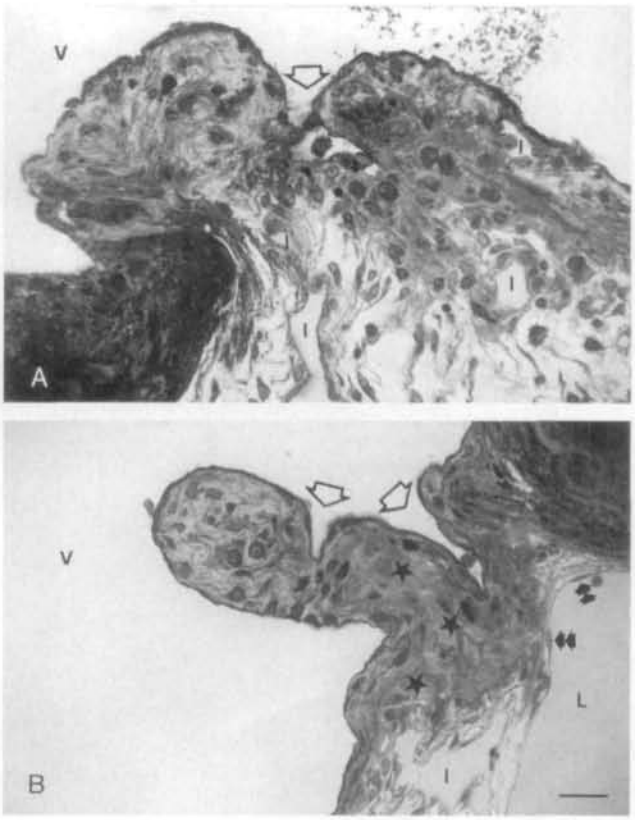

FIGURE 4. Light micrographs of semithin sections of the choroid from a normal chicken after enucleation and en bloc fixation (**A**), and from an experimental chicken subjected to paracentesis of the anterior eye chamber, followed by perfusion fixation and microwave irradiation (**B**). R = retina; S = sclera. (**A**) The lumina of the blood vessels (a, a_1 = arterioles; v, v_1 = venules; c = capillaries) contain numerous blood cells. Some erythrocytes (*arrows*) also are seen in one of the large lacunae (L). Three venules (v_1) and an arteriole (a_1) merge into the capillaries. l = small lacunae; mf = membrana fusca; m = melanocytes. (**B**) Both the large lacunae (L) and their smaller branches (l) appear engorged with blood cells. Because of the perfusion fixation, all the blood vessels (a = arteriole; v = venules) are completely cell free and clear. The arteriole and one of the venules (v_1) communicate with capillaries (c, *open block arrows*). The outer part of the suprachoroidea (sc) is extremely dilated, and the membrana fusca is not evident. Scale bar = 100 μm.

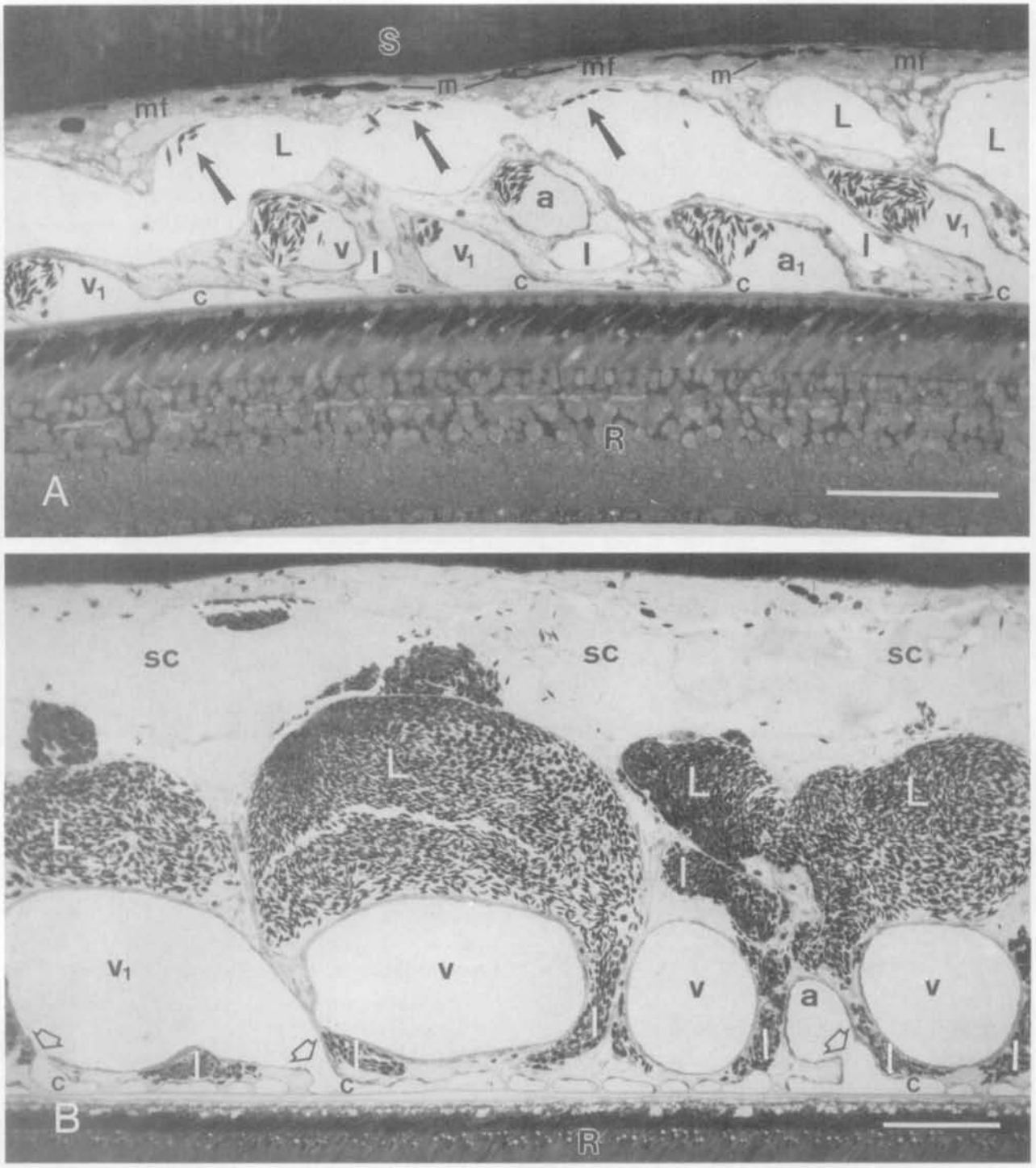

FIGURE 5. Light micrographs showing details of blood vessels and lacunae of the eye choroid from experimental chickens subjected to paracentesis of the anterior eye chamber, followed by perfusion fixation and microwave irradiation. Bm = Bruch's membrane. (**A**) Branches (l) of large lacunae (L), filled with blood cells, reach the choriocapillaris. The blood vessels (v, v_1 = venules; a_1 = arteriole) are clear after perfusion. One venule (v_1) and one arteriole (a_1) open into the capillaries. (**C**) (*double block arrows*) Nuclei of the lacunar endothelial cells. (**B**) Anterior part of the eye bulb. Here the choroid shows only small and sparse lacunae (l); the blood capillaries (C) are enlarged. Lacunae are filled with blood cells, and the suprachoroidea (sc) is dilated. (*double block arrow*) Nucleus of a lacunar endothelial cell. R = retina. Scale bar = 25 μm.

patchy. In specimens post-fixed in the microwave oven, this precipitate is more dense and homogeneous and fills the entire lacunar lumen (Fig. 1B). This precipitate lacks in both arterioles and veins, whose content was replaced completely or almost completely by fixative during perfusion fixation.

Communication between arteries and lacunae was never observed. By contrast, at the points in which the vortex veins leave the choroid, a consistent aggregation of lacunae is present (Fig. 2). Both large- and small-sized lacunae surround the area in which the medium-sized veins approach the sclera, and small lacunae enter the sclera itself and reach the outer perimeter of the wall of large veins. In the eyeballs fixed in situ (i.e., without perfusion), we noted occasional erythrocytes in the lacunae (Fig. 4A). This observation suggested that lacunae are connected with veins and that blood cells may have backflowed into the lacunae because of a drop of the IOP after decapitation. To investigate this hypothesis, we performed a paracentesis of the anterior chamber, a procedure known to cause a dramatic drop in the IOP.[112] We observed in vivo that, during the 10 minutes of paracentesis, the iris blood vessels in the eyes operated on were clearly dilated. After fixation of these experimental eyes, all the lacunae, large and small, appeared engorged completely with blood cells in a series of semithin sections covering the entire eyeball perimeter (Figs. 4B, 5A, 5B); the outer portion of the suprachoroidea was extremely dilated (Fig. 4B), whereas the choroidal arteries and veins did or did not contain blood cells, depending on whether the chickens were killed by decapitation or by perfusion (Figs. 4B, 5A, 5B). In the contralateral control eyes of chickens fixed by perfusion, the choroidal structure was compact and the lacunae contained an acellular precipitate and were clear of blood cells, as observed in normal specimens.

