Microvasculature of the Rat Optic Nerve Head

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Purpose. To describe the arterial blood supply, capillary bed, and venous drainage of the rat optic nerve head.

Methods. Ocular microvascular castings from 6 Wistar rats were prepared by injection of epoxy resin through the common carotid arteries. After polymerization, tissues were digested with 6 M KOH, and the castings washed, dried, and coated for scanning electron microscopy.

Results. Immediately posterior to the globe, the ophthalmic artery trifurcates into the central retinal artery and two posterior ciliary arteries. The central retinal artery directly provides capillaries to the nerve fiber layer and only contributes to capillary beds in the neck of the nerve head. The remainder is supplied by branches of the posterior ciliary arteries that are analogous to the primate circle of Zinn–Haller. Arterioles arising from these branches supply the capillaries of the transitional, or laminar, region of the optic nerve head. These capillaries are continuous with those of the neck and retrobulbar optic nerve head. All optic nerve head capillaries drain into the central retinal vein and veins of the optic nerve sheath. A flat choroidal sinus communicates with the central retinal vein, the choriocapillaris, and with large veins of the optic nerve sheath.

Conclusions. The microvasculature of the rat optic nerve head bears several similarities to that of the primate, with a centripetal blood supply from posterior ciliary arteries and drainage into the central retinal and optic nerve sheath veins. Association of nerve sheath veins with the choroid represents an important difference from the primate. (*Invest Ophthalmol Vis Sci.* 1999;40: 1702–1709)

Animal models are powerful tools for studying potential mechanisms of glaucomatous optic nerve damage.[1] Experimentally elevated intraocular pressure (IOP) and altered optic nerve blood flow in normal animals can reveal cellular mechanisms by which each of these factors mediate damage.[2–5] These findings may then be used in studies of human tissue to help understand the mechanisms underlying optic nerve damage in glaucoma.

Nonhuman primates are anatomically the most appropriate animals for studying human disease.[6,7] However, their expense and limited supply restricts their use in careful experiments requiring large numbers of animals and prompts the search for more cost-effective models of experimental optic nerve damage.

To develop such a model in laboratory rats, we have successfully created methods for measuring IOP in Brown Norway rats using the Tono–Pen tonometer[8–11] and for sclerosing the aqueous humor outflow pathways of these animals to increase IOP.[12,13] These pressure levels produce characteristic nerve fiber damage and connective tissue alterations within the optic nerve head,[14–16] which can be prevented by controlling IOP with topical glaucoma agents.[17] This model may thus prove useful for understanding the mechanisms of pressure-induced optic nerve damage and for evaluating current and future agents designed to protect optic nerve fibers directly.

Because vascular mechanisms may also contribute to glaucomatous optic neuropathy,[18,19] it is important to understand optic nerve head perfusion in this model. We have begun by analyzing the microvascular anatomy of rat optic nerve heads using scanning electron microscopy of ocular corrosion castings. When compared with the primate anatomy, the findings demonstrate important similarities and differences, which provide an important foundation for subsequent studies of the pathology and physiology of the rat optic nerve head.

Materials and Methods

All animal experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Six adult Wistar rats with normal ocular examinations were cannulated through the heart under deep surgical anesthesia and the jugular veins severed. A plastic mixture consisting of 10% acetone, 60% Araldite CY 223, and 30% hardener HY 2267 (Ciba–Geigy) was injected by thumb pressure and continued until resin flowed from the severed jugular veins. Tissues were allowed to rest for 1 hour and the eyes enucleated and fixed in 10% formaldehyde overnight.

Tissues were digested in 33% KOH at 40°C for 12 hours. Castings were gently washed with running tap water and digested a second time to remove residual tissue. The castings

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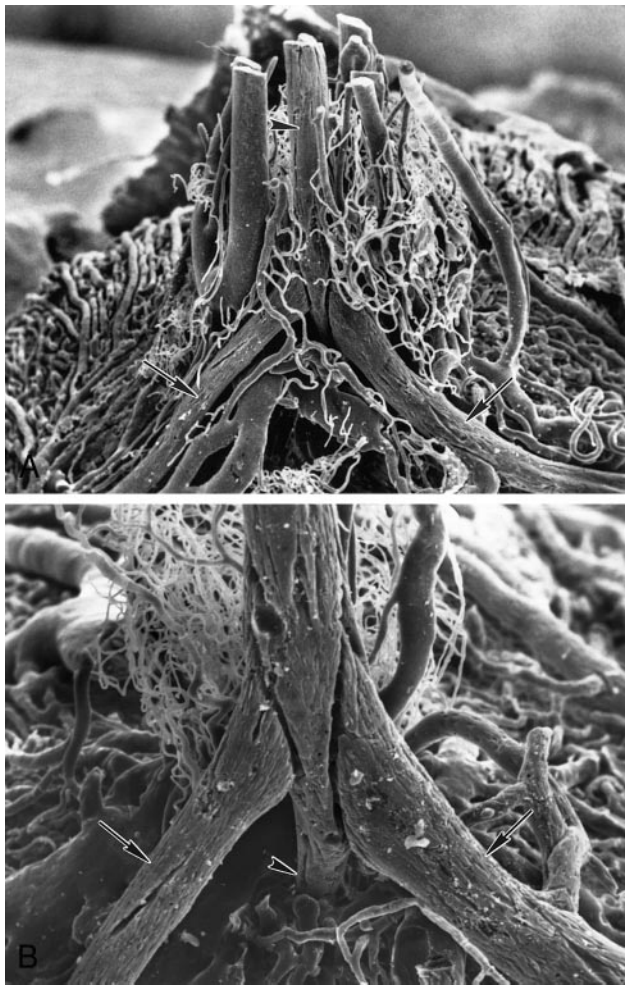

