

Optic Nerve Hydropic Axonal Degeneration and Blocked Retrograde Axoplasmic Transport

Histopathologic Features in Human High-Pressure Secondary Glaucoma

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Objective: To describe histopathologic features of anterior optic nerves of 12 eyes enucleated for sustained high ocular pressure from iris-ciliary body melanomas in 10 and choroidal melanomas with chronic angle closure in 2.

Methods: In this retrospective study, we analyzed cases indexed in 2 eye pathology laboratories and reviewed the pertinent literature. Cases were identified from diagnostic indexes; microscopic study of slides stained with hematoxylin-eosin and Verhoeff-van Gieson, Mallory trichrome, periodic acid-Schiff, alcian blue, or colloidal iron for acid mucopolysaccharide; review of available clinical documentation; and analysis of features and photography. The main outcome measures were description of optic nerve heads, prelaminar atrophy, laminar posterior bowing, locations and density of hydropic axonal degeneration, blocked retrograde axoplasmic transport, posterior atrophy, and optic nerve disorganization with glial proliferation.

Results: Hydropic axonal degeneration was present in front of, within, and posterior to the lamina cribrosa in all 12 eyes. This degeneration extended diffusely and posteriorly from the peripheral lamina and was most dense centrally in 10 eyes. Retrolaminar changes compatible with blockage of retrograde axoplasmic transport were seen in 9 eyes. Posterior atrophy with disorganization and glial proliferation was seen in 10 eyes. No eye had classic glaucomatous atrophic cupping.

Conclusions: Diffuse and centrally intense hydropic axonal degeneration and central blocked retrograde axoplasmic transport explain loss of central acuity, generalized contraction of visual field, and generalized optic atrophy without glaucomatous cupping in eyes with prolonged high-pressure secondary glaucoma.

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IT HAS BEEN OBSERVED AND GENERALLY accepted that sustained moderately high intraocular pressure from secondary glaucoma or prolonged angle-closure glaucoma causes reduced visual acuity, contracted visual fields, and generalized optic disc pallor in humans.¹⁻⁵ Current pathophysiologic concepts of early glaucoma are based primarily on experimentally induced elevations of ocular pressure in laboratory primates. The modern era of experimental glaucoma began in the mid 1960s.⁶⁻¹⁰ Kalvin et al⁶ in 1966 reported their findings from 34 owl monkeys in whom ocular pressure elevations were induced by injections of α -chymotrypsin into the posterior chambers and measured by Schiotz tonometers. They concluded that pressures of 40 mm Hg or

higher, sustained for longer than 14 days, were most likely to produce optic disc cupping and small round areas of degeneration in the lamina cribrosa. Longer periods of elevated pressure produced changes that they considered early cavernous degeneration. In a second series of experiments, Kalvin et al⁷ developed evidence that blood supply to the nerve head was reduced in these animals.

The next major contribution came in 3 articles by Zimmerman et al,⁸ Lampert et al,⁹ and Lampert.¹⁰ Using the technique of Kalvin et al to raise ocular pressure in owl monkeys, they studied, by light and electron microscopy, abnormalities in the lamina cribrosa, which they labeled "hydropic axonal degeneration" (HAD). These changes were described as follows: individual axons have