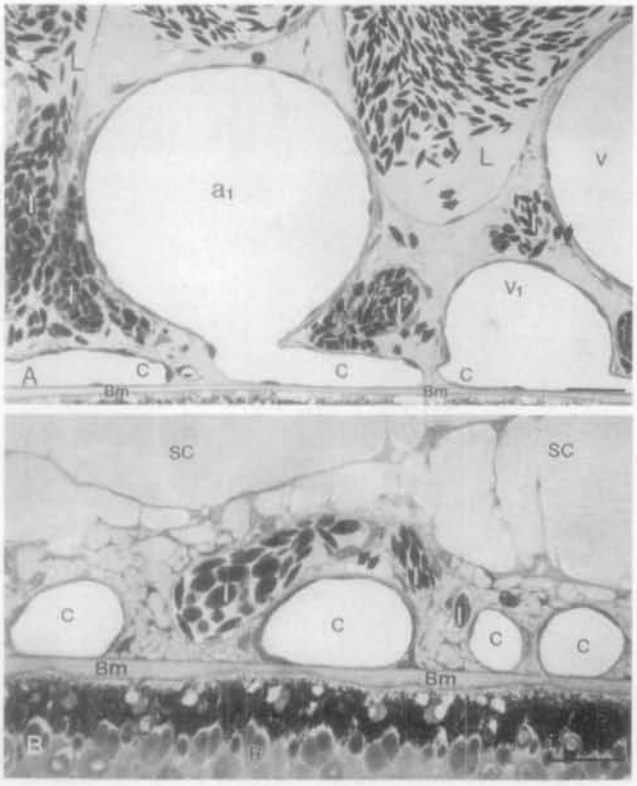

FIGURE 6. Cell plugs, or villi, located in the wall of large veins (V), may represent the sites where lacunae open into veins. The plugs contain various types of cell, including plasma cells, which are round or ovoidal and appear darkly stained, and smooth muscle cells, which are lightly stained and appear irregular in shape. (**A**) Light micrograph from a section serial to the one depicted in Figure 3A. The cell plug, located at a branching point of a large vein, bulges into the vessel's lumen. Small lacunae (l) are situated deeply within the cell aggregate. A diverticulum of the venous lumen is indicated (*open block arrow*). (**B**) Light micrograph from a section serial to the one depicted in Figure 3B. This cell plug, whose root is formed mostly by strands of smooth muscle cells (stars), is approached by a large lacuna (L) and a small lacuna (l) with a scalloped perimeter. (*open block arrows*) Diverticula of the venous lumen (V). (*double block arrows*) Endothelial cell nuclei. Scale bar = 20 μm.

Both the blood vessels and the lacunae are surrounded by a network of connective tissue and smooth muscle cells that forms bridges, termed trabeculae, which support the vessels in a loosely arranged structure (Figs. 1B, 2, 3A).

The outer portion of the suprachoroidea is occupied by the membrana fusca, a multilayer of thin and elongated cells closely apposed to each other, that separates the soft choroidal coat from the hard sclera (Figs. 1, 4A). Among the flat cells, we also observed fusiform melanocytes (Figs. 1B, 4A), occasional extravasated blood cells, and bundles of varisized myelinated axons.

Electron Microscopy

Capillaries, Arterioles, and Venules. In this section, we describe only briefly the organization of the capillaries and the small blood vessels in the choroid. For a detailed ultrastructural description, refer to a previous article.[108] The avian choriocapillaris consists primarily of a single layer of fenestrated capillaries (Fig. 7). The fenestrations are organized primarily in clusters and appear distinctly polarized, occurring almost exclusively on the aspect of the endothelial lining facing the retina (inset to Fig. 7A).[108]

Arterioles and venules consist of an inner layer of endothelial cells, surrounded by a thin muscular tunica that usually is represented by one (venules, Fig. 7A) or more (arterioles, Fig. 7B) layers of circular smooth muscle cells, whose processes overlap and contact each other. The circular muscular tunica is not always continuous, and large gaps between one cell and another often are observed (Fig. 7B). Collagen

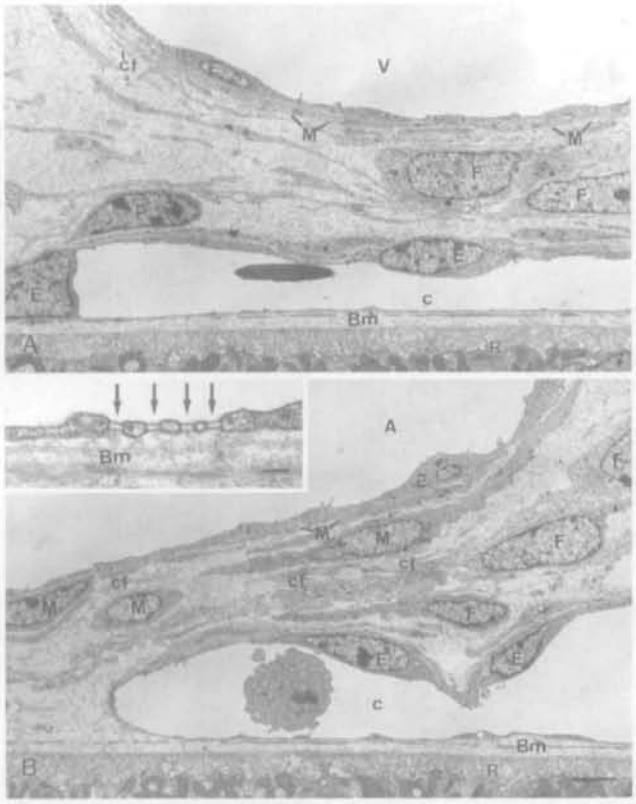

FIGURE 7. Electron micrographs showing portion of a venule (**A**) and an arteriole (**B**) in the stromal layer of the choroid. V = venule; A arteriole. Both vessels are located close to the choriocapillaris. The inner wall of the capillaries (c) faces the retina (R) and lies adjacent to Bruch's membrane (Bm), whereas the outer wall contains the endothelial cell nuclei. The venule shows a thin endothelium partially underlined by slim processes of smooth muscle cells, whereas in the wall of the arteriole, the endothelium bulges slightly into the vessel's lumen and the muscular coat is more prominent. Fibroblasts (F) and bundled or isolated collagen fibers (cf) fill the spaces among the vessels. E = endothelium; M = muscle cells; P = pericyte. Scale bar = 2 μm. (*inset*) Clusters of fenestrations (*arrows*) are distributed along the inner side of a capillary endothelium. Bm = Bruch's membrane. Scale bar = 0.1 μm.

fibers and fibroblasts are the common components of the tunica adventitia (Fig. 7).

Lacunae

The lacunae are distinctly different from ordinary blood vessels. They are lined exclusively by a thin endothelial wall (Figs. 8, 9A, 10), not only in the suprachoroidea but also where they penetrate among the blood vessels in the middle portion of the choroid. As mentioned above, their size and shape are variable, ranging from large lacunae (Fig. 9A), which are distributed primarily in the area closest to the membrana fusca, here referred to as the suprachoroidea, to small lacunae (Fig. 8A), which are localized primarily in the choriocapillaris or in the wall of large blood vessels. The endothelial cells are extremely thin, and the only thick region of their cell bodies is the perinuclear portion (Figs. 8). The nuclei are elongated and surrounded by scarce cytoplasm containing mitochondria, small portions of the Golgi apparatus, vesicles of different types, occasional Weibel–Palade bodies, slender cisterns of rough endoplasmic reticulum, and free polyribosomes (Fig. 8B). The endothelial cell becomes abruptly velate in addition to the perinuclear region and lacks a continuous basal lamina (Figs. 8, 9A, 10). Occasionally, small bundles of microfilaments are distributed underneath the plasma membrane of the thin cellular portions. Basal lamina components are observed in correspondence to these points (Fig. 10B), suggesting that these are spots of interaction between extracellular matrix, endothelial plasmalemma, and cytoskeleton. The thin edges of adjacent cells processes overlap for long tracts or interdigitate with different degrees of complexity (Fig. 9B). These processes usually contact each other at many points along the appositional area through small, macular, adherent junctions and occasional punctiform gap junctions (Fig. 9C). Both large and small lacunae show fenestrations, randomly distributed along the vessels perimeter, that open in the surrounding connective tissue (Fig. 8B, inset to Fig. 8B). The fenestrations are much less numerous than those occurring in the blood capillaries, are always monodiaphragmatic, and usually are not clustered.

The endothelium of the larger lacunae is bordered by elements of the stroma. Both melanocytes (Fig. 9A) and fibroblasts may lie close to the lacunae, and their processes follow the endothelial cell lining for long tracts. The trabecular smooth muscle cells also approach the lacunae and are sometimes oriented parallel to the endothelium (Figs. 9A, 10), but they never form a continuous layer or tunica. The smooth muscle cells may send short appendages toward the endothelial cells and abut them at points (Fig. 10). Bundles of unmyelinated axons and clusters of synaptic boutons, usually flanking stromal smooth muscle cells, are observed occasionally near the lacunae (Fig. 10A). Bundles of collagen and elastic fibers are distributed randomly along the endothelial cell perimeter, often disposed in between the endothelium and the nearby smooth muscle cells, fibroblasts, and melanocytes (Figs. 8A, 9A). These aggregates may contribute to maintain patency of the thin-walled lacunae. The lacunae situated in the scleral matrix have a fine structure similar to those situated in the suprachoroidea and in the stromal layer, with the exception that their endothelial cells are slightly thicker, the fenestrations less numerous, and the interdigitations formed by the processes of two adjacent endothelial cells more complex.