FIGURE 1. Ventral views of an optic nerve head casting, showing the ophthalmic artery beneath the optic nerve. (**A**) Nasal and temporal posterior ciliary arteries (*arrows*) originate from the ophthalmic artery (*arrowhead*; magnification, ×50). (**B**) Removal of surrounding optic nerve sheath vessels reveals central retinal artery (*arrowhead*) extending from the ophthalmic artery, between the posterior ciliary arteries (*arrows*; magnification, ×100).

were allowed to air-dry, and then the posterior pole regions with attached optic nerve were dissected free and mounted on metal scanning EM stubs and sputter-coated with gold palladium for scanning EM. Using an AMR-1000 and an AMR-1600 scanning electron microscope (Amray, Bedford, MA), castings were examined under low and high powers, using sequential dissection of superficial vascular connections.[20]

RESULTS

As in other animals, the rat optic nerve head can be divided into distinct prelaminar, laminar, and postlaminar regions.[21,22] In the narrow prelamina, referred to by some authors as the "neck," unmyelinated optic nerve fiber bundles are surrounded by columns of astrocytes. Posterior to this, the nerve expands, due to gradual myelination of the nerve fibers and the addition of densely packed astrocytes oriented horizontally across the scleral opening, whose processes are intimately related to the unmyelinated axons. This region, the "transition" zone, contains vascular connective tissue bands whose composition is identical to that of primate laminar beams.[23–25] Its posterior limit marks the beginning of the postlaminar intraorbital optic nerve, in which the majority of nerve fibers are fully myelinated.

All castings demonstrated complete filling of the major arteries and veins of the optic nerve head, with extensive filling of the fine capillary beds in a majority of specimens. The observations presented here were consistently observed in all specimens that allowed detailed dissection and observation. The rat optic nerve head microvasculature originates from the ophthalmic artery, which lies inferior to the optic nerve. Immediately posterior to the globe, the ophthalmic artery trifurcates into the central retinal artery and nasal and temporal posterior ciliary arteries on either side (Fig. 1). Although these

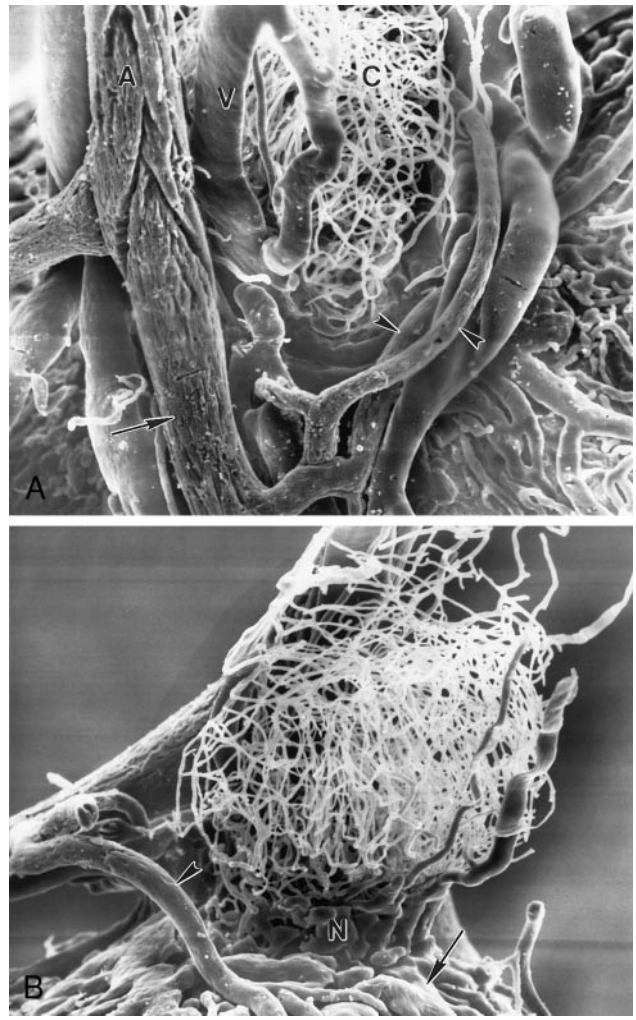

FIGURE 2. (**A**) Partially dissected casting shows that arterioles (*arrowheads*) arise from a posterior ciliary artery (*arrow*) wraparound optic nerve head capillaries (C), similar to the circle of Zinn–Haller in the primate. A, ophthalmic artery; V, central retinal vein (magnification, ×100). (**B**) Side view of specimen after removal of nerve sheath veins reveals arteriole (*arrowhead*) from the arterial circle entering the choroidal vascular bed (*arrow*) adjacent to the neck of the optic nerve head (N; magnification, ×100).

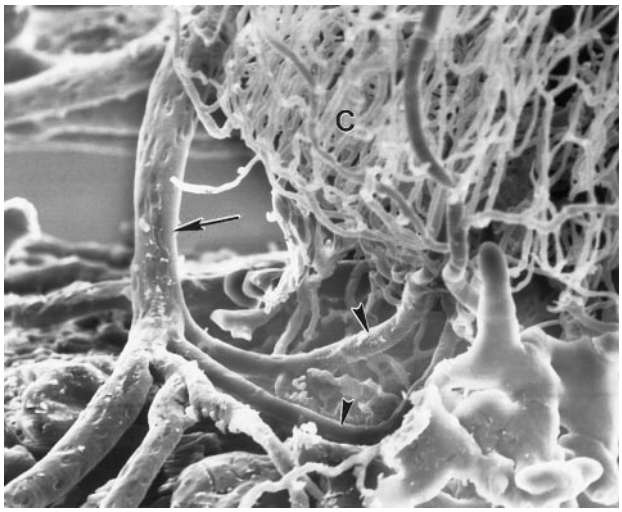

FIGURE 3. Branches of an arteriole (*arrow*) from the arterial circle mingle with choroidal vessels, some of which have been removed to reveal penetrating arteriolar branches (*arrowheads*) entering the neck and transition zone of the optic nerve head capillary bed (C; magnification, ×100).