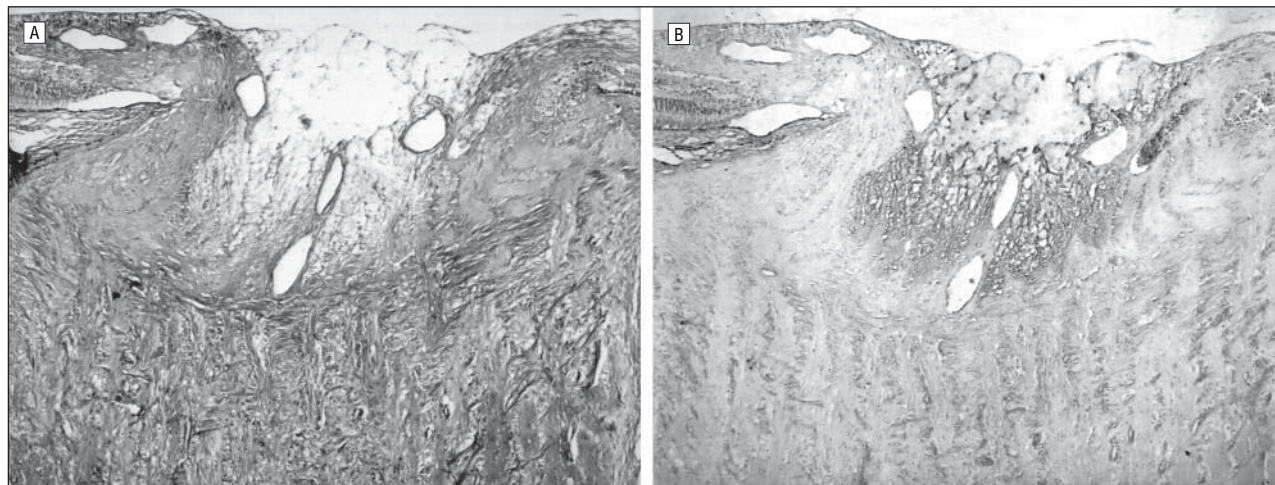


Figure 1. A, Posterior bowing of the lamina and prelaminar nerve atrophy. B, Positive stain for acid mucopolysaccharide with alcian blue (original magnification $\times 20$).

become markedly swollen and exhibit an increased affinity for eosin, fuchsin, and Schiff reagent after treatment with periodic acid. Distended axons showed granular disintegration, clumping of neurotubules, and vesicles. Seven days after onset of high pressure, macrophages from capillaries and veins were seen within distended myelin sheaths. Three and 4 weeks after onset, glaucomatous cupping, necrosis, and marked gliosis with numerous microglia were seen. These changes occurred centrally, nasally, and, least frequently, in the temporal optic nerve. In long-term experiments, some eyes developed lesions that were considered early Schnabel cavernous atrophy.⁹

The next phase of experiments focused on the roles of ocular pressure and vascular perfusion pressure as they affected both orthograde and retrograde axoplasmic transport in optic nerve fibers at the lamina cribrosa. Anderson and Hendrickson¹¹ raised and measured ocular pressure manometrically with saline reservoirs connected by 27-gauge needles inserted into the anterior chambers of owl monkeys. They measured arterial pressure by cannulas connected to pressure transducers, enabling them to calculate a perfusion pressure by subtracting ocular pressure from arterial pressure. Anderson and Hendrickson injected tritiated leucine into monkey posterior preretinal vitreous and, by autoradiography, determined where leucine localized in the retina, lamina cribrosa, optic nerve, and lateral geniculate body. They demonstrated that when ocular perfusion pressures were lower than 25 mm Hg, leucine accumulated mainly in the posterior lamina where nerve fibers were swollen with mitochondria, other cytoplasmic organelles, and inclusions.¹¹ Accumulation of leucine in the lamina was considered a result of blocked orthograde axoplasmic transport. They also observed that in eyes with low perfusion pressure there was a markedly reduced uptake of leucine in retinal ganglion cells. Quigley and Anderson,¹² by a different method, produced similar changes.

Reduced uptake of leucine in retinal ganglion cells was explained by Minckler et al,^{13,14} who demonstrated

in experiments similar to those of Anderson and Hendrickson that horseradish peroxidase injected into the lateral geniculate bodies of rhesus monkeys accumulated within the lamina cribrosa but not in ganglion cells of animals with low perfusion pressures. Minckler¹⁵ has also demonstrated, by transmission electron microscopy, organelles in axons at the lamina scleralis level of experimental primates and in 1 autopsy eye with chronic open-angle glaucoma. This localization and decreased ganglion cell function were explained as a result of blocked retrograde axoplasmic transport of substances necessary for normal ganglion cell function.¹³⁻¹⁵ Gaasterland and Kupfer¹⁶ and Gaasterland et al¹⁷ refined these experiments and demonstrated, by electron microscopy, changes in optic nerve axons at the lamina cribrosa.

Establishing that high ocular and low perfusion pressures interfered with both orthograde and retrograde axoplasmic transport did not define the relative roles of mechanical and ischemic factors. Shearing or compression of axons by lamina fibers has been proposed.¹⁸ Reduced but not complete interruption of the blood supply may have interfered with transport by astrocytes of nutrients from capillaries to intraneural axons.

