Chronic experimental glaucoma in primates
II. Effect of extended intraocular pressure elevation on optic nerve head and axonal transport

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Intraocular pressure (IOP) elevations lasting from 2 to 42 days were produced in 13 primate eyes by anterior chamber injections of autologous, fixed red blood cells. The retina, optic nerve head, and optic nerves were studied by electron microscopy, and ganglion cell rapid axonal transport was examined after IOP elevations for various durations. Transport of axonal material was blocked at the scleral lamina cribrosa by IOP elevations to 50 mm Hg. With IOP elevation for less than 1 week, return to normal IOP restored normal transport in some axons. However, in other axons IOP elevation for less than 1 week initiated ganglion cell degeneration. The process of cellular death involved a rapid ascending degeneration from nerve head to brain, followed 3 to 4 weeks later by descending degeneration of the ganglion cell body and its attached axon. Axons of the superior and inferior optic nerve head and nerve seem to be damaged more extensively than those in the nasal and temporal optic nerve. Two to four days after IOP elevation, axons of the superficial optic nerve head were swollen with accumulating axonal material, leading to histologic disk edema. In those eyes with IOP elevation longer than 1 week, the loss of anterior disk nerve fibers combined with posterior and lateral movement of the lamina cribrosa lead to an increase in optic disk cupping. Astrocytes and capillaries of the optic nerve head seem to tolerate elevated IOP well and were relatively spared.

Key words: glaucoma, intraocular pressure, optic nerve head, erythrocyte

There are a number of unanswered questions regarding glaucomatous optic nerve damage which can be approached by the short-term modeling of glaucoma. In early glaucoma, optic disk cup size increase precedes visual field loss in many eyes. More information is needed about the cellular events which lead to abnormal cupping. In acute glaucoma, optic disk edema (papilledema) occurs shortly after the IOP increase, to be replaced by abnormal cupping only with extended IOP elevation. What cellular events occur to cause first the swelling of the disk, then its excavation? Is the disk edema of acute glaucoma quantitatively or qualitatively different from the disk swelling associated with increased intracranial pressure? Finally, glaucoma blindness results from irreversible retinal ganglion cell degeneration. The course and pattern of selectivity of this degeneration may elucidate the mechanism of neuronal damage.

In a previous report, we described a method for the production of extended IOP elevation in primate eyes using the injection
Fig. 1. Autoradiographs of optic nerve head showing radioactive protein moving in rapid phase axonal transport as round white dots (dark field illumination). In each photomicrograph, the vitreous surface of the nerve head is at top, and the termination of the scleral lamina cribrosa (myelinated portion of optic nerve) is indicated by an arrow. Upper left, Mild transport blockade in an eye (No. 1G) which had 2 days at IOP 50 mm Hg followed by 3-day gradual return to normal IOP. Transport obstruction is evident by lack of autoradiographic gains below arrow. Upper right, Eye, No. 7R which had 7 days of IOP elevation, then 11 days of normal IOP. Legend continued
Table I. Data summary

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<th>Increased disk cup</th>
<th>Ganglion cell loss</th>
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*G, Cynomolgus monkey with ghost cell injection. R, Squirrel monkey with fixed red blood cell injection.
†Degree of ascending degeneration in nerve quadrants: S, Superior; I, Inferior; N, Nasal; T, Temporal.

Methods

The method for preparation of fixed RBC and GBC was previously described, along with the methods used for monitoring IOP.1 Briefly, each animal's own processed RBC were injected into the anterior chamber with IOP monitoring by a pneumatonograph specifically calibrated for the animal eyes studied. Both squirrel (Saimiri sciureus) and cynomolgus (Macaca fascicularis) monkeys were used.

IOP elevations of various durations were produced (Table I). On the day of sacrifice, rapid-phase axonal transport was studied by the following procedure. Anesthesia was induced by intramuscular phencyclidine and intraperitoneal pentobarbital. Systemic blood pressure was monitored by means of a femoral artery catheter connected to a pressure transducer. IOP was set at the prevailing IOP for that eye during the preceding 24 hr with an anterior chamber needle connected to a variable height reservoir. An intravitreal injection of 0.1 ml (100 /μCi) of 3H-leucine (L-leucine-4, 5-3H(N), 30 to 50 Ci/m mole, New England Nuclear) was given and the entry site sealed with epoxy glue. The IOP was held constant for 4 hr, after which 4% buffered parafomaldehyde was infused retrograde into the arterial system via the aorta.