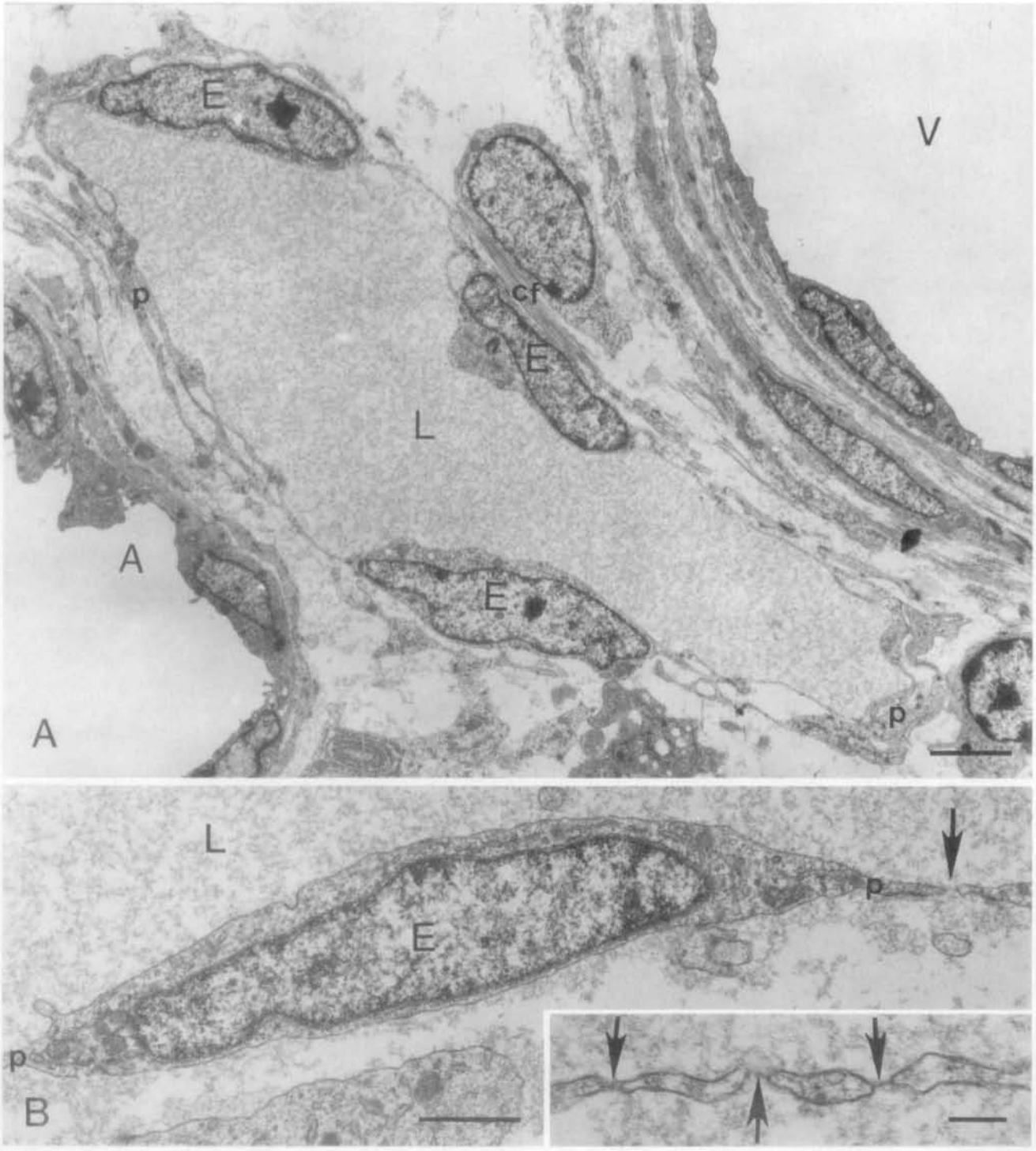

FIGURE 8. (**A**) Electron micrograph showing a small lacuna (L) of the stromal layer, situated between an arteriole (A) and a venule (V). The lacunar capillary is lined by an extremely thin endothelium (E). The processes (p) of adjacent endothelial cells loosely overlap for long tracts, and the nuclei bulge into the lumen. A thick bundle of collagen fibers (cf) lies underneath a portion of the endothelium. Scale bar = 2 μm. (**B**) Higher magnification of a lacunar endothelial cell (E). The heterochromatic nucleus occupies a large portion of the cell body, and the cell becomes abruptly thinner (p) at both paranuclear regions. The cytoplasm contains free ribosomes, short cisterns of rough endoplasmic reticulum, and few mitochondria. The arrow points to a fenestration. The endothelial cell is surrounded by a flocculent precipitate but lacks a basal lamina. L = lacunar lumen. Scale bar = 1 μm. (*inset*) Fenestrations (*arrows*) are scattered along a thin process of a lacunar endothelial cell. Scale bar = 0.1 μm.

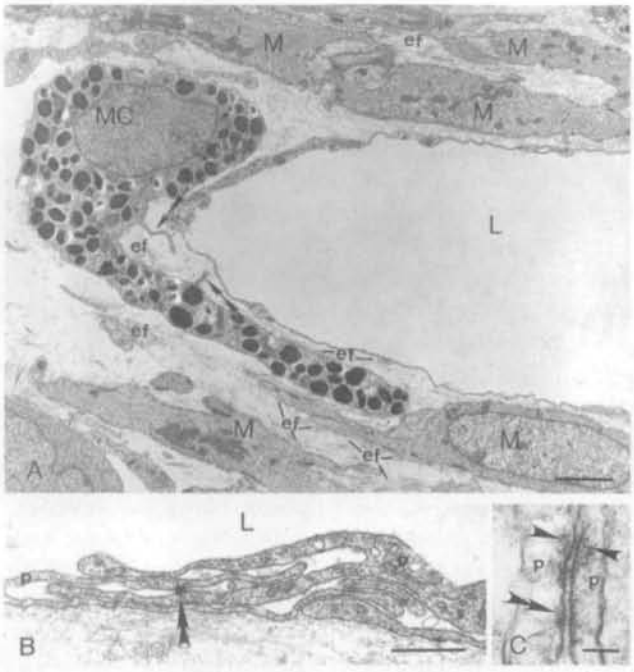

FIGURE 9. (A) This lacuna (L) of the choroidal stroma is approached by a large melanocyte (MC), which underlines with one cell process the thin endothelium. The melanocyte approaches the lymphatic endothelium with two finger-like appendages (*double arrows*). Smooth muscle cells (M) of the stromal-supporting tissue approach the lacuna. Small bundles of elastic fibers (ef) are present between the endothelium and the melanocyte and among the neighboring smooth muscle cells. Scale bar = 2 μm. (**B**) Complex interdigitations between the processes (p) of two adjacent endothelial cells of a large lacuna (L) in the suprachoroidal layer. A small punctum adherens is indicated (*double arrowhead*). Scale bar = 0.5 μm. (**C**) A punctiform gap junction (*arrowheads*) and an adherens junction (*double arrowhead*) are established between two overlapping processes (p) of neighboring endothelial cell of a lacuna. Scale bar = 0.1 μm.

Some of the lacunae intrude into the wall of large veins entering the sclera (Fig. 11) and give rise to smaller branches that lie close to the venous endothelium. These lacunar branches differ from those in the choriocapillaris layer by having slightly thicker endothelial cells whose cytoplasm is enriched in pinocytotic vesicles, by displaying a more substantial, but still discontinuous, basal lamina, and by the rarity or absence of fenestrations. Where the thin lacunar branches approach the lumen of the large veins, the two endothelia abut each other at points that also show interruptions of the basal lamina (inset to Fig. 11B). The lacunae found inside the venous wall also may emanate side branches that protrude into the venous lumen (Fig. 11B). At the angles between the protruding lacunae and the venous endothelium, the latter appear differentiated morphologically (Fig. 12). The modified endothelial cells have large, highly indented, and heterochromatic nuclei and protrude toward the intima (Fig. 12) from which they are separated by a distinct basal lamina (Fig. 12B); moreover, their cell cytoplasm is less electron dense and richer in microfilaments than that of standard venous endothelium, and the luminal and intimal sides of the cells are provided with numerous microvilli (Fig. 12B). The lateral portions of the modified cells interdigitate with neighboring standard endothelial cells (Fig. 12B). In correspondence to the modified cells, the connective tissue is enriched with collagen fibers (Fig. 12). Several small lacunar branches also penetrate into the cell plugs, described in the light microscopy section above, and approach the vessel's endothelium. The cell plugs are characterized by an intricate net of different types of cell, such as fibroblasts, extravasated lymphocytes, and plasma cells, and, occasionally, also flat cells of the membrana fusca and melanocytes embedded in a highly collagenous and elastic matrix (not illustrated).