The purpose of this report is to describe the histopathologic features of 12 human eyes with secondary glaucoma caused by uveal melanomas. Ten eyes had iris-ciliary body melanomas and 2 eyes, choroidal melanomas and neovascular angle-closure glaucoma. We compared findings in these eyes with those produced by experimental glaucoma in primate eyes and documented histopathologic features in human chronic open-angle glaucoma.

METHODS

This retrospective study is based on 12 eyes, which were enucleated because of advanced, unresectable tumors and persistent elevated ocular pressure. Five eyes were processed in the Eye

Pathology Laboratory of the Wills Eye Hospital. Six eyes were processed in the Wm. R. Green Eye Pathology Laboratory of the Wilmer Eye Institute. One of these was specially handled and studied by Harry A. Quigley, MD, as part of a separate report.¹⁹ Sections from 1 eye were accessed from Martha Farber, MD, of Albany, NY, after her presentation at the 2003 meeting of the Verhoeff-Zimmerman Society. Two eyes have been reported previously.^{18,19}

Eleven eyes were opened horizontally and microtome sectioning was performed in the transverse horizontal plane through the optic nerve head. The nerve head studied by Quigley and Green¹⁹ was excised from the globe and cut into 2 temporal quarter sections and a nasal half section. Hematoxylin-eosin, Verhoeff-van Gieson, periodic acid-Schiff, and phosphotungstic acid-hematoxylin stains were used.

These 12 eyes were identified by review of diagnostic indexes. Slides were retrieved from storage, studied, and photographed for publication.

RESULTS

Ocular pressures in this group of eyes ranged from 20 mm Hg in 1 eye medically treated for glaucoma to 32 to 60 mm Hg in 11 eyes.

Prelaminar nerve head atrophy was present in 4 eyes (**Figure 1**). In 3 of these eyes, prelaminar atrophy stained positively for acid mucopolysaccharide with alcian blue in 2 eyes and with colloidal iron in 1 eye. Alcian blue stains were negative in 2 eyes. In eyes, which stained positively for acid mucopolysaccharide, no area of HAD stained positively, indicating that these areas are not infarctions.

The lamina cribrosa was bowed posteriorly in 6 eyes (**Figure 1A** and **Figure 2A**). In no eye was there an appearance compatible with glaucomatous cupping.

Hydropic axonal degeneration, as defined by Lampert et al,⁹ was present in all 12 eyes, anterior, within, and closely posterior to the lamina and diffusely from nasal to temporal origins of the lamina from the sclera. Four eyes had HAD more nasally than temporally and 5 eyes had more HAD centrally than temporally.

Hydropic axonal degeneration was characterized by varying sized, horizontally clustered, small, adjacent, or coalescing spaces, which took no stain. High-power microscopic examination revealed that some axonal spaces appeared empty, some contained degenerating amorphous material, and others were filled with normal axonal material (**Figure 3B**). Empty spaces are the remains of axon channels without axonal material.¹⁰ Hydropic axonal degeneration tended to occur in groups independent of nerve bundles, often extending from temporal to nasal lamellar junctions or under sclera in the periphery of optic nerves.

Blocked retrograde axoplasmic transport, shown in **Figure 2A** and **Figure 4**, was present in 9 eyes. These lesions were posterior and characterized by slightly bulbous, enlarged nerve bundles containing amorphous, poorly staining material having few nuclei. The largest diameters of these areas occurred more posterior to the lamina. **Figure 4B** shows posterior accumulation of blocked axoplasmic transport. **Figure 2A** shows areas of both HAD and blocked retrograde axoplasmic transport.