The eyes and optic nerves were divided into specimens including retina, optic nerve head, and optic nerve. These were postfixed in 2% phosphate-buffered osmium tetroxide and embedded in epoxy resin. One-micron sections on glass slides were examined by phase contrast microscopy and processed for autoradiography by a previously described method.2 The pattern of retinal ganglion cell incorporation of radioactive amino acid as well as transport of labeled proteins was examined by light microscopy. The same material was thionined for electron microscopy, stained with uranyl acetate and lead citrate, and examined with a JEOL 100B instrument.

Fig. 1. cont’d. Transport is almost completely obstructed at the scleral lamina, again despite IOP normalization. Lower left, Normal transport in an eye (No. 6G) with 4 days at IOP 45 mm Hg followed by 1 month of normal IOP. Though some axonal loss had occurred (See Fig. 4), transport apparently had returned to normal in surviving nerve fibers. Lower right, Normal transport in an eye (No. 13G) with 42 days at average IOP 24 mm Hg. At this IOP level, most axons seem to function normally for at least this duration of IOP elevation. (Unstained sections, dark field illumination. Upper right ×25, others ×125.)
Results

Axonal transport. The normal pattern of ganglion cell incorporation of radioactive amino acid and its transport via the optic nerve have been summarized elsewhere.\(^3\) With experimentally increased IOP, an abnormal accumulation of autoradiographic grains representing transported protein occurs at the scleral lamina cribrosa.\(^2\) Coinciding with this autoradiographic finding, ganglion cell axons at this level of the optic nerve head exhibit an abnormal accumulation of intracellular organelles, chiefly smooth vesicles and mitochondria. These two findings define a blockade of rapid phase axonal transport.

In the animals studied, the axonal transport findings varied depending upon the height and duration of IOP elevation (Table I). Some were sacrificed after 2 or 4 days IOP elevation (eyes 1, 3 to 5). Others had IOP rises for 2 or 4 days followed by spontaneous return to normal IOP for a period of 1 month (eyes 2 and 6). A third group had IOP rises for longer than 1 week at a relatively high IOP level (eyes 7, 8, 9, and 11). The fourth
group had mild IOP elevation for longer than 1 week (eyes 10, 12, and 13).

With continuous IOP elevation for either 2 to 4 days or 1 week and longer, there was significant blockade of rapidly transported material, judged by autoradiographic or electron microscopic findings (Figs. 1 and 2). In eyes with 2- or 4-day IOP elevation followed by 1 month of normal IOP, no transport interruption was defined in the optic nerve, though it was obvious that some axons had undergone degeneration during the period of normal IOP (Fig. 1). With moderate IOP elevation for 1 to 6 weeks, no definite interruption of transport could be seen, although again the loss of some axonal fibers was noted (Fig. 1).

In some eyes, increased size of the physiologic cup of the nerve head had already occurred at the time of transport study. In these eyes, radioactive material accumulated at the neuroretinal disk rim (Fig. 3). In other eyes in which shorter durations of elevated IOP were studied, the optic nerve head was histologically swollen. At the level of the disk rim there were dense accumulations of autoradiographic grains over the peripapillary nerve fiber layer (Fig. 3).

*Induced optic disk changes.* In eyes with 2- to 4-day increases in IOP, the most common light microscopic finding was swelling of the superficial disk axonal tissue, with lateral crowding of the peripapillary retina and retinal pigment epithelium (Figs. 4 and 5). This swelling was clearly due to swollen axons, seen ultrastructurally to be filled with intracytoplasmic organelles (Fig. 5). In eyes with similar 2- to 4-day IOP rises followed by 1 month of normal IOP, no swelling was evident (Figs. 4 and 6). Similarly, no disk swelling was observed in eyes with longer-lasting IOP elevations. In those longer elevations the disk was either normal or abnormally cupped (Figs. 4 and 6).