DISCUSSION

We have shown that although several aspects of the vasculature of the avian choroid are clearly similar to

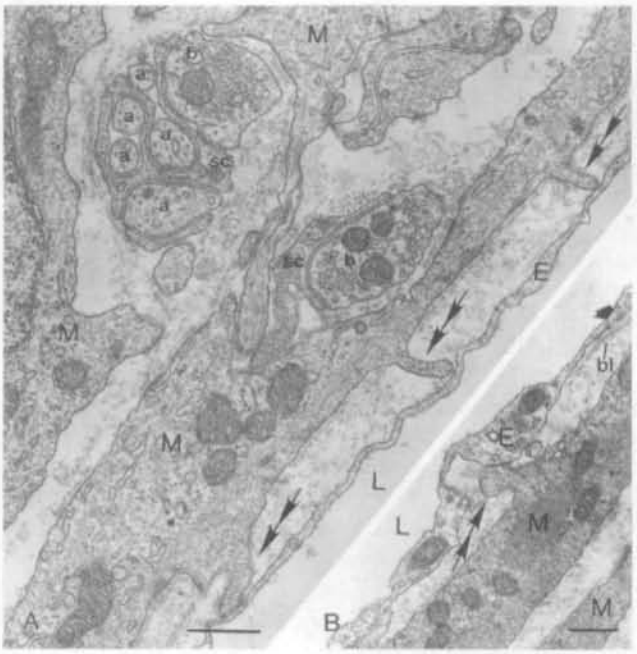

FIGURE 10. Smooth muscle cells (M) of the supporting tissue in the stromal layer approach the endothelium (E) of two lacunae (L) with thin appendages (*double arrows*). (**A**) Preterminal axons (a) and terminal boutons (b) containing synaptic vesicles are situated close to smooth muscle cells and are surrounded partially by Schwann cells processes (sc). (**B**, *block arrow*) Site at which an endothelial cell process appeared at higher magnification to contain a small bundle of microfilaments abutting the plasma membrane, in correspondence to extracellular basal lamina material (bl). Scale bar = 0.5 μm.

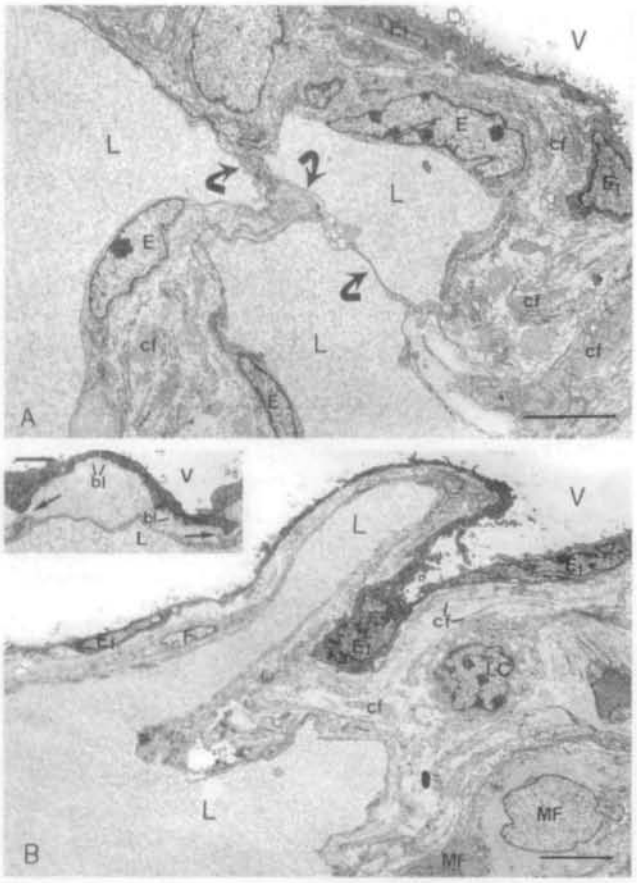

FIGURE 11. Electron micrographs showing branches of lymphatic lacunae (L) distributed in the wall of a large scleral vein (V). (**A**) A lymphatic lacuna splits into three smaller branches, whose endothelial cells (E) processes merge and overlap one another (*curved arrows*). Numerous bundles of collagen fibers (cf), oriented at different angles, are distributed in the surrounding connective tissue. (**B**) A lymphatic lacuna branches and protrudes into the vein's lumen. E_1 = endothelial cells; MF = cells of the membrana fusca; F = fibroblast; LC = lymphocyte; cf = collagen fibers. Scale bar = 5 μm. (*inset*) The endothelial of a lymphatic lacuna and a large vein abut each other (*arrows*). bl = basal lamina. Scale bar = 1 μm.

those in the mammalian choroid, there is a major difference; namely, the avian choroid contains a conspicuous system of thin-walled lacunae, which, in all probability, represent short lymphatic vessels of the posterior eye bulb.

Blood Vessels of the Choriocapillaris

The layering of the avian choroid is not greatly different from that described in mammals.[6,7] The fenestrated vessels of the choriocapillaris form a single layer immediately adjacent to the Bruch's membrane, whereas numerous arterioles and venules are situated in the stromal layer, ensuring a large blood supply. Nourishment of the retina and dissipation of heat caused by light stimulation of the photoreceptors, therefore, may be accomplished as done with mammals.[1-4,9-12] Fenestrated capillaries are found in organs, such as glands and kidney glomeruli, in which there are functional requirements for rapid movement of fluids into or out of the vessels.

In mammals, tissue fluids in the choroid have a high content of plasma proteins and engender a gradient of oncotic pressure, 12 to 14 mm Hg in rabbit,[13] that promotes the filtration of fluids from the retina into the choroid.[1,2,4] Because the retinal hydrostatic pressure is slightly higher than in the choroid, fluids filter in the same direction.[1,2] The capillaries control the net flow balance in the choroid: when the blood flow in the choroid vessels is reduced, the fall of hydrostatic pressure facilitates the resorption of tissue fluids into the capillaries; the opposite condition reverses this tendency. Endothelial fenestrations ensure that this pathway is patent even to large molecules such as proteins. Paracellular diffusion also may occur between adjoining endothelial cells, because the tight junctions in the choriocapillaries are of the discontinuous, or rather leaky, type.[113] Fluids in the choroid stroma (e.g., local tissue fluid, retinal fluids, and amounts of aqueous humor from the anterior chamber) that are not reabsorbed into the capillaries leave the eye seeping into areas with a lower hydrostatic pressure, such as scleral tissue and perivascular and perineural spaces, until they reach the episcleral tissues.[1,4,5,17-21]

No corresponding data are available for the avian eye, which has an avascular retina. Birds, however, have a deep source of fluids from the vascular system of the pecten oculi. This unique structure consists primarily of capillaries and pigmented stromal cells[114] and may have evolved in relation to the high level of metabolic requirements and visual acuity of the avascular avian retina.[96] The arterial and venous system of the pecten are separated completely from that of the choroid and are associated with extrabulbar specializations, such as the rete mirabile pectinis and ophthalmicum and arteriovenous anastomoses, that may ensure constant pressure. The pecten basilar vein opens into a sinus surrounding the optic nerve. The rich vasculature of the pecten provides nutrients to the inner layers of the retina without impairing visual acuity. In addition, the pigmented stromal cells presumably have a secretory function. Thus, the pecten complements the ciliary processes in providing a continual production of aqueous humor. This also is indicated by the analogous alterations of these two structures in chickens treated with acetazolamide.[115]

To our knowledge, the gradient of extracellular fluid pressure in the avian retina and the parameters of tissue fluid movement in the avian choroid have not been measured, and we can only speculate about the functional implications of our data. The presence

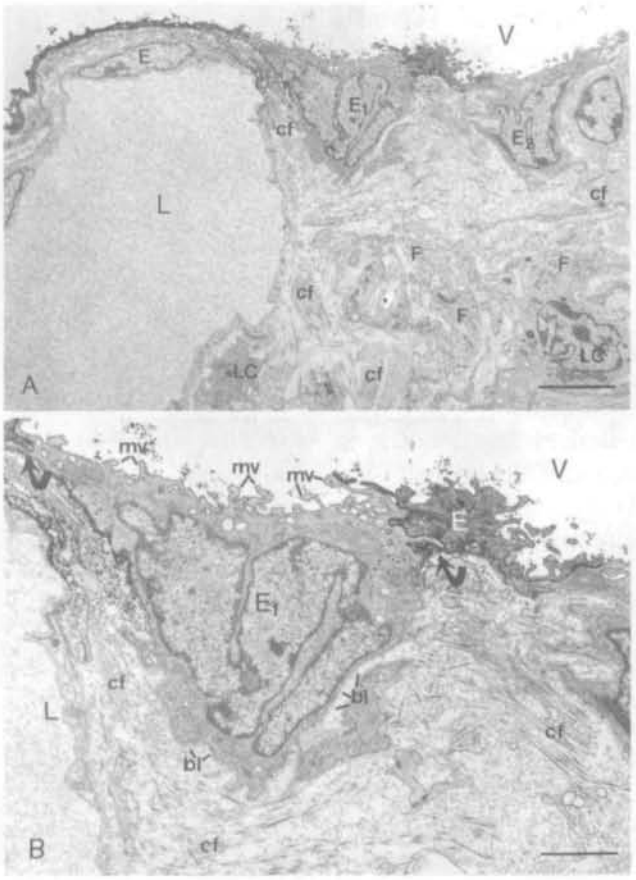

FIGURE 12. Electron micrographs showing specializations of the endothelium of a large scleral vein (V). (**A**) Two special cells (E_1 and E_2) form part of the endothelial lining of the large vein in proximity of a lymphatic lacuna (L). Their indented, highly heterochromatic nuclei protrude toward the surrounding connective tissue. LC = lymphocytes; F = fibroblast processes; cf = collagen fibers. Scale bar = 5 μm. (**B**) Higher magnification of the cell labeled E_1 in **A**. The edges of the cell interdigitate with those of the neighboring standard endothelial cells (E, *curved arrows*). The luminal side of the plasma membrane shows several microvilli (mv), and the rest of the cell surface is indented irregularly and flanked by a thick and continuous basal lamina (bl). L = lacuna; cl = collagen fibers. Scale bar = 2 μm.

of fenestrations in the vessels of the choriocapillaris would suggest that oncotic pressure is higher in the choroid than in the retina in birds also. It is possible that the endothelial lining's tight junctions of the avian choriocapillaris vessels are also of the "very leaky" type, as are those in the human choriocapillaris.[113]

In another article, we show that fenestrations occur at high linear density (nearly 1/μm), but usually only on the side of the capillaries facing the retina.[108] The fenestrations must represent the main functional "pores" in the endothelium of the choriocapillaris vessels. Because fenestrations face the retina, we propose that their primary function is to exchange fluids with the retina. As argued below, the presence, in the avian eye, of another prominent system of thin-walled and fenestrated vessels, the lacunae, which is thought to have a lower luminal pressure than that of the choriocapillaries, leads us to hypothesize that resorption of fluids takes place predominantly through this second system.