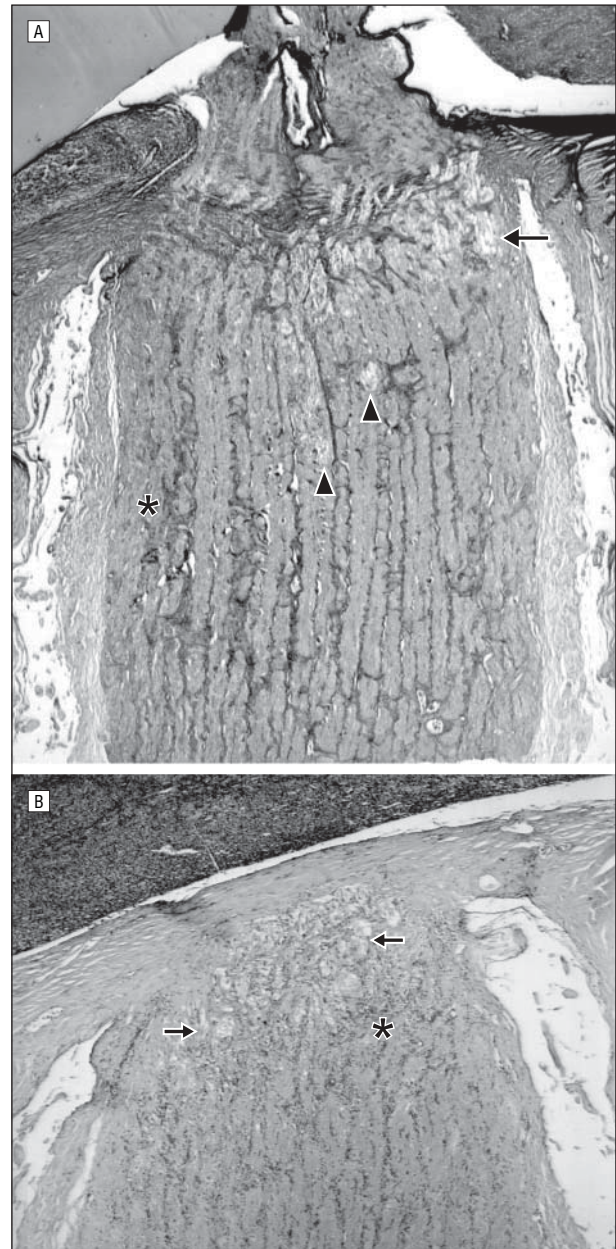


Figure 2. A 49-year-old man with known choroidal melanoma (13.5 × 4-mm under the fovea and partially around the optic nerve) for 7 years, with an ocular pressure of 56 mm Hg and closed iridocorneal angles. A, Paralamellar and intralaminar hydropic axonal degeneration (arrow), dilated nerve bundle areas of blocked retrograde axoplasmic transport (arrowheads), and glial hypercellular disorganization nasally (asterisk) (hematoxylin-eosin, original magnification ×5). B, Diffuse hydropic axonal degeneration under the lamina scleritis (arrows) and hypercellular disorganization (asterisk) (hematoxylin-eosin, original magnification ×10).

Disorganization of optic nerves, characterized by loss of definition of optic nerve bundles, absence of columns of glial cells, and proliferation of glial cells, was present in 10 eyes (**Figures 2B** and **4B** and **Figure 5B**). Glial hyperplasia and disorganization were seen adjacent to both HAD and areas of blocked retrograde axoplasmic transport.

Posterior optic nerve atrophy is demonstrated in **Figure 5C**. In cross-sections, this atrophy was charac-