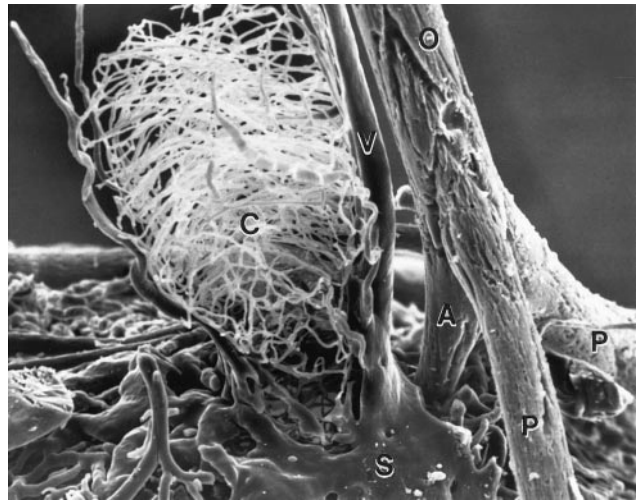

FIGURE 5. Side view of a casting at the level of the junction of the optic nerve with the globe after removal of optic nerve sheath veins. This shows that the central retinal artery (A) supplies no distinct vessels to the transition zone capillaries (C). V indicates central retinal vein, which receives capillaries from the transition zone and connects with a choroidal sinus (S) overlying the choroid. O, the ophthalmic artery; P, posterior ciliary arteries (magnification, ×100).

arteries extend on either side of the globe to supply the anterior uvea,[26] posteriorly they contribute branches to the optic nerve head and adjacent choroid. Branches from the posterior ciliary arteries supply the majority of the optic nerve head, and the central retinal artery supplies its anterior regions.

Posterior Ciliary Arteries

Arterioles arise from the posterior ciliary arteries at the junction of the optic nerve with the posterior sclera (Fig. 2). These arterioles border the optic nerve head, analogous to the primate circle of Zinn–Haller. Multiple branches emerge from these arterioles and enter the posterior choroid, where, internal to the choroidal veins, they arborize into branches that enter the neck of the optic nerve head (Fig. 3) These branches primarily supply capillaries to the transition region of the optic nerve head (Fig. 4). This capillary bed is continuous posteriorly with that of the retrobulbar optic nerve and anteriorly with capillaries of the superficial optic nerve head.

Central Retinal Artery

The central retinal artery, which extends from the ophthalmic artery in between the posterior ciliary arteries, enters the eye inferior to the optic nerve but does not penetrate the substance of the optic nerve or nerve head. It does not supply

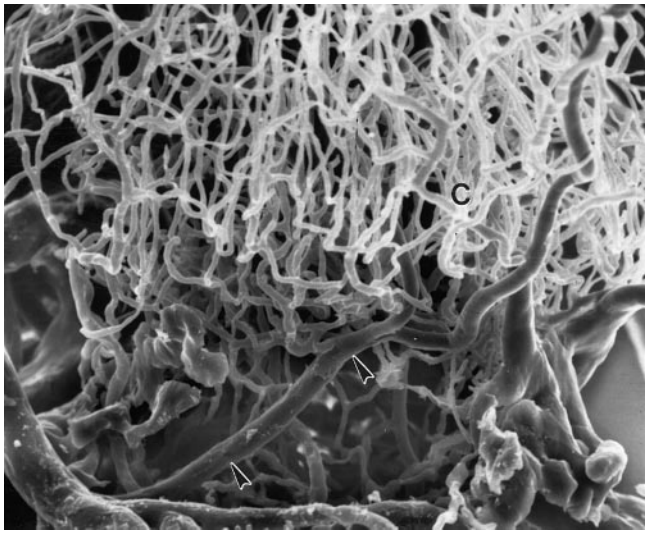

FIGURE 4. Side view of specimen in Figure 3 shows arteriolar branches (*arrows*) supplying capillaries (C) of the optic nerve head transition zone (magnification, ×370).

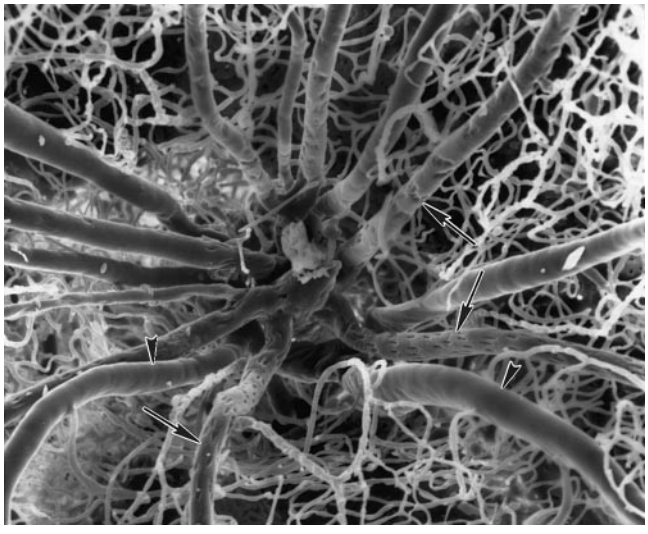

FIGURE 6. Anterior view of optic nerve head, showing retinal arteries (*arrows*) and veins (*arrowheads*) with intervening retinal capillary bed (magnification, ×200).