Lymphatic Vessels

Walls[99] observed in the avian choroid a thickened region, lying between the pigmented lamina fusca and the choriocapillaris, that had a "sinusoidal" structure, and he left open the question on the nature of these vessels. More recently, Meriney and Pilar,[95] in their study on the distribution of cholinergic fibers in the chick choroidal coat, made a brief morphologic reference to the lacunae, which make up ". . .most of the choroidal volume" and ". . .are connected to arterioles by narrow openings formed by endothelial cells wrapped by innervated smooth muscle cells." They suggested that ". . .the lacunae serve as a liquid reservoir and regulate IOP by filtering fluid out of the blood vessels." Our studies are in partial agreement with their observations and conclusions. We confirm the importance of the lacunae as a fluid space in the choroid, but we observed presumptive communications of the lacunae with scleral veins, and not with choroidal arterioles and scleral arteries. This confirmation also is based on the observations that the lacunar content was not removed by the fixation perfusate delivered through the arterial system under moderate pressure and that backflow of blood into the lacunae occurred when the IOP was decreased because of paracentesis.

Our observations led us to the novel interpretation of the lacunae as a system of short lymphatics devoted primarily to the drainage of the back of the eye bulb as follows:

1. All the lacunae, irrespective of their caliber, have a fine structure identical to that of lymphatic vessels.[116,117] The extremely thin endothelium; the absence of well-defined basal lamina, muscular tunica, and innervation; and the presence of fenestrations scattered along the entire vascular perimeter are major morphologic characteristics that differentiate these lacunae from those of blood vessels.
2. The lacunae lay mostly outside the stromal layer, which contains a substantial number of blood vessels of varying caliber. Although the wider lacunae originate from smaller branches that ramify and penetrate toward the innermost part of the choroidal coat, they can be considered primarily as part of the suprachoroidea.
3. In addition to the inner, small lacunar branches

that reach the choriocapillaris, there are small lacunar branches in the wall of the blood vessels, especially the large scleral veins. These outer branches are ramifications of large lacunae that pierce the sclera and actually may open into the lumen of large scleral veins through villous structures reminiscent of arachnoidal villi, in which the virtual communications are difficult to show morphologically. The finer mechanisms for the communication between lymphatics and veins of the avian eye and its functional regulation, thus, remain to be clarified.

4. There are observations that in perfused specimens post-fixed in the microwave, the lacunae contain an acellular precipitate that fills the entire lumen and may represent coagulated lymph. These observations contribute to their classification as lymphatics. We exclude that this acellular precipitate represents stromal proteins filtered through the fenestrations and translocated by microwave irradiation, because no such precipitate was ever observed in the vessels of the choriocapillaris, which are densely fenestrated. Furthermore, there are no reports of protein translocation into vascular lumina in the microwave fixation literature.[109] The lacunar precipitate also was observed in several specimens prepared by perfusion fixation under moderate delivery pressure.
5. The lacunae become progressively smaller and less numerous toward the optic nerve, the pecten oculi, and the anterior chamber angle, and thus they do not represent an extension of the Schlemm's canal.
6. In mammals, including nonhuman and human primates, a "lymphatic" pathway is represented by the Schlemm's canal and its tributaries, but this serves primarily the anterior portion of the eye.

In a study on the effects of paracentesis on the blood–aqueous barrier in the primate eye, Raviola[112] showed that after lowering the IOP by emptying the anterior chamber, there is a distinct backflow of blood cells and an accumulation of intravenously injected human retinal pigment in the Schlemm's canal. Earlier, Abelsdorf and Wessely[99] had found that when the anterior chamber of a bird's eye was drained, the choroid thickened enormously through engorgement, as demonstration of the high plasticity of this structure. In addition, in our experiments, after paracentesis, a massive blood backflow occurred in all the choroidal lacunae, including the smallest lacunar branches near the choriocapillaris; furthermore, the region between the choroid and the sclera, in correspondence to the membrana fusca, became enormously dilated. The latter phenomenon may be the result of an outflow of fluids between the lacunar endothelial cells and through their fenestrations, under the pressure of the sudden blood influx. The lacunae were engorged with blood cells even if the birds were fixed by perfusion, whereas arteries and veins had completely clear lumina, suggesting that a valve-like apparatus separates the lacunae from the veins. In the contralateral eyes, used as control specimens, the lacunae usually were devoid of blood cells in both perfused and nonperfused chickens.

Taken together, these results strongly support the identification of the lacunae in the avian choroid as lymphatic vessels and indicate that the avian choroid is substantially different from the mammalian choroid. Furthermore, these data support the notion that the lacunae and the veins merge together at some point. This might happen either on the eye's outer surface, because large lacunae occasionally were seen to penetrate the sclera, exiting from the eye bulb in the vicinity of large veins, or in the outer portion of the choroid, because small lacunar branches enter deeply into the villous structures of the venous walls, approach the vessels endothelium, and protrude, in a characteristic way, into the lumen of large veins. The diverticula of the venous lumen that penetrate into the villi and the lacunar branches that abut the venous intima may mediate communication between lacunae and veins. The specialized endothelial cells that occur at the sites of contact between the lacunar branches and the venous endothelial lining also may be part of a complex valvular apparatus yet to be understood in finer detail. Obviously, the connections between lacunae and veins should be shown directly with a dynamic method. Yet, our data strongly suggest that the lacunar vessels represent a well-developed system of short lymphatics, situated at the choroidoscleral interface and provided with a large fluid-carrying potential. This conclusion is schematized in the block diagram illustrated in Figure 13. The pressure propelling the lymph along these vessels and into the scleral veins may derive from various mechanisms (also see section below). The vis a tergo from the blind-ended lymphatic capillaries may be of primary importance. It is probable that the densely innervated smooth muscle cells that make up a substantial proportion of the stroma of the avian choroid[108] and the short lymphatics, both of which are absent in the primate eye, are functionally related, and that contraction of the trabeculae helps move the lymph into the veins. Data in the literature suggest that accommodation has a complex effect on the conventional and uveoscleral routes of fluid removal, and it is possible that this process could be involved in lymph circulation in the lacunar system. During accommodation in birds, the IOP increases by approximately 3 mm Hg,[118] which might alter the pressure

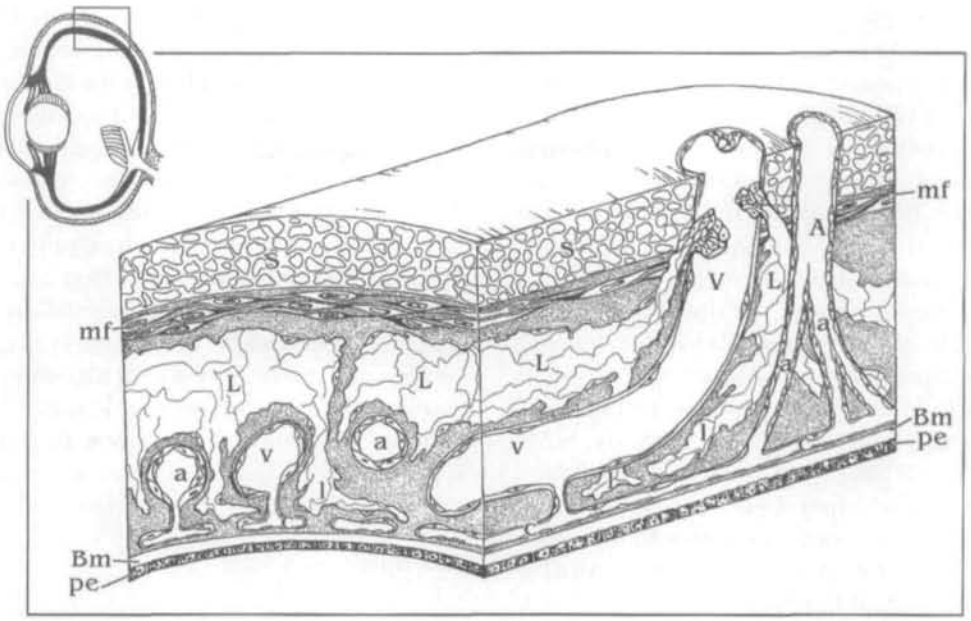

FIGURE 13. This block diagram illustrates a new interpretation of the organization of the vasculature in the choroidal coat of the avian eye. Veins (V) and arteries (A) traverse the eye wall from the sclera (S) through the suprachoroidal and stromal layers, where they branch into smaller arterioles (a) and venules (v), to the choriocapillaris, where they form a network of polarized capillaries (c) facing the Bruch's membrane (Bm). Large lymphatic vessels, or lacunae (L), occupy the suprachoroidea and branch into lymphatic capillaries (l) that reach the choriocapillaris. The lacunae also pierce the sclera, as do the blood vessels, and branch extensively, entering the wall of large veins, where they end in cellular plugs reminiscent of arachnoidal villi. According to this view, the lacunae represent a system of short lymphatics. mf = membrana fusca; pe = retinal pigment epithelium.