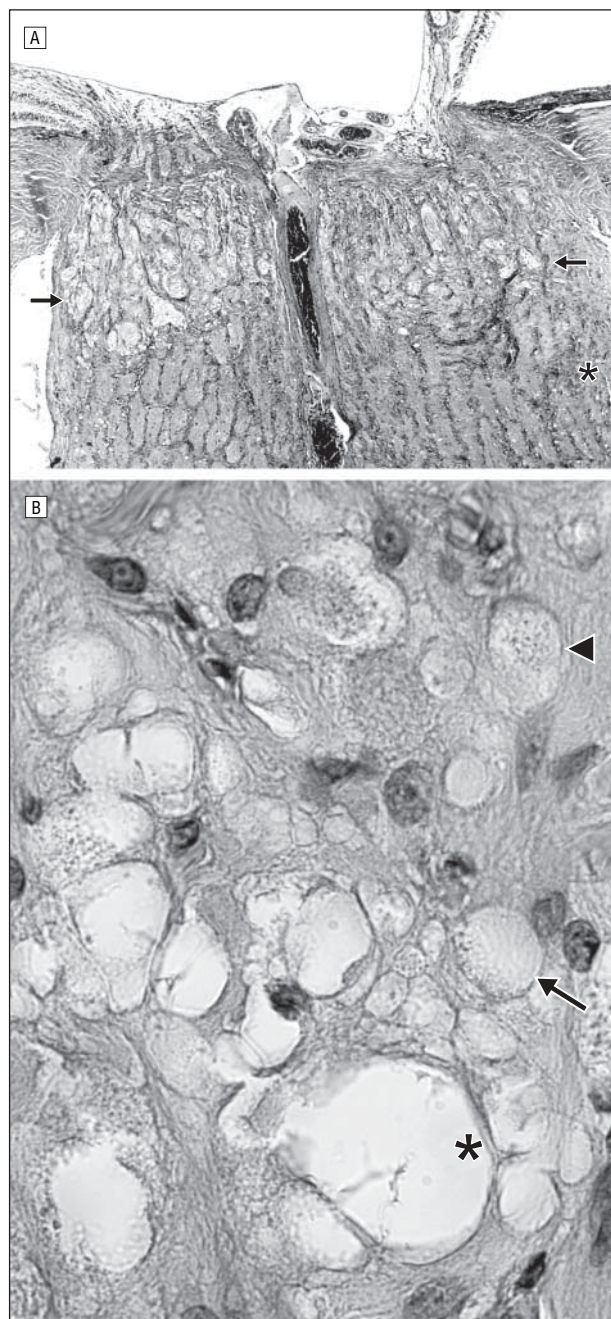


Figure 3. A 60-year-old man with iris melanoma and an ocular pressure of 62 mm Hg. A, Small intralaminar hydropic axonal degeneration (arrows), and nasal hypercellular disorganization (asterisk) (hematoxylin-eosin, original magnification $\times 10$). B, Normal axon (arrow), degenerating axon (arrowhead), and empty axon channel (asterisk) (hematoxylin-eosin, original magnification $\times 100$).

terized by absence of myelin and collapse of nerve bundles, which occurred centrally near major vessels, extending diffusely to the periphery. Cross-sections of 9 nerves were available for study. The case previously reported by Quigley and Green¹⁹ as their case H 204, Figure 9, had severe atrophy more inferiorly and nasally than temporally.

In cross-sections in the present series, 4 eyes had mainly nasal atrophy. Three eyes had peripheral subpial atro-

phy of the whole outer circumference of the optic nerve. One eye had temporal atrophy. In 1 eye, cross-sections were so far posterior to central vessels that the nasal or temporal side could not be determined. Posterior optic nerves of 2 eyes were not available.

COMMENT

This report, demonstrating that HAD and blocked retrograde axoplasmic transport occur in human eyes, reaffirms that the early and primary locus of glaucoma damage is in the area of the lamina cribrosa, with ganglion cell death a secondary phenomenon. We show hydropic axonal degeneration, as defined by Lampert et al,⁹ in human tissue, to our knowledge, for the first time. It was seen diffusely from peripheral origins of the lamina, centrally and nasally more than temporally. It was shown by Quigley and Green¹⁹ in an insert to their Figure 9.

Degeneration of axons, near and within the lamina cribrosa, is thought by many to be the basic damage in glaucoma. Permanent loss of axonal material starts with increased mitochondria and multilaminated organelles, as observed by Lampert et al,⁹ Quigley and Anderson,¹² and Minckler et al¹³⁻¹⁵ in experiments lasting only 4 hours. The experiments of Gaasterland and colleagues^{16,17} lasted as long as 1 month.

The experiments of Anderson and Hendrickson,¹¹ Quigley and Anderson,¹² and Minckler et al¹³⁻¹⁵ established that low perfusion pressure (blood pressure minus ocular pressure) is a major factor in the production of glaucomatous optic nerve damage. A logical hypothesis proposes that reduced blood supply, without causing infarction, interferes with nutrition and the normal physiology of axons in the area of the lamina cribrosa. This hypothesis can be amplified by arguing that low perfusion pressure of the blood supply interferes with glial astrocytic transmission from capillaries of nutrients, such as adenosine triphosphate, essential to the physiology of axons.^{9,10}

Evidence against a strictly ischemic (infarction) mechanism comes first from McNaught et al,¹ who reported in 1974 on their studies of 25 patients who had been surgically treated for angle-closure glaucoma. They described that contracted visual fields seen in their patients were markedly different from altitudinal defects seen with ischemic optic neuropathies. In some patients, contracted fields later expanded. Primary optic atrophy persisted in some. These authors, using clinical-based logic, concluded "it is most unlikely that the damage to the optic nerve head is the result of infarction."^{18(p414)}

Most clinical events labeled ischemic (infarction) are of sudden onset, severe, and complete. Figure 3B, demonstrating normal axons, degenerating axons, and empty axon channels, is evidence of a progressive, multifocal process.