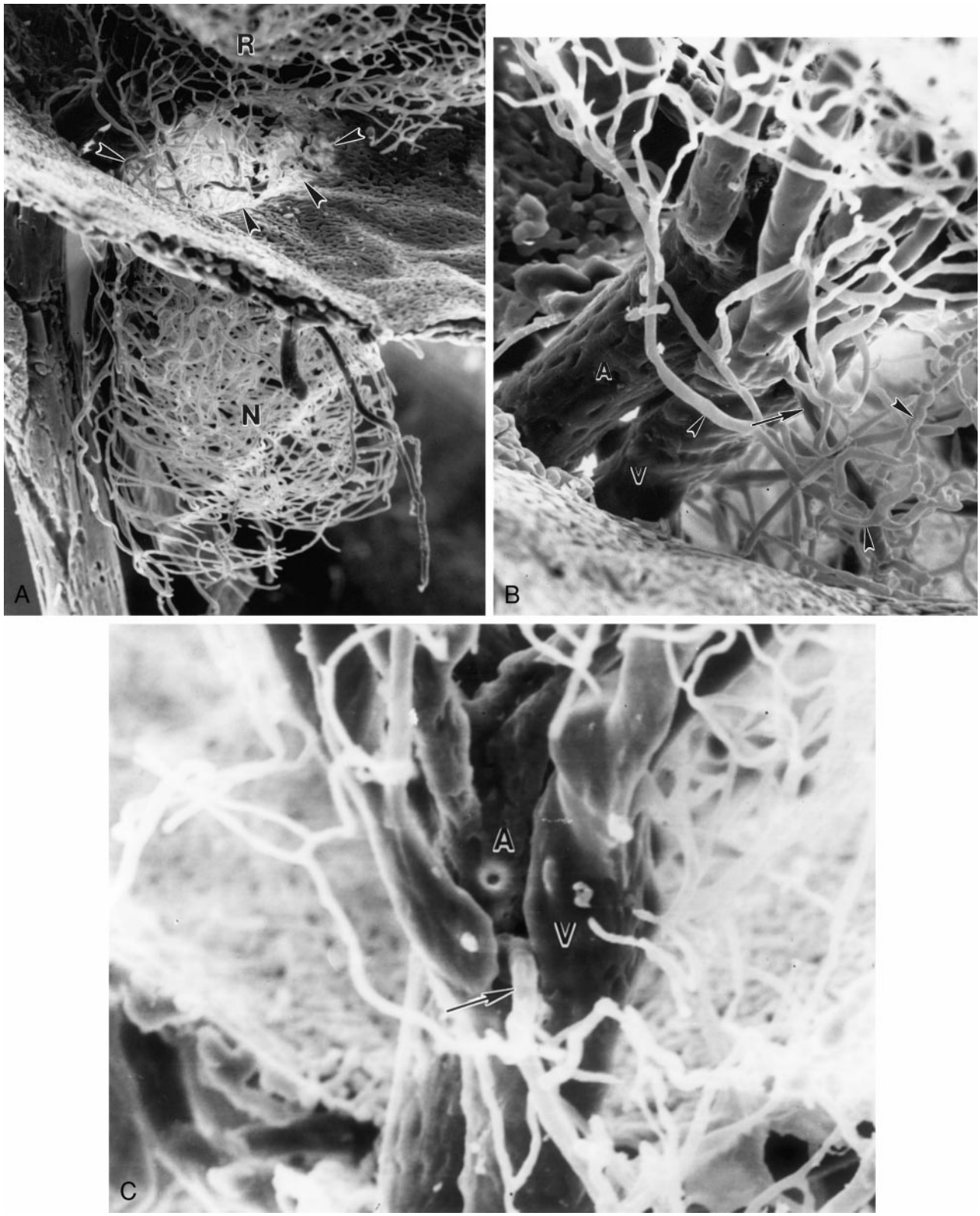

FIGURE 7. (**A**) Casting viewed with optic nerve down shows apparent continuity between capillaries of the optic nerve (N) and retina (R) through the opening of the choriocapillaris (*arrowheads*; magnification, ×100). (**B**) High power shows that this continuity consists of capillaries (*arrowheads*) associated with a small arteriole (*arrow*). Central retinal artery (A) and vein (V) appear to left. Magnification, ×600. (**C**) Arteriole (*arrow*) originates from the central retinal artery (A) immediately beneath its arborization on the surface of the optic nerve head (magnification, ×400).

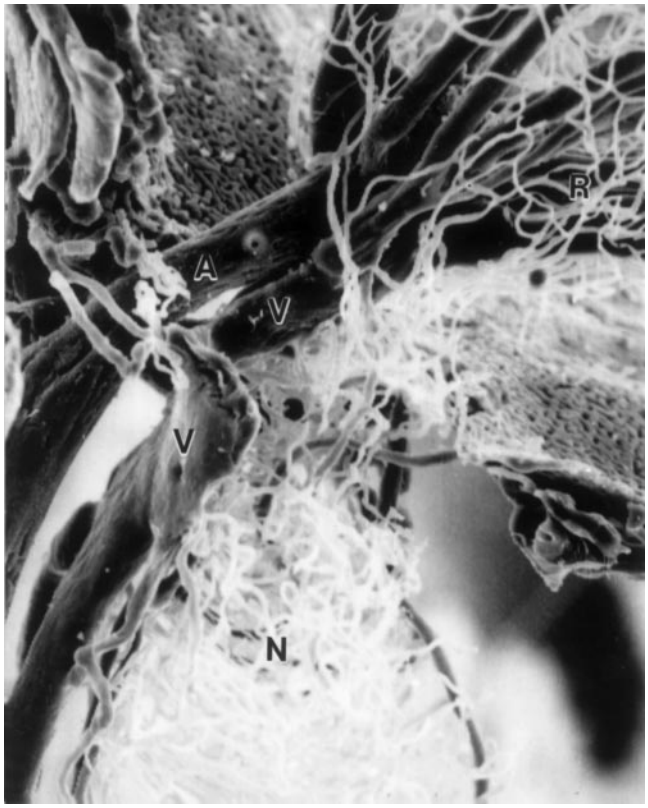

FIGURE 8. Dissected casting with the choroid and capillary bed removed to improve access to the vessels of the neck of the optic nerve head. Note continuity of central retinal vein (V) and central retinal artery (A) from the retina (R), through the optic nerve head and onto the ventral aspect of the optic nerve (N) (magnification, ×100).

direct connections to the transition region of the optic nerve head (Fig. 5).