Increase in the optic disk cup occurred only in eyes with IOP elevations lasting longer than 1 week. The increase in the physiologic cupping seemed to result from a combination of posterior and lateral movement of the lamina cribrosa and loss of axonal fibers of the anterior optic nerve head (Figs. 4, 6, and 9). No eye had gross or fine structural cup size increase due to posterior lamina movement alone. Eyes with some loss of axons due to 2- to 4-day IOP elevations followed by 1 month of normal IOP had a mild collapse of the disk tissues without abnormal cupping (Figs. 6 and 10), an appearance similar to optic nerve heads after experimental optic nerve degeneration induced by orbital nerve trauma. Eyes with IOP increases to a mean of 24 to 45 mm Hg for 2 to 6 weeks...
Fig. 4. Junction of retina and optic nerve head. Compared to normal rim (upper left), 4 days of IOP elevation (upper right) leads to swelling of peripapillary nerve fiber layer and lateral crowding of the outer retina and pigment epithelium, indicating disk edema (eye No. 4C). Lower left. With sustained IOP elevation for 2 weeks, there is a relative loss of rim tissue and backward bowing of lamina cribrosa (eye No. 9G). Lower right. After 3 weeks of IOP elevation, few nerve fibers remain passing over the nerve head rim and the lamina is bowed considerably backward (eye No. 11R). (Paraphenylenediamine, phase contrast, lower left X25, others X90.)

showed mild axonal loss without definite cup size increase.

Astrocytes and capillaries of the optic nerve head were not demonstrably damaged by IOP levels which gave rise to axonal degeneration (Figs. 9 to 11). Both of these cellular elements appeared ultrastructurally normal, and astrocytes incorporated radioactive amino acid quite readily in all eyes (Fig. 11).

Ganglion cell degeneration. None of the optic nerves studied were completely intact. Even in eyes with 2- to 10-day IOP elevations, significant ascending optic nerve degeneration was evident, beginning at the scleral lamina cribrosa and extending toward the brain (Fig. 12). In these eyes, however,
no detectable loss of ganglion cell bodies in
the retina was found (Fig. 13). Ganglion cell
body death did occur in eyes with either 2- to
4-day elevations followed by 1 month of nor-
amal IOP or in eyes with IOP elevations las-
ting 4 weeks or longer, where mean IOP was
greater than 40 mm Hg. Histologically, there
was atrophy in ganglion cell and nerve fiber
layers of the retina, without other retinal
damage (Fig. 13). Those eyes with mean IOP
lower than 40 mm Hg showed no significant
ganglion cell loss despite up to 6 weeks of
abnormally high IOP.

The selectivity of damage at the level of the
optic nerve was examined in cross sections
oriented to identify superior/inferior and
nasal/temporal axes of the tissues. In eight of
13 nerves, the pattern of ascending degen-
eration was not uniform in the optic nerve.
The temporal quadrant of the nerve was
either least affected or not damaged in all eight of these nerves (Fig. 12). The superior
quadrant was among the most damaged areas
in seven of the eight nerves, being somewhat
more affected overall than the inferior and
nasal quadrants, respectively. However, the
longer the time of study after the initial IOP
increase, whether sustained or transient, the
more uniform was the damage pattern of the
optic nerve.

There was a cystic increase in the extracel-
lar space of the retrobulbar optic nerve
(cavernous atrophy) in only one specimen,
5R, which had the highest peak and mean
IOP of those studied.

Discussion

Previous studies of the effects of IOP elev-
ations of less than 24 hr duration have
defined a number of features of axonal dam-
age.2 4-5 Such acute experiments demon-
strated a blockade of normal axonal transport

Fig. 5. With 4 days of IOP elevation, axons of the peripapillary nerve fiber layer are swollen
with accumulated organelles and neurofilaments (see Fig. 3, lower, and Fig. 4, upper right).
(Eye No. 4C, ×10,900.)
within many ganglion cell fibers at the level of the scleral lamina cribrosa. This acute insult involved both rapid orthograde and retrograde axonal transport and possibly slow phase transport. The induced rapid transport blockade was reversible after 4 hr of IOP elevation. There was slightly greater interruption of transport in the superior and inferior quadrants of the nerve head compared to the nasal and temporal quadrants. There are strong suggestions that the cause of the transport blockade is more likely to be mechanical compression of the axons within the nerve head, rather than vascular insufficiency.