Blocked retrograde axoplasmic transport, defined in primate experiments by Minckler et al,^{13,14} is emphasized in the current study for the first time, to our knowledge, in human tissue. As shown in Figure 4 and Figure 5A, these retrolaminar areas of swollen, amor-

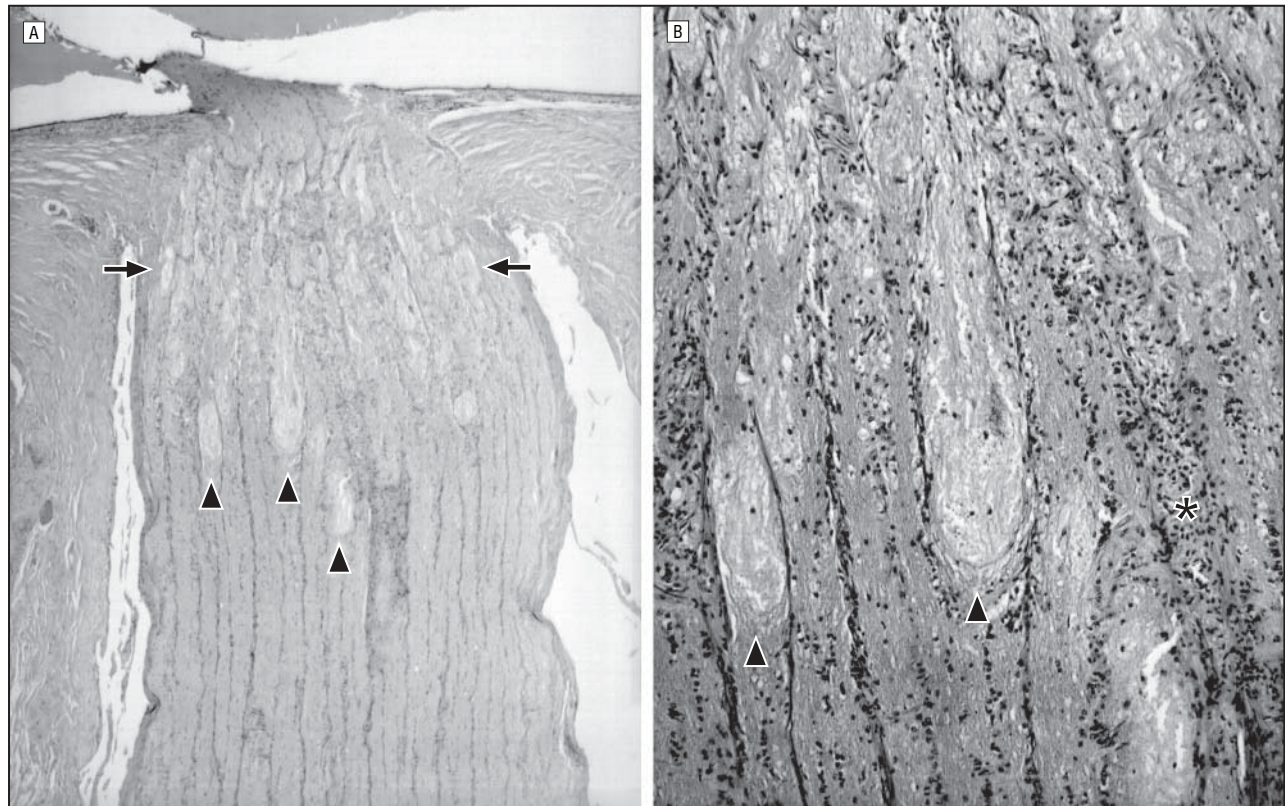


Figure 4. A 55-year-old man with complete retinal detachment, large choroidal melanoma (12 × 10 mm), ocular pressure of 65 mm Hg, and closed iridocorneal angles. A, Hydronic axonal degeneration at all layers of the lamina and 1.5 mm posterior (arrows) and blocked retrograde axoplasmic transport (arrowheads) (hematoxylin-eosin, original magnification ×5). B, Blocked retrograde axoplasmic transport (arrowheads) and hypercellular glial disorganization (asterisk) (hematoxylin-eosin, original magnification ×100).