Within the eye, the central retinal artery branches into several major retinal arteries, in a characteristic "wagon-wheel spoke" configuration (Fig. 6). These retinal arteries supply capillaries to the nerve fiber layer of the optic nerve head, which are continuous with capillaries in the neck that arise from the central retinal artery immediately posterior to its branching on the surface of the optic nerve head (Fig. 7). These capillaries are also continuous with those of the more posterior transition zone of the optic nerve head.

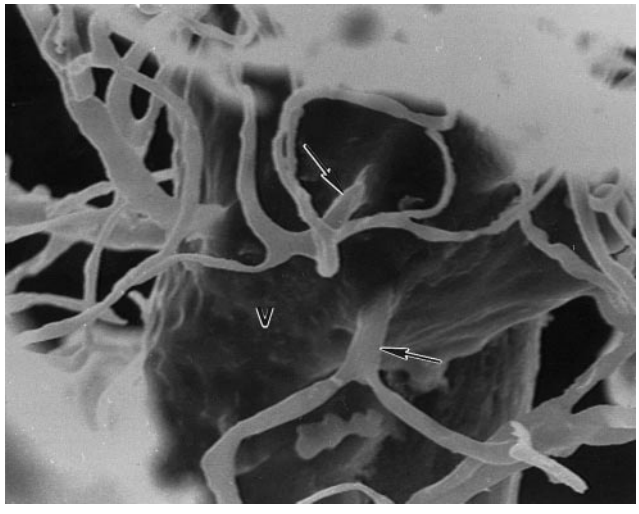

FIGURE 9. Nerve fiber layer capillaries (*arrows*) connect with the central retinal vein (V) immediately beneath the retinal surface (magnification, ×700).

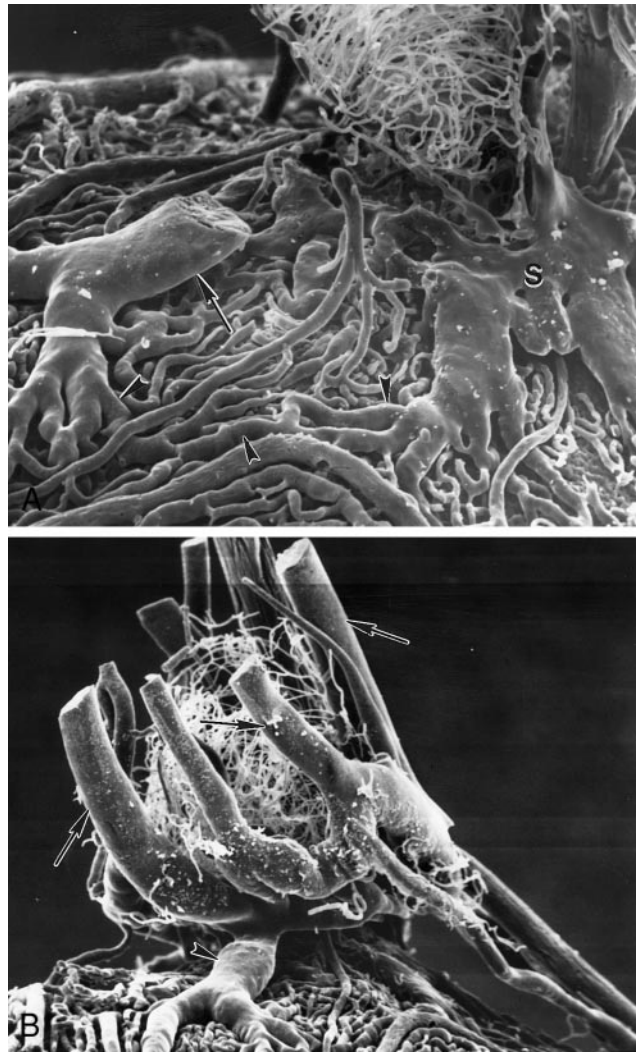

FIGURE 10. Dissected specimen at the junction of the optic nerve with the globe after removal of large choroidal veins. (**A**) Flat sinus (S) at the base of the optic nerve head (shown in Fig. 5 connecting with the central retinal vein) communicates (*arrowheads*) with a partially dissected choroidal vein (*arrow*). (**B**) Same specimen before removal of the choroidal veins demonstrates connections of the choroidal vein in (**A**; *arrowhead*) with veins of the optic nerve sheath shown (*arrows*). Magnification, **A**, ×100; **B**, ×50.

Venous Drainage

The central retinal vein of the rat eye originates from the confluence of the major retinal veins at the optic nerve head (Fig. 6). This vein travels posteriorly, beneath the optic nerve head with the central retinal artery. (Fig. 8).