The more extended IOP elevations in the present study have added further information to the effects of IOP-induced axonal transport interruption. First, the transport blockade seen after 8 to 24 hr of IOP elevation obviously continues with IOP increases lasting for periods up to 2 weeks. Second, if an IOP elevation lasts 2 to 4 days and is followed by normal IOP for 1 month, no significant transport blockade is observed. In part, this must occur by return to normal transport in axons.
which are not irreversibly damaged and in part by the death of other axons which do not tolerate the initial IOP rise. With IOP elevations lasting longer than 1 week, significant loss of axons from the nerve head to the lateral geniculate has occurred. In this circumstance, the remaining ganglion cell bodies incorporate radioactivity and transport it to the rim of the nerve head, where it stops for lack of axon continuity. By 1 month after a significant insult to the axons at the disk, their ganglion cell bodies degenerate, as seen in a previous study of optic nerve descending degeneration. In this case there is a major decrease in incorporated and, therefore, transported radioactivity. Thus ability of axons to return to normal transport function can be seen to depend on the duration and height of IOP elevation. Although the number of eyes studied here is relatively small, the conclusion seems justified that many axons are permanently damaged by IOP between 45 and 70 mm Hg for as short a duration as 2 to 4 days. Since rapid axonal transport is completely blocked in many axons under these conditions, it is not surprising that they do not survive. Just as correctly, some axons tolerate such insults and return to normal function if normal IOP is restored. From the pattern of ascending degeneration, it would seem that the upper and lower poles of the optic nerve near the nerve head are most susceptible to irreversible damage. It remains to be determined whether this is due to a difference in the degree of insult to which these fibers are subjected or to a difference in the tolerance of fibers from various disk quadrants to the same insult.

The swelling of the optic nerve head tissue observed with acute IOP increase corresponds to axons distended with cytoplasmic organelles. In the first 12 hr of IOP increase, the buildup of organelles is limited to the lamina cribrosa at the level of the sclera. It seems likely that the later swelling of axons in the more superficial nerve head represents simply a backup of material which cannot pass the deeper obstruction. The high density of autoradiographic grains overlying such swollen axons confirms that the accumulating material is at least in part due to obstructed rapid-phase axonal transport. It will require further studies of slow-phase axonal transport in similarly treated eyes to determine the degree to which blockade of slowly transported materials contributes to the axonal swelling. The axonal swelling observed with extended IOP increase appears superficially similar to that found in disk edema from increased intracranial pressure and ocular hypotony. However, there seem to be real differences in the disturbances in axonal function between the latter forms of disk edema and short-term IOP increase. While IOP increase causes an immediate blockade of rapid transport, increased intracranial pressure or hypotony appear to produce more of a slowdown of transport, and rapid-phase transport is disturbed only after actual axonal swelling has taken place. Thus the pathogenesis of two similar-appearing end results may be quite different.

The increase in optic disk cup size observed in some of the nerve heads in this study seemed to result from two histologic changes. In every nerve head with increased cupping there was a decrease in the thickness of the disk rim. Detailed ultrastructural examination disclosed significant axonal degeneration occurring in these areas. The second altered element was posterior and lateral
displacement of the scleral lamina cribrosa itself. We were not able to detect with present histologic techniques any nerve head with posterior laminar movement without associated loss of axons. There were nerve heads with axonal loss, but without posterior laminar displacement when short duration IOP increase was followed by return to normal IOP. These nerve heads were identical to nerve heads after experimental optic atrophy induced by orbital trauma,6 in which the optic disk was pale but not abnormally cupped. Seemingly, then, it is the combination of axonal atrophy and posterior laminar movement which gives rise to characteristic glaucomatous cupping. It is well known that after a brief acute glaucoma attack the optic disk may be pale, even with apparently normal vision, but is rarely severely cupped.17 By analogy to the eyes in this study that had brief, high IOP elevations and sufficient time (1 month) for descending degeneration to occur, the same mild optic nerve degeneration must explain the pale, noncupped appearance of these human eyes in the clinical setting. It is only the continued elevation of IOP that causes posterior laminar movement in addition to neuronal atrophy, leading to glaucomatous cupping.