phous, and hypocellular nerve bundles are mostly central in optic nerves but can occur close to the peripheral retrolaminar nerve. To our knowledge, they have not been reported before. We believe that these distended optic nerve bundles are the result of blockage at the lamina cribrosa where axons cannot distend because of dense connective tissue and that the continued inflow of axoplasmic substances causes swellings morphologically similar to widening upstream from a dam on a creek or river. Similar swelling was not seen anterior to the lamina cribrosa.

No physiologic or glaucomatous cupping was seen histopathologically in any of these 12 eyes. In spite of high pressure over months or years, the nerve head appears resistant to cupping. In the fourth edition of *Glaucoma*,² Epstein et al emphasized that high ocular pressure (40-60 mm Hg) over weeks may cause pure atrophy with minimal cupping. They also considered “whether eyes with unusually large physiologic cups are extraordinarily vulnerable.”²

Central, and often nasal, optic nerve changes seen in 11 of the 12 eyes in this series demonstrate that temporal nerve defects were not the first in these eyes. The classic concept of glaucoma damage occurring first temporally was not valid in these eyes either because their optic nerves were not predisposed by large physiologic cups with thin temporal rims or because the height and duration of elevated ocular pressures were different than those in chronic open-angle glaucoma.

Optic atrophy, characterized by absence of myelin and axonic material; disorganization and collapse of nerve bundles; and glial cell proliferation was seen in 10 eyes. Disorganization of nerve bundles is a result of axon collapse, loss of columns, and proliferation of glia. Glial cell proliferation has been defined by Hernandez and coworkers²⁰⁻²² and Lampert et al⁹ in experimental glaucoma studies as a proliferation of astrocytes. Diffuse glial astrocytic proliferation and disorganization can be used as strong, but not exclusive, histopathologic indicators of glaucoma. The absence of HAD in atrophic disorganized nerves can be explained by glial cell proliferation, which, over time, obliterated empty myelin channels. Mechanisms by which glaucoma induces glial cell proliferation are not well understood at this time. Glial cell proliferation occurs with other atrophic processes but not after optic nerve ischemia or infarctions.²³

Prelaminar nerve head atrophy, seen in 4 eyes, is best explained by high ocular pressure interference with the blood supply to this tissue or to astrocytes, which are prominent in this area.^{20,21} The presence of acid mucopolysaccharide in wedge-shaped prelaminar lesions of 3 eyes can be explained either by high pressure driving hyaluronic acid from vitreous into necrotic areas⁹ or because infarcted tissue in optic nerves develops positive staining by alcian blue or colloidal iron.^{9,23}

Cavernous infarction was present in both the prelaminar and postlaminar nerve in only 1 case (**Figure 6**).