The central retinal vein receives blood from several capillary beds. These include nerve fiber layer capillaries and

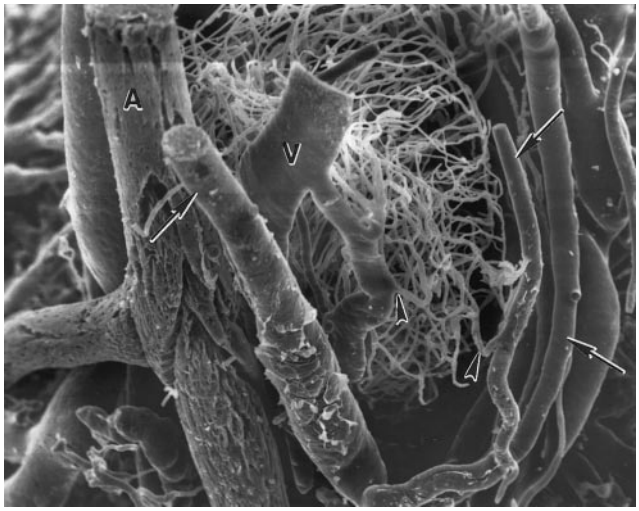

FIGURE 11. Posterior view of intraorbital optic nerve shows ophthalmic artery (A), central retinal vein (V), and surrounding optic nerve sheath veins (*arrows*). *Arrowheads* indicate dual drainage of optic nerve capillaries into a tributary of the central retinal vein and a vein in the optic nerve sheath (magnification, ×100).

capillaries of the transition zone, or lamina cribrosa region of the optic nerve head (Fig. 9). A large flat sinus located within the choroid also empties into the central retinal vein (Figs. 5, 10). This sinus also communicates with choroidal veins that drain the choriocapillaris and are continuous with several large optic nerve sheath veins. Posteriorly, within the intraorbital optic nerve, capillary beds drain into the central retinal vein and into veins within the optic nerve sheath (Fig. 11).

DISCUSSION

Laboratory rats offer several advantages for determining cellular mechanisms of optic nerve damage. Aside from their relatively modest expense, a large body of literature based on rats already exists on the cell biology of optic nerve damage, which may provide important insights into mechanisms of injury when applied to glaucoma models using these animals.

Although the rat posterior segment microvasculature has previously been studied using microvascular castings, the results have concentrated on the capillary beds of the retina and their response to experimental disease states.[27–31] To the best of our knowledge, this is the first detailed description of the blood supply, capillaries, and venous drainage of the optic nerve head in rats. Our observations describe several important similarities and differences between the microvasculature of the rat and primate optic nerve head (Fig. 12)

The arterial blood supply to the rat optic nerve head arises from the ophthalmic artery, which trifurcates just posterior to the globe into the central retinal artery and two posterior ciliary arteries. Because previous casting studies have shown that the posterior ciliary arteries also supply the anterior choroid, iris, and ciliary body,[26] the ophthalmic artery in rats, as in other species, is the major source of blood for the entire eye.

Branches arising from the posterior ciliary arteries form an arterial circle around the optic nerve head that resembles the primate circle of Zinn–Haller.[18,32] From this circle, arterioles supply capillaries of the transition zone of the optic nerve head, just as analogous arterioles in primates and other animals serve the lamina cribrosa.[33,34] This observation, along with its previously mentioned connective tissue[23] and astrocytic characteristics, supports our proposition that the transition zone of the rat optic nerve head corresponds to the primate lamina cribrosa.

Previous studies of rat eyes with chronically elevated IOP have shown that initial nerve fiber damage occurs within the transitional region of the optic nerve head[13] and that more extensive injury includes abnormal deposition of extracellular matrix materials at this same level.[14] Further analysis of this region in this model may reveal important clues to the mechanism of pressure-induced optic nerve damage.

We have also found that the rat central retinal artery directly supplies only the anterior portions of the optic nerve head, as in other mammals.[33,34] Current techniques for evaluating optic nerve head blood flow in humans primarily sample the anterior nerve fiber layer region.[35,36] Because deeper portions of the nerve head rely on the posterior ciliary system, it is unclear how much information these techniques provide about perfusion of the lamina cribrosa. However, these methods have recently been adapted to the study of the rat optic nerve head and retina.[37] This development, and our demonstration that blood supply to the anterior optic nerve head in the rat relies primarily on the central retinal artery, opens the possibility of developing and noninvasively studying experimental models of ischemia in these small eyes and understanding how experimental glaucoma in rats[13] might affect optic nerve perfusion.

As with the primate, venous return from capillaries at all levels of the nerve head is primarily through the central retinal vein, which lies adjacent to the central retinal artery at its exit from the globe, although a distinct primate-like centripetal pattern is not apparent. Extensive structural alterations of the optic nerve head caused by elevated IOP[14–16] could severely affect perfusion of this entire region.

The prominent sinus overlying the choroidal vasculature represents a potentially significant departure from the primate microvasculature. The drainage of optic nerve capillaries into veins of the optic nerve sheath and the central retinal vein via this sinus underscores the importance of avoiding the sheath when severing or crushing the nerve to study axonal degeneration, because obstruction of these vessels could secondarily congest the optic nerve head. In addition, congestion of the choroidal vasculature after inadvertent obstruction of vortex veins could back up into capillaries of the transitional optic nerve head. The resulting engorgement of these capillaries could damage axons by mechanical compression and altered perfusion. However, physiologic responses could be different from those suggested by our anatomic findings and direct physiological measurements of blood flow in these situations would be necessary to resolve this possibility.