gradient between lacunae and veins, whereas fluid drainage through the conventional route would be allowed to continue by the accompanying dilation of the ciliary venous sinus through an active pull on the inner lamellae of the cornea and the outer wall of the sinus by the anterior muscle group of the ciliary region.[119]

Speculations on the Drainage of Intraocular Fluids in Normal and Abnormal Conditions

In the mammalian choroid, there are no lymph vessels, although the choroid itself is involved in the route of the aqueous humor drainage termed uveoscleral.[1-3,5,7,18-22,24-26] The aqueous humor, which is produced by the ciliary processes,[13,16,22,24,28] flows from the posterior chamber into the the anterior chamber from which it is removed continuously. The largest proportion of aqueous humor is drained into the Schlemm's canal at the iridocorneal chamber angle[2,3,5,7,21,22,24-26,120-122] and then passes through collector channels into "aqueous veins."[1-3,5-7] The aqueous veins,[27,28] which are filled with a clear fluid, connect the canal of Schlemm and its outlets to the deep scleral meshwork (intrascleral and episcleral veins). In the uveoscleral route, instead, the humor diffuses through the chamber angle tissue and the ciliary muscles into the supraciliary and suprachoroidal spaces, where it mixes with local tissue fluids. From here, fluids leave the eye bulb, filtering into the scleral tissue or into the spaces around large blood vessels and nerves, including the optic nerve.[1-3,17-19,26] Although the rate of aqueous outflow by way of the uveoscleral route usually is smaller than the one estimated for the Schlemm's canal, the state of contraction of the ciliary muscles is of great importance for its contribution to fluid removal: contraction of the muscles almost blocks the uveoscleral flow.[123] This relation indicates that the uveoscleral outflow is regulated partially by the process of accommodation.[20] Moreover, it has been shown that the rate of aqueous humor formation and its outflow by way of the Schlemm's canal is pressure dependent: an increase of the IOP results in a reduction of aqueous humor formation and in a parallel increase of fluid drainage from the anterior chamber,[20,24,123,124] whereas it has only a small effect on the outflow facility through the uveoscleral route.[20,21]

The corresponding processes of fluid removal in birds remain a matter of speculation. Birds, like mammals, have ciliary processes and a Schlemm's canal, also named ciliary venous sinus[97-99]; they differ from mammals, however, in having the peculiar, highly vas-

cularized pecten in the posterior portion of the eye bulb and, as shown here and in a separate article,[108] a large system of lymphatic vessels and stromal smooth muscle cells in the choroid. In birds, the aqueous humor outflow is high (2 to 2.5 μl/min),[125] but there are no data that specify the amount of aqueous humor that leaves the eye through either the conventional or the unconventional route. The choroidal lymphatics represent a truly substantial system, and we favor the hypothesis that they are useful not only for removing transretinal and local tissue fluids but also for draining part of the aqueous humor produced by the ciliary processes. This fluid drainage route may be of special auxiliary importance in diving or predatory birds, whose eye bulbs are subjected to shifting pressures, and may play a role in experimental eye conditions.

Recent studies have pointed out that in the recovery period after experimental myopia, the avian choroid expands considerably within a few days. Thickening of the choroid has the effect of moving the retina forward in compensation for the defocus.[66] The thickening seems to involve the suprachoroidea particularly. Because this sublayer corresponds to the region that contains the largest lacunae, as shown in this article, it is likely that the compensatory choroidal thickening involves a swelling of the system of short lymphatics. During recovery, after wearing a partial diffuser, the choroid expands only in the myopic region, indicating that the compensatory choroidal thickening is controlled locally.[67,126] The local mechanism might include an increase in vascular permeability; the entrance of osmotically active molecules in the lumina of the lymphatic vessels, which would draw fluid from the extracellular spaces; the contraction of the trabecular tissue with consequent dilation of the thin-walled lymphatics; or an increase of the venous pressure with fluid backflow into the lacunae. Our demonstration that the lacunae swell considerably after paracentesis, moving the retina forward, also might offer a parsimonious explanation for the observation that form-deprived eyes that received daily intravitreous injections of control saline solutions were less myopic than were eyes that were not injected.[83] No matter how careful, penetration of the cornea by a needle may produce loss of a variable amount of fluid from the anterior chamber.

The work of Meriney and Pilar[95] indicates that both vasculature and stromal tissue in chicks are well-enough developed to sustain an important role in normal and abnormal eye growth, and the current study shows yet another fluid compartment that could be part of the compensatory mechanisms for experimental ametropia. Detailed quantitative analysis of the development of the chick choroid in normal and abnormal conditions, therefore, may be helpful in clarifying these processes.

In conclusion, we have shown that the avian choroid, in addition to stromal smooth muscle cells, has an extensive choroidal lymphatic system, the counterpart of which is absent in mammals. This finding might represent the morphologic substrate for unequal preferential routes for the outflow of intraocular fluids in these species. Differences in the functional anatomy of the eye between birds and mammals must be taken into consideration when one chooses birds as animal models for the study of pathologic processes, such as experimental ametropia and glaucoma, that involve perturbation of the ocular fluid balance. Our studies also indicate that sophisticated histologic procedures are required to analyze possible changes in the structure of the avian choroid during experimental conditions.

Key Words

aqueous humor, choroid, intraocular pressure, lacunae, paracentesis

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References

1. Bill A. Blood circulation and fluid dynamics in the eye. *Physiol Rev.* 1975;55:383–417.
2. Bill A. Circulation in the eye. In: Renkin EM, Michel CC, eds. *The Microcirculation, Part 2. Handbook of Physiology, The Cardiovascular System.* Baltimore: The American Physiological Society; 1984:1001–1034.
3. Bill A. Some aspects of the ocular circulation. *Invest Ophthalmol Vis Sci.* 1985;26:410–424.
4. Bill A, Sperber G, Ujiie K. Physiology of the choroidal vascular bed. *Int Ophthalmol.* 1983;6:101–107.
5. Davson H. *Physiology of the Eye.* 4th ed. New York: Academic Press; 1980:3–81.
6. Duke-Elder S, Wybar KC. *The Anatomy of the Visual System.* London: Kimpton H; 1961.
7. Hogan MJ, Alvarado J, Weddell JE. *Histology of the Human Eye.* Philadelphia: Saunders Company; 1971.
8. Alm A, Bill A. The oxygen supply to the retina, II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal flow in cats. *Acta Physiol Scand.* 1972;84:306–319.
9. Auker CR, Parver LM, Doyle T, Carpenter DO. Choroidal blood flow. I. Ocular tissue temperature as a measure of flow. *Arch Ophthalmol.* 1982;100:1323–1326.
10. Parver LM, Auker C, Carpenter DO. Choroidal blood flow as a heat dissipating mechanism in the macula. *Am J Ophthalmol.* 1980;89:641–646.
11. Parver LM, Auker CR, Carpenter DO, Doyle T. Choroidal blood flow. II. Reflexive control in the monkey. *Arch Ophthalmol.* 1982;100:1327–1330.
12. Parver LM, Auker CR, Carpenter DO. Choroidal