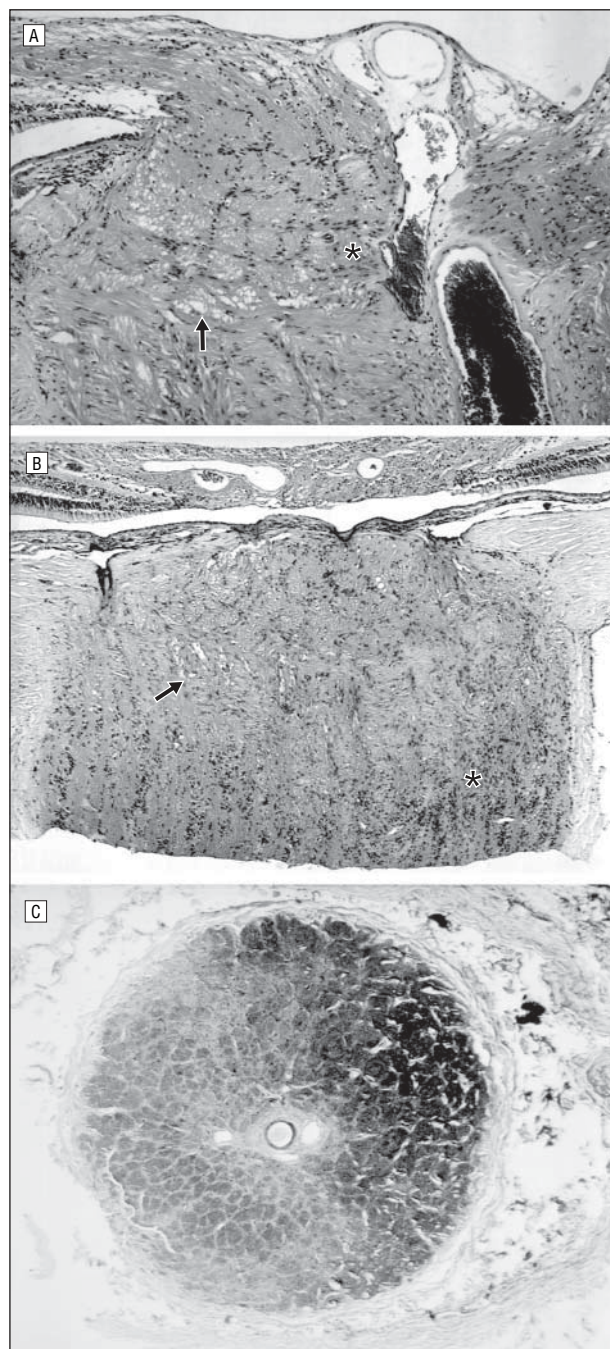


Figure 5. An enlarged iris nevus followed up for 15 years. A, Ocular pressure of 32 mm Hg, a 5 × 7 × 2-mm melanoma, small intralamellar hydropic axonal degeneration, larger retrolamellar hydropic axonal degeneration (arrow), posterior bowing of the lamina, and hypercellular disorganization (asterisk) (hematoxylin-eosin, original magnification ×20). B, Diffuse small hydropic axonal degeneration (arrow), just posterior to the lamina choroidalis; hypercellular glial proliferation; and disorganization posteriorly and temporally (asterisk) (hematoxylin-eosin, original magnification ×20). C, Verhoeff-van Gieson-stained cross-section with patchy atrophy around the central vessels, sweeping atrophic extensions to the upper and lower nasal nerve edge, and nasal subpial dense atrophy (original magnification ×7.5).

Colloidal iron staining was positive for acid mucopolysaccharide. Adjacent to this infarction was HAD, which did not stain with alcian blue. It is assumed that infarction obliterated the HAD. This case is evidence that glau-

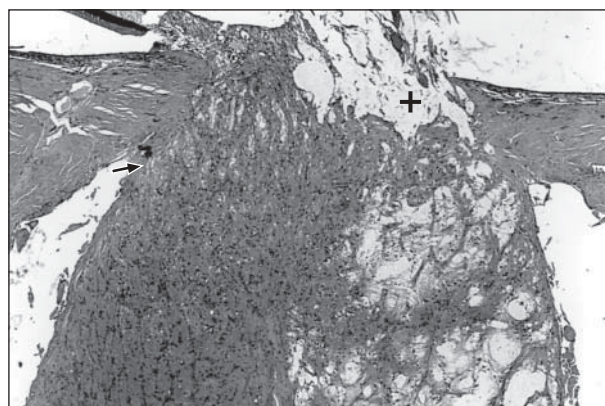


Figure 6. Long-standing glaucoma with hydropic axonal degeneration (arrow) on one side and prelaminar and postlaminar cavernous infarction, positively stained with colloidal iron (cross), on other side (original magnification ×10).

coma and infarction can occur in the same eye. Hydropic axonal degeneration and blocked retrograde axoplasmic transport do not resemble acute, subacute, or chronic ischemic lesions, as described by Knox et al,²³ and were not seen in the eyes studied for their report. The areas of retrolaminar blocked retrograde axoplasmic swelling do not resemble “crush artifacts” seen in eyes enucleated for choroidal melanomas and other disorders.

Major defects in this retrospective report include that only 1 patient had documented visual field testing,¹⁹ ocular pressures were measured and recorded infrequently, blood pressures were not recorded, and enucleated eyes were processed with an emphasis on melanomas, not optic nerves. The paucity of optic nerve sections limited availability for additional histochemical stains such as Verhoeff-van Gieson for myelin, Mallory trichrome for connective tissue, silver for axons, and alcian blue for acid mucopolysaccharides.

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