Although these anatomic observations do not in themselves predict vascular physiology, the similarities observed here with the microvasculature of the primate optic nerve head support the relevance of using rat models to study mechanisms of glaucomatous optic nerve damage.[13,38,39] Finally, they provide an important foundation for using rats to analyze the role of blood flow in many pathologic conditions of the

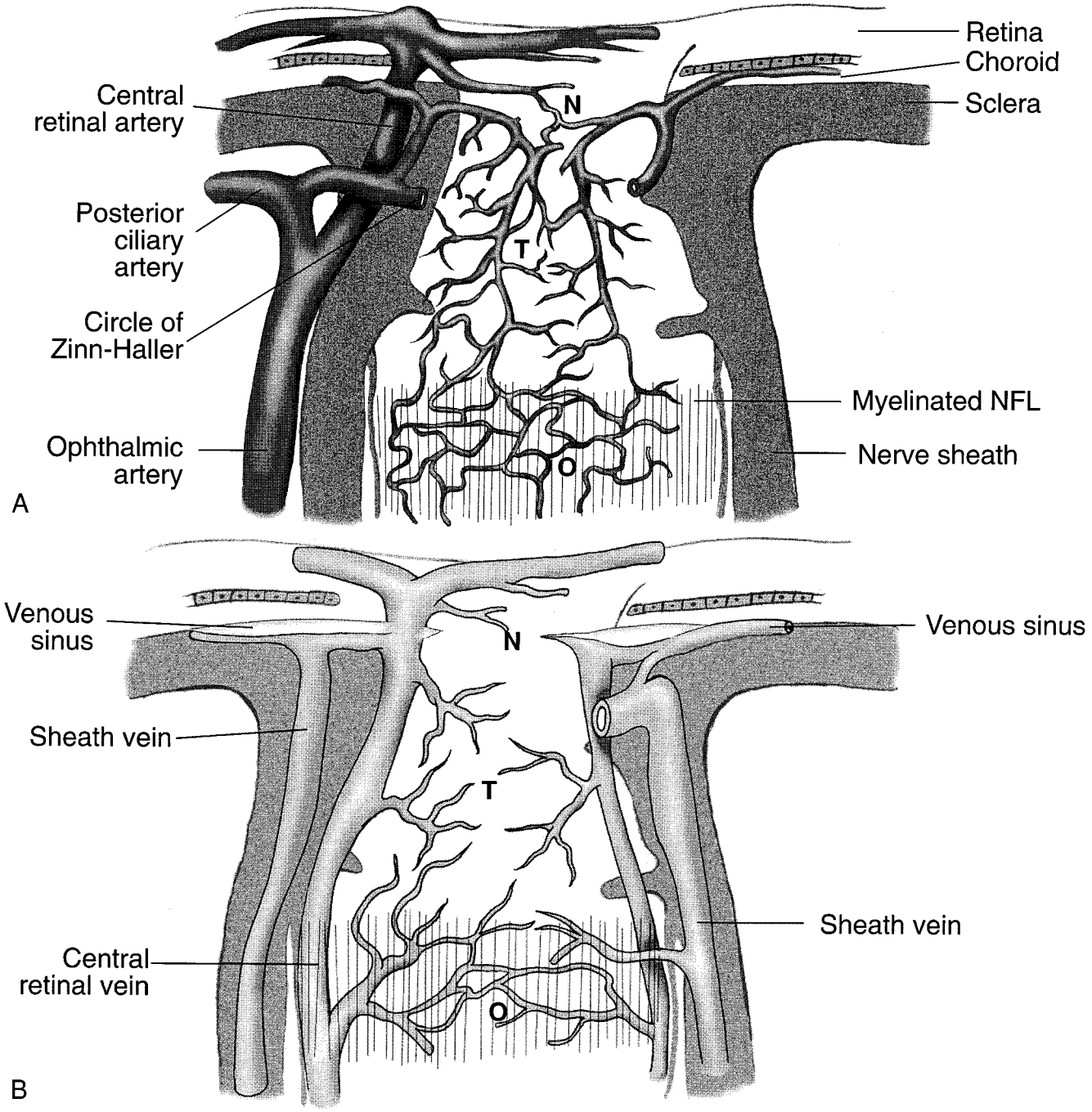

FIGURE 12. Schematic representation of the rat optic nerve head microvasculature, showing the arterial supply (**A**) and venous drainage (**B**), along with their relationship to the posterior sclera, and the neck (N), transition (T), and myelinated optic nerve (O) regions of the optic nerve head.

optic nerve, including ischemia[40] and neurogenic damage, and elevated IOP.

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References

1. Morrison JC, Johnson EC, Cepurna W. Animal models in glaucoma research. *Ophthalmic Pract.* 1998;16:12–20.
2. Orgul S, Cioffi GA, Bacon DR, Van Buskirk EM. An endothelin-1 model of chronic optic nerve ischemia in rhesus monkeys. *J Glaucoma.* 1996;5:135–138.
3. Orgul S, Cioffi GA, Wilson DJ, Van Buskirk EM. An endothelin-1 induced model of optic nerve ischemia in the rabbit. *Invest Ophthalmol Vis Sci.* 1996;37:1860–1869.
4. Quigley HA, Hohman RM. Laser energy levels for trabecular meshwork damage in the primate eye. *Invest Ophthalmol Vis Sci.* 1983;24:1305–1307.
5. Radius RL, Pederson JE. Laser-induced primate glaucoma, II: histopathology. *Arch Ophthalmol.* 1984;102:1693–1698.