blood flow. III. Reflexive control in human eyes. *Arch Ophthalmol.* 1983;101:1604–1606.
13. Bill A. A method to determine osmotically effective albumin and gammaglobulin concentrations in tissue fluids, its application to the uvea and a note on the effects of capillary "leaks" on tissue fluid dynamics. *Acta Physiol Scand.* 1968;73:511–522.
14. Korte GE, Burns MS, Bellhorn RW. Epithelium-capillary interactions in the eye: The retinal pigment epithelium and the choriocapillaris. *Int Rev Cytol.* 1989; 114:221–248.
15. Bito LZ. The physiology and pathophysiology of intraocular fluids. In: Bito LZ, Davson H, Fenstermacher JD, eds. *The Ocular and Cerebrospinal Fluids.* London: Academic Press; 1977:273–289.
16. Cole DF. Secretion of the aqueous humor. In: Bito LZ, Davson H, Fenstermacher JD, eds. *The Ocular and Cerebrospinal Fluids.* London: Academic Press; 1977: 161–176.
17. Bill A. The aqueous humor drainage mechanism in the cynomolgus monkey (Macaca Irus) with evidence for unconventional routes. *Invest Ophthalmol.* 1965; 4911–4919.
18. Bill A. Movement of albumin and dextran through the sclera. *Arch Ophthalmol.* 1965b;74:248–252.
19. Bill A. Aqueous humor dynamics in monkeys (Macaca irus and Cercopitecus ethiops). *Exp Eye Res.* 1971;11: 195–206.
20. Bill A. Basic physiology of the drainage of aqueous humor. In: Bito LZ, Davson H, Fenstermacher JD, eds. *The Ocular and Cerebrospinal Fluids.* London: Academic Press; 1977:291–304.
21. Bill A. Drainage of intraocular fluids. In: Cunha VC, ed. *The Blood–Retinal Barriers.* New York: Plenum Press; 1980:179–193.
22. Bill A. Formation and drainage of aqueous humor in cats. *Exp Eye Res.* 1966;5:185–190.
23. Bill A, Phillips CI. Uveoscleral drainage of aqueous humor in human eyes. *Exp Eye Res.* 1971;12:275–281.
24. Bill A. Conventional and uveo-scleral drainage of aqueous humor in the cynomolgus monkey (*Macaca irus*) at normal and high intraocular pressures. *Exp Eye Res.* 1966;5:45–54.
25. Bill A. The routes for bulk drainage of aqueous humor in the vervet monkey (*Cercopithecus ethiops*). *Exp Eye Res.* 1966;5:55–57.
26. Bill A, Hellsing K. Production and drainage of aqueous humor in the cynomolgus monkey (*Macaca irus*). *Invest Ophthalmol.* 1965;4:920–926.
27. Asher KW. Aqueous veins: Preliminary note. *Am J Ophthalmol.* 1942;25:31–38.
28. Asher KW. Aqueous veins. Their status eleven years after their detection. *Arch Ophthalmol.* 1953;49:438–451.
29. Reiner A, Karten HJ, Gamlin PDR, Erichsen JT. Parasympathetic ocular control. Functional subdivisions and circuitry of the avian nucleus of Edinger–Westphal. *Trends Neurosci.* 1983;6:140–145.
30. Fitzgerald MEC, Gamlin PDR, Zagvazdin Y, Reiner A. Central neural circuits for the light-mediated reflexive control of choroidal blood flow in the pigeon eye: A laser Doppler study. *Vis Neurosci.* 1996;13:655–669.
31. Stjernschantz, J, Bill A. Effect of intracranial stimulation of the oculomotor nerve on ocular blood flow in the monkey, cat and rabbit. *Invest Ophthalmol Vis Sci.* 1979;18:99–103.
32. Stjernschantz J, Bill A. Vasomotor effects of facial nerve stimulation: Non-cholinergic vasodilation in the eye. *Acta Physiol Scand.* 1980;109:45–50.
33. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. *Nature (Lond).* 1990;347:768–770.
34. Yamamoto R, Bredt D, Snyder S, Stone R. The localization of nitric oxide synthase in the eye and related cranial ganglia. *Neuroscience.* 1993;54:189–200.
35. Mann RM, Riva CE, Stone RA, Barbes GE, Cranston SD. Nitric oxide and choroidal blood flow regulation. *Invest Ophthalmol Vis Sci.* 1995;36:925–930.
36. Nilsson SFE. Nitric oxide as a mediator of parasympathetic vasodilation in ocular and extraocular tissues in the rabbit. *Invest Ophthalmol Vis Sci.* 1996;37:2110–2119.
37. Behar–Cohen FF, Goureau O, D'Hermies F, Courtois Y. Decreased intraocular pressure induced by nitric oxide donors is correlated to nitrite production in the rabbit eye. *Invest Ophthalmol Vis Sci.* 1996;37:1711–1715.
38. Goldstein IM, Ostwald Roths P. Nitric oxide: A review of its role in retinal function and disease. *Vision Res.* 1996;36:2979–2994.
39. Yanagisawa M, Kirihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond).* 1988;214:241–244.
40. MacCumber MW, Japel HD, Snyder SH. Ocular effects of the endothelins: Abundant peptides in the eye. *Arch Ophthalmol.* 1991;109:705–709.
41. Wiesel T, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature (Lond).* 1977; 266:66–68.
42. Raviola E, Wiesel T. An animal model of myopia. *N Engl J Med.* 1985;312:1609–1615.
43. Raviola E, Wiesel TN. Neural control of eye growth and experimental myopia in primates. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:22–44.
44. O'Leary DJ, Millodot M. Eyelid closure causes myopia in humans. *Experientia.* 1979;35:1478–1479.
45. Tigges M, Tigges J, Fernades A, Eggers HM, Gammon JA. Postnatal axial eye elongation in normal and visually deprived rhesus monkeys. *Invest Ophthalmol Vis Sci.* 1990;31:1035–1046.
46. Bradley DV, Fernades A, Tigges M, Boothe RG. Diffuser contact lenses retard axial elongation in infant Rhesus monkeys. *Vision Res.* 1996;36:509–514.
47. Bartmann M, Schaeffel F. A simple mechanism for emmetropization without cues for accommodation or colour. *Vision Res.* 1994;34:873–876.
48. Sherman SM, Norton TT, Casagrande VA. Myopia in the lid-sutured tree shrew (*Tupaia glis*). *Brain Res.* 1977;124:154–157.

49. McBrien NA, Norton TT. The development of experimental myopia and ocular component dimensions in monocularly lid-sutured tree shrews (*Tupaia belangeri*). *Vision Res.* 1992;32:843–852.
50. Criswell MH, Goss DA. Myopia development in nonhuman primates: A literature review. *Am J Optom Physiol Opt.* 1983;60:250–268.
51. Norton TT. Experimental myopia in tree shrews. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:178–199.
52. Troilo D, Judge SJ. Ocular development and visual deprivation myopia in the common marmoset (*Callithrix jacchus*). *Vision Res.* 1993;33:1311–1324.
53. Cottrial CL, McBrien NA. The M_1 muscarinic antagonist pirenzepone reduces myopia and eye enlargement in the tree shrew. *Invest Ophthalmol Vis Res.* 1996;37:1368–1379.
54. Guggenheim JA, McBrien NA. Form deprivation myopia induces activation of scleral matrix metalloprotease 2 in tree shrew. *Invest Ophthalmol Vis Res.* 1996; 37:1380–1395.
55. Schaeffel F, Howland HC. Guest editorial. *Vision Res.* 1995;35:1135–1139.
56. Goss DA, Criswell MH. Myopia development in experimental animals: A literature review. *Am J Optom Physiol Opt.* 1981;58:859–869.
57. Yinon U. Myopia induction in animals following alteration of the visual input during development: A review. *Curr Eye Res.* 1984;3:677–690.
58. Troilo D. Neonatal eye growth and emmetropisation: A literature review. *Eye.* 1992;6:154–160.
59. Van Alphen GWHM. Choroidal stress and emmetropization. *Vision Res.* 1986;26:723–734.
60. Van Alphen GWHM. Emmetropization in the primate eye. In: Block Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:115–125.
61. Sivak JG, Barrie DL, Callender MG, Doughty MJ, Seltner RL, West JA. Optical causes of experimental myopia. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:160–177.
62. Pilar GR, Johnson DA. Model cholinergic systems: The avian ciliary ganglion. In: Whittaker VP, ed. *Handbook of Experimental Pharmacology.* Berlin; Heidelberg: Springer–Verlag; 1988:41–54.
63. Pilar G, Tuttle JB. A simple neuronal system with a wide range of uses: The avian ciliary ganglion. In: Hanin I, Goldberg AM, eds. *Progress in Cholinergic Biology: Model Cholinergic Synapses.* New York: Raven Press; 1982:213–247.
64. Wallman J, Turkel J, Trachtman J. Extreme myopia produced by modest changes in early visual experience. *Science.* 1978;201:1249–1251.
65. Wallman J. Retinal influences on sclera underlie visual deprivation myopia. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:126–148.
66. Wallman J, Wildsoet C, Xu A, et al. Moving the retina: Choroidal modulation of refractive state. *Vision Res.* 1995;35:35–50.
67. Wildsoet C, Wallman J. Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Res.* 1995;35:1175–1194.
68. Nickla DL, Wildsoet C, Wallman J. Myopia defocus, but not form-deprivation, causes large phase shifts in diurnal rhythms of choroid thickness and of axial length. ARVO Abstracts. *Invest Ophthalmol Vis Sci.* 1996;37:S687.
69. Hodos W, Kuenzel WJ. Retinal-image degradation produces ocular enlargement in chicks. *Invest Ophthalmol Vis Sci.* 1984;25:652–659.
70. Hodos W. Avian models of experimental myopia: Environmental factors in the regulation of eye growth. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:149–159.
71. Wilkinson JL, Hodos W. Intraocular pressure and eye enlargement in chicks. *Curr Eye Res.* 1991;10:163–168.
72. Irving EL, Callender MG, Sivak JG. Inducing ametropias in hatching chicks by defocus- Aperture effects and cylindrical lenses. *Vision Res.* 1995;35:1165–1174.
73. Irving EL, Callender MG, Sivak JG. Inducing myopia, hyperopia, and astigmatism in chicks. *Optom Vis Sci.* 1991;68:364–368.
74. Irving EL, Sivak JG, Callender MG. Refractive plasticity of the developing chick eye. *Ophthalmic Physiol Opt.* 1992;12:448–456.
75. Li T, Troilo D, Glasser A, Howland HC. Constant light produces severe corneal flattening and hyperopia in chickens. *Vision Res.* 1995;35:1203–1210.
76. Liang H, Crewther DP, Crewther SG, Barila AM. A role for photoreceptor outer segments in the induction of deprivation myopia. *Vision Res.* 1995;35:1217–1246.
77. Rada JA, Breza HL. Increased latent gelatinase activity in the sclera of visually deprived chicks. *Invest Ophthalmol Vis Sci.* 1995;36:1555–1565.
78. Reiner A, Shin Y–F, Fitzgerald MEC. The relationship of choroidal blood flow and accommodation to the control of ocular growth. *Vision Res.* 1995;35:1227–1245.
79. Stone RA, Lin T, Desai D, Capehart C. Photoperiod, early post-natal growth, and visual deprivation. *Vision Res.* 1995;35:1195–1202.
80. Troilo D, Li T, Glasser A, Howland HC. Differences in eye growth and the response to visual deprivation in different strains of chickens. *Vision Res.* 1995;35:1211–1216.
81. McBrien NA, Moghaddam HO, Cottriall CL, Leech EM, Cornell LM. The effects of blockade of retinal cell action potentials on ocular growth, emmetropization and form deprivation myopia in young chicks. *Vision Res.* 1995;35:1141–1152.
82. Murphy CJ, Howland M, Howland HC. Raptors lack lower-field myopia. *Vision Res.* 1995;35:1135–1156.
83. Roher B, Spira AW, Stell WK. Apomorphine blocks form-deprivation myopia in chickens by dopamine D_2-receptor mechanism acting in retina or pigmented epithelium. *Vis Neurosci.* 1993;10:447–453.
84. Roher B, Stell WK. Basic fibroblast growth factor