It has been previously hypothesized that loss of astrocytes of the optic nerve head might participate in the early cup size in-
Fig. 9. As axons are lost with sustained IOP elevation, portion of nerve head opposite choroid shows few remaining axons, with most processes in this zone belonging to astrocytes. Capillary (C) and associated pericyte are unaffected. (Eye No. 9G, ×10,000.)
crease in glaucoma. Furthermore, there are reports of astrocyte compression with acute IOP elevation in primates. Another ultrastructural study of experimentally produced cupping found no damage to nerve head astrocytes. In our study, not only were disk astrocytes histologically normal, but they continued to incorporate the injected radioactive amino acid into intracellular protein by autoradiography. In addition, a light and electron microscopic study of 21 human eyes from known glaucoma patients demonstrated no selective loss of nerve head astrocytes. We conclude that the evidence is quite convincing that astrocyte loss does not significantly participate in the process of glaucomatous cupping.

The increase in cup size observed here occurred quite rapidly in eyes subjected to IOP greater than 40 mm Hg. Previous reports of experimental IOP elevations in primates with laser treatment of the angle have demonstrated increased cupping after a similar duration and height of IOP elevation. This seems to be more rapid than the rate at which increased cupping occurs in adult human eyes with similar IOP exposure. The threshold for nerve head damage in primates may differ from that in human eyes, despite the similarity between their optic nerve head tis-

Fig. 10. After 4-day IOP elevation followed by 1 month of normal IOP, the nerve head at the level of the choroid shows a relative decrease in the number of axons compared to astrocytes (darker processes running left to right). Dark globules interpreted as degeneration products of axons are seen at left within astrocytes. Compare to the more extensive loss of axons in an eye with sustained IOP elevation, Fig. 9. (Eye No. 6G, x8,400.)
sues. There is, however, a striking similarity between the histologic events in these primate eyes and the available findings in human glaucoma eyes. The experimental primate glaucoma models seem to represent the production of similar nerve head changes with a compressed time frame.

Cavernous optic nerve degeneration describes the finding of cystic spaces containing hyaluronic acid within the optic nerve substance, usually in the setting of an event which has caused severe optic atrophy. Cavernous degeneration was a frequent finding in a group of animals with extremely high experimental IOP increase induced by alpha-chymotrypsin injection into the posterior chamber. This finding was uncommon in our study, as well as in other studies of experimental acute IOP elevation in primates. The major differences between the study of Zimmerman and the more recent reports seems to be the severe IOP elevations induced by enzyme injection (IOP = 70 to 80 mm Hg, Schiotz). In addition, these eyes demonstrated retinal edema, optic nerve head hemorrhages, and some nonperfusion of major retinal vessels. Cavernous degeneration seems, then, to occur at the
Fig. 12. Example of selective loss of axons in retrobulbar optic nerve (eye No. 7R). Upper left, normal cross-sectioned profiles of myelinated axons in temporal optic nerve compared to superior quadrant degeneration (upper right) in same nerve. Lower electron micrograph illustrates axon degeneration in superior optic nerve. (Light micrographs. Paraphenylene-diamine, phase contrast, ×190. Electron micrograph, ×7,200.)
Fig. 13. After 4 days of increased IOP (upper left), the retinal ganglion cell layer is normal despite axon damage at the nerve head (eye No. 3R). However, after 3 weeks of IOP elevation (upper right and lower), descending degeneration of ganglion cell bodies begins (arrows). (Eye No. 11R. Upper light micrographs, ×380, phase contrast, paraphenylenediamine. Electron micrograph, ×11,300.)
high end of the spectrum of IOP-induced damage. The mucopolysaccharide present in the optic nerve substance in cavernous degeneration is most likely derived from contents of the vitreous humor which follow the extreme pressure gradient from within the eye outward via the optic nerve head extracellular space. By a number of investigative approaches, material within the vitreous cavity has been demonstrated to follow this route. There may be a movement of fluid and larger molecules from vitreous to optic nerve under normal IOP which is increased with IOP elevation. In this context, cavernous degeneration would occur when extremely high IOP levels lead to more material movement into the extracellular space of the optic nerve than could be removed by the normal exit pathways for such substances.

REFERENCES
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