6. Anderson DR. Ultrastructure of human and monkey lamina cribrosa and optic nerve head. *Arch Ophthalmol.* 1969;82:800–814.
7. Anderson DR. Ultrastructure of the optic nerve head. *Arch Ophthalmol.* 1970;83:63–73.
8. Moore CG, Milne S, Morrison JC. Non-invasive measurement of rat IOP with the TonoPen. *Invest Ophthalmol Vis Sci.* 1993;34:363–369.
9. Moore CG, Epley D, Milne ST, Morrison JC. Long-term non-invasive measurement of intraocular pressure in the rat eye. *Curr Eye Res.* 1995;14:711–717.
10. Moore CG, Johnson EC, Morrison JC. Circadian rhythm of intraocular pressure in the rat. *Curr Eye Res.* 1996;15:185–191.
11. Mermoud A, Baerveldt G, Minckler DS, Lee MB, Rao NA. Intraocular pressure in Lewis rats. *Invest Ophthalmol Vis Sci.* 1994;35: 2455–2460.
12. Morrison JC, Fraunfelder FW, Milne ST, Moore CG. Limbal microvasculature of the rat eye. *Invest Ophthalmol Vis Sci.* 1995;36: 751–756.
13. Morrison JC, Moore CG, Deppmeier LMH, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. *Exp Eye Res.* 1997;63:85–96.
14. Johnson EC, Morrison JC, Farrell SK, Deppmeier LMH, Moore CG, McGinty MR. The effect of chronically elevated intraocular pressure on the rat optic nerve head extracellular matrix. *Exp Eye Res.* 1996;62:663–674.
15. Hernandez MR, Andrzejewska WM, Neufeld AH. Changes in the extracellular matrix of the human optic nerve head in primary open-angle glaucoma. *Am J Ophthalmol.* 1990;109:180–188.
16. Morrison JC, Dorman–Pease ME, Dunkelberger GR, Quigley HA. Optic nerve head extracellular matrix in primary optic atrophy and experimental glaucoma. *Arch Ophthalmol.* 1990;108:1020–1024.
17. Morrison JC, Nylander KB, Lauer AK, Cepurna WO, Johnson EC. Glaucoma drops control intraocular pressure and protect optic nerves in a rat model of glaucoma. *Invest Ophthalmol Vis Sci.* 1998;39:526–531.
18. Hayreh SS. Blood supply of the optic nerve head and its role in optic atrophy, glaucoma and oedema of the optic disc. *Br J Ophthalmol.* 1969;53:721–748.
19. Goldberg I, Hollows FC, Kass MA, Becker B. Systemic factors in patients with low-tension glaucoma. *Br J Ophthalmol.* 1981;65: 56–62.
20. Morrison JC, Van Buskirk EM. Sequential microdissection and scanning electron microscopy of ciliary microvascular castings. *Scanning Electron Microsc.* 1984;2:857–865.
21. Ffrench–Constant C, Miller RH, Burne JF, Raff MC. Evidence that migratory oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells are kept out of the rat retina by a barrier at the eye-entrance of the optic nerve. *J Neurocytol.* 1988;17:13–25.
22. Hildebrand C, Remahl S, Waxman G. Axo-glial relations in the retina-optic nerve junction of the adult rat: electron-microscopic observations. *J Neurocytol.* 1985;14:597–617.
23. Morrison JC, Farrell SK, Johnson EC, Deppmeier LMH, Moore CG, Grossmann E. Structure and composition of the rodent lamina cribrosa. *Exp Eye Res.* 1995;60:127–135.
24. Morrison JC, Jerdan JA, L'Hernault NL, Quigley HA. The extracellular matrix composition of the monkey optic nerve head. *Invest Ophthalmol Vis Sci.* 1988;29:1141–1150.
25. Morrison JC, L'Hernault NL, Jerdan JA, Quigley HA. Ultrastructural location of extracellular matrix components in the optic nerve head. *Arch Ophthalmol.* 1989;107:123–129.
26. Morrison JC, DeFrank MP, Van Buskirk EM. Comparative microvascular anatomy of mammalian ciliary processes. *Invest Ophthalmol Vis Sci.* 1987;28:1325–1340.
27. Seke R. Differences between three-dimensional posterior and peripheral retinal angioarchitecture of rats. *Acta Soc Ophthalmol Jpn.* 1987;91:1281–1285.
28. Bhutto IA, Amemiya T. Corrosion cast demonstration of retinal vasculature of normal Wistar-Kyoto rats. *Acta Anat.* 1995;153: 290–300.
29. Bhutto IA, Amemiya T. Vascular changes in retinas of spontaneously hypertensive rats demonstrated by corrosion casts. *Ophthalmic Res.* 1997;29:12–23.
30. Pannarale L, Onori P, Ripani M, Gaudio E. Precapillary patterns and perivascular cells in the retinal microvasculature: a scanning electron microscope study. *J Anat.* 1996;188:693–703.
31. Fulcher T, Beigi B, Eustace P, Bannigan J. Early changes in the choroidal vasculature of rats occurring with experimentally induced hypertension. *Ophthalmic Res.* 1996;28:255–259.
32. Lieberman MF, Maumenee BA, Green WR. Histologic studies of the vasculature of the anterior optic nerve. *Am J Ophthalmol.* 1976; 82:405–423.
33. Cioffi GA, Van Buskirk EM. Microvasculature of the anterior optic nerve. *Surv Ophthalmol.* 1994;38(suppl):S107–S116.
34. Sugiyama K, Bacon DR, Morrison JC, Van Buskirk EM. Optic nerve head microvasculature of the rabbit eye. *Invest Ophthalmol Vis Sci.* 1992;33:2251–2261.
35. Riva CE, Harino S, Petrig BL, Shonat RD. Laser doppler flowmetry in the optic nerve. *Exp Eye Res.* 1992;55:499–506.
36. Michelson G, Schmauss B. Two dimensional mapping of the perfusion of the retina and optic nerve head. *Br J Ophthalmol.* 1955;79:1126–1132.
37. Chauhan BC, Yu DY, Cringle SJ, Carlsson AM, Su EN. Confocal scanning laser ophthalmoscopy and doppler flowmetry of the rat retina [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4): **S**274. Abstract nr 1273.
38. Shareef SR, Garcia–Valenzuela E, Salierno A, Walsh J, Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats. *Exp Eye Res.* 1995;61:379–382.
39. Garcia–Valenzuela E, Shareef SR, Walsh JB, Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp Eye Res.* 1995;61:33–44.
40. Cioffi GA, Orgul S, Onda E, Bacon DR, Van Buskirk EM. An in vivo model of chronic optic nerve ischemia: the dose-dependent effects of endothelin-1 on the optic nerve microvasculature. *Curr Eye Res.* 1995;14:1147–1153.