(bFGF) and transforming factor beta (TGF-b) act as stop and go signals to modulate postnatal ocular growth in the chick. *Exp Eye Res.* 1994;58:553–562.
85. Pickett–Seltner RL, Sivak JG, Pasternak JJ. Experimentally induced myopia in chicks: Morphometric and biochemical analysis during the first 14 days after hatching. *Vision Res.* 1988;28:323–328.
86. Pickett–Seltner RL, Stell WK. The effect of vasoactive intestinal peptide on development of form deprivation myopia in the chick: A pharmacological and immunocytochemical study. *Vision Res.* 1995;35:1265–1270.
87. Roher B, Iuvone PM, Stell WK. Stimulation of dopaminergic amacrine cells by stroboscopic illumination or fibroblast growth factor (bFGF, FGF-2) injections: Possible roles in prevention of form-deprivation myopia in the chick. *Brain Res.* 1995;686:169–181.
88. Shih YF, Fitzgerald MEC, Norton TT, Gamlin PDR, Hodos W, Reiner A. Reduction in choroidal blood flow occurs in chicks wearing goggles that induce eye growth toward myopia. *Curr Eye Res.* 1993a;12:219–227.
89. Shih YF, Fitzgerald MEC, Reiner A. Choroidal blood flow is reduced in chicks with ocular enlargement induced by corneal incisions. *Curr Eye Res.* 1993;12:229–237.
90. Troilo D. Experimental studies of emmetropization in the chick. In: Bock G, Widdows K, eds. *Myopia and the Control of Eye Growth.* Ciba Foundation Symposium 155. Chichester: Wiley; 1990:89–114.
91. Zhu X, Lin T, Stone RA, Laties AM. Sex differences in chick eye growth and experimental myopia. *Exp Eye Res.* 1995;61:173–179.
92. Lauber JK. Avian models of experimental myopia. *J Ocul Pharmacol.* 1991;7:259–276.
93. Guglielmone R, Cantino D. Autonomic innervation of the ocular choroid membrane in the chicken. *Cell Tissue Res.* 1982;222:417–431.
94. Kajikawa J. Beiträge zur Anatomie und Physiologie des Vogelauges. *Albrecht v Graefes Arch Klin Exp Ophthalmol.* 1923;112:260–346.
95. Meriney SD, Pilar G. Cholinergic innervation of the smooth muscle cells in the choroid coat of the chick eye and its development. *J Neurosci.* 1987;7:3827–3839.
96. Meyer DB. The avian eye and its adaptations. In: Crescitelli ed. *The Visual System in Vertébrates.* Berlin–Heidelberg–New York: Springer–Verlag; 1977:549–611.
97. O'Rahilly R. The development of the sclera and the choroid in staged chick embryos. *Acta Anat (Basel).* 1962;48:335–346.
98. Rochon–Duvigneaud A. *Les Yeux et la Vision des Vertébrés.* Paris: Masson; 1943.
99. Walls GL. *The Vertebrate Eye and its Adaptive Radiation.* Bloomfield Hills, Michigan: Cranbrook Institute of Science; 1942.
100. Coulombe JN, Nishi R. Stimulation of somatostatin expression in developing ciliary ganglion neurons by cells of the choroid layer. *J Neurosci.* 1991;11:553–562.
101. Coulombe JN, Schwall R, Parent AS, Eckenstein FP, Nishi R. Induction of somatostatin immunoreactivity in cultured ciliary ganglion neurons by activin in choroid cell-conditioned medium. *Neuron.* 1993;10:1–20.
102. Epstein ML, Davis JP, Gellman LE, Lamb JR, Dahl JL. Cholinergic neurons of the chicken ciliary ganglion contain somatostatin. *Neuroscience.* 1988;25:1053–1060.
103. Gray DB, Pilar GR, Ford MJ. Opiate and peptide inhibition of transmitter release in parasympathetic nerve terminals. *J Neurosci.* 1989;9:1683–1692.
104. Gray DB, Zelazny D, Manthay N, Pilar G. Endogenous modulation of ACh release by somatostatin and the differential roles of Ca^{2+} channels. *J Neurosci.* 1990;10:2687–2698.
105. Gray DB, Scranton V, Pilar G. Somatostatin inhibits transmitter release via nitric oxide and cGMP-dependent kinase in choroid nerve terminals. *Soc Neurosci Abstr.* 1993;1177:485.11.
106. De Stefano ME, Ciofi Luzzatto A, Mugnaini E. Neuronal ultrastructure and somatostatin immunolocalization in the ciliary ganglion of chicken and quail. *J Neurocytol.* 1993;22:868–892.
107. Marwitt R, Pilar G, Weakly JN. Characterization of two ganglion cell populations in avian ciliary ganglia. *Brain Res.* 1971;25:317–334.
108. De Stefano ME, Mugnaini E. Fine structure of the choroidal coat of the avian eye. II. Vascularization, supporting tissue and innervation. *Anat Embryol (Berl).* 1997 (in press).
109. Boon ME, Kok LP. In: Boon ME, Kok LP, eds. *Microwave Cookbook of Pathology. The Art of Microscopic Visualization.* Leyden: Coulomb Press; 1988.
110. Marti R, Wild P, Schraner EM, Mueller M, Moor H. Parathyroid ultrastructure after aldehyde fixation, high-pressure freezing, or microwave irradiation. *J Histochem Cytochem.* 1987;35:1415–1424.
111. Hiruma T. Formation of the choroidal arteries in the chick embryo: Observations of corrosion casts by scanning electron microscopy. *Anat Embryol (Berl).* 1996;193:585–592.
112. Raviola G. Effects of paracentesis on the blood–aqueous barrier: An electron microscope study on *Macaca mulatta* using horseradish peroxidase as a tracer. *Invest Ophthalmol.* 1974;13:828–858.
113. Spitznas M, Reale E. Fracture faces of fenestrations and junctions of endothelial cells in human choroidal vessels. *Invest Ophthalmol.* 1975;14:98–107.
114. Raviola E, Raviola G. A light and electron microscopic study of the pecten of the pigeon eye. *Am J Anat.* 1967;120:427–462.
115. Seaman AR, Himelfarb TM. Correlated ultrafine structural changes of the avian pecten oculi and ciliary body of *Gallus domesticus:* Preliminary observations on the physiology: I. Effects of decreased intraocular pressure induced by intravenous injection of acetazolamide (Diamox). *Am J Ophthalmol.* 1963;56:278–296.
116. Leak LV, Burke JF. Fine structure of the lymphatic capillary and the adjoining connective area. *Am J Anat.* 1966;118:785–810.
117. Leak LV, Burke JF. Ultrastructural studies on the lymphatic anchoring filaments. *J Cell Biol.* 1978,36:129–149.
118. Glasser A, Troilo D, Howland HC. The mechanism of

corneal accommodation in chicks. *Vision Res.* 1994; 34:1549–1566.
119. Murphy CJ, Glasser A, Howland HC. The anatomy of the ciliary region of the chicken eye. *Invest Ophthalmol Vis Sci.* 1995;36:889–896.
120. Tripathi RC. Mechanism of the aqueous outflow across the trabecular wall of Schlemm's canal. *Exp Eye Res.* 1971;11:116–121.
121. Tripathi RC. Ultrastructure of the exit pathway of the aqueous in lower mammals (a preliminary report on the 'angular aqueous plexus'). *Exp Eye Res.* 1971;12:311–314.
122. Tripathi RC, Tripathi BJ. The mechanism of aqueous outflow in lower mammals. *Exp Eye Res.* 1972;14:73–79.
123. Bill A, Wailinder PE. The effects of pilocarpine on the dynamics of aqueous humor in a primate (*Macaca irus*). *Invest Ophthalmol.* 1966;5:170–175.
124. Bill A. Effects of norepinephrine, isoproterenol and sympathetic stimulation on aqueous humor dynamics in vervet monkeys. *Exp Eye Res.* 1970;10:31–46.
125. Lauber JK, Boyd JE, Boyd TAS. Intraocular pressure and aqueous outflow facility in light-induced avian buphthalmos. *Exp Eye Res.* 1970;9:181–187.
126. Wallman J, Gottlieb MD, Rajaram V, Fugate-Wentzek LA. Local retinal regions control local eye growth and myopia. *Science.* 1987;237:73–77.