Swelling and Loss of Photoreceptors in Chronic Human and Experimental Glaucomas

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Objective: To determine whether outer retinal changes occur in chronic, presumed primary open-angle glaucoma (POAG).

Methods: The outer retinas from 128 human eyes with a diagnosis of chronic glaucoma (presumably POAG in most cases) and 90 control eyes were examined histologically by 3 masked observers for photoreceptor swelling and loss. Retinas from 9 rhesus monkeys with glaucoma induced experimentally by laser trabecular destruction were compared with 7 fellow (control) eyes. The mean pressure elevations in the eyes with laser trabecular destruction ranged from 26.6 to 53.6 mm Hg with durations varying from 7 to 33 weeks.

Results: Swelling of the red- and green-sensitive cones was observed in a statistically significantly greater proportion of human eyes with presumed POAG compared with the control eyes. Patchy loss of red/green cones and rods was also found in some of the glaucomatous retinas. In a subset of the human eyes with end-stage disease, cone swelling was a variable finding. Although no photoreceptor loss was found in the 9 monkey eyes with experimental glaucoma, 8 had swelling of their red/green cones that was remarkably similar to that seen in the human eyes. Swelling was not present in any of the control monkey eyes.

Conclusions: The photoreceptors are affected by chronically elevated intraocular pressure.

Clinical Relevance: These findings may explain some of the abnormalities of color vision and the electrophysiological effects that have been observed in patients with POAG.


Despite advances in medical and surgical treatment, glaucoma remains the second leading cause of blindness worldwide after cataract. Unlike cataract, its effects are irreversible. Visual loss from primary open-angle glaucoma (POAG) occurs because the retinal ganglion cells die, probably by apoptosis. Traditionally, elevated intraocular pressure (IOP) is thought to gradually and irreversibly damage the ganglion cell axons in the optic nerve and thereby cause retrograde degeneration of their cell bodies. The mechanism by which elevated pressure causes death of the optic nerve fibers has been assumed to be direct—either by reducing the blood flow to the nerve or by causing an outward deformation of the lamina cribrosa with the subsequent interruption in axoplasmic flow. However, electrophysiological studies and the observed pattern of color vision loss suggest that the process may be more complex, perhaps involving the outer retina.

The electroretinogram, which is generated mostly by the photoreceptors and the bipolar cells and is not altered by optic nerve transection, should not be affected if glaucomatous damage is limited to the optic nerve and inner retina. Recent work using careful quantification and proper selection of controls now provides strong evidence of outer retinal effects in humans. Furthermore, Weiner et al found that foveal cone electroretinogram amplitude was subnormal in a significant proportion of glaucomatous eyes. On the other hand, outer retinal electroretinographic changes were not seen in a monkey model of experimental glaucoma. Deficits in color vision that arise from optic nerve disease usually result in confusion along the red-green color axis, while those disorders that affect the outer retina usually cause blue-yellow defects (Kollner’s rule). Although the underlying mechanisms for this “rule” are not completely understood, it has been repeatedly confirmed for specific diseases by numerous studies over the past century (for...
METHODS
HUMANS

The tenets of the Declaration of Helsinki were followed. Approval by our institutional human subjects committee was granted for this study.

To learn whether similar anatomical changes develop in humans with naturally occurring glaucoma, retinas from 128 eyes from 64 deceased donors who had a clinical diagnosis of glaucoma were examined. These were compared with 90 eyes thought to be free of retinal or optic nerve disease from 45 deceased donors. Sex and race distributions were similar for both groups (Table 1), although there were differences in age and autolysis time (time from death to placement of globes into fixative) (see the "Results" section).

The eyes were enucleated after death, fixed in 10% formalin for 24 to 72 hours, and stored in 0.1-mol/L phosphate buffer at 4°C. Each globe was sectioned in a coronal plane between the equator and the ora serrata. The retinas and optic nerves were then inspected with a dissecting microscope for signs of disease other than glaucoma. Those that had obvious retinal disease (eg, age-related macular degeneration, diabetic retinopathy, vascular occlusive disease) were excluded from the study. Segments of retina from the inferotemporal macula were removed and processed in a manner similar to that used for the monkey eyes. In addition, we used enzyme histochemical analysis for carbonic anhydrase (CA) to distinguish the red- and green-sensitive cones from the blue-sensitive cones (the procedure is not effective for rhesus monkeys).13,27

The diagnosis of POAG can be difficult to make. For example, the Baltimore Eye Survey found that about 3% of whites and 12% of blacks older than 70 years may have the disease—perhaps half of whom are undiagnosed.29 With all of our subjects, a diagnosis of glaucoma had been made by either an ophthalmologist or an optometrist. In only a minority of cases were we able to independently confirm the diagnosis due to missing or inadequate medical records. Complicating matters, some of the subjects may have had good therapeutic control of their disease, while others had obviously poor control (judging from progression of visual field loss). Finally, even with good records, there was usually a significant lapse between the last ocular examination and when the patient died, making it impossible to be certain which eyes had active disease at the time of death. For these reasons, and because we were interested in determining only if there was a statistical association, rather than determining prevalence, we included all of the eyes that had been clinically diagnosed with glaucoma and compared them with eyes in which such a diagnosis had not been made.

Because complete records were not available for all of the subjects, we could not rule out the possibility that a few had normal-tension glaucoma. However, given that normal-tension glaucoma is less common than POAG with elevated pressure, and because we had no indication that any of the subjects had acute, high-pressure glaucoma (eg, angle closure, particle, or inflammatoty glaucomas, which are also relatively uncommon), we believe our sample contained mostly eyes with POAG. For simplicity, we will use term "POAG" when referring to these subjects.

To determine if the observed cone swelling was statistically associated with the diagnosis of glaucoma in the human eyes, 3 of us (T.M.N., G.L.P., and R.W.N.) performed masked gradings of the 218 eyes. One (T.M.N.) is a trained pathologist and retinal specialist, one (G.L.P.) is a review see Pokorny and Smith12). Work from our laboratory suggests that blue-yellow discrimination loss in retinal detachment and diabetic retinopathy is due to increased susceptibility of the blue cones compared with the red and green cones.13,14

A peculiar aspect of the vision loss in patients with POAG is the type of color vision deficit observed. Given the putative involvement of the optic nerve in the etiology of glaucoma, it would be expected that color vision loss would be along the red-green axis. Patients with glaucoma, by contrast, confuse colors along the blue-yellow axis.15-19 This is often explained by assuming that those axons responsible for conveying blue-yellow opponent data are more susceptible to damage than those transmitting red-green information. However, such an explanation remains controversial.

We were interested in exploring whether blue-yellow color problems could be based on photoreceptor damage as we found in retinal detachment11 and diabetes.12 Histopathologic evidence for photoreceptor injury in high-pressure glaucoma is unequivocal. Anderson and Davis20 observed focal dropout of photoreceptors in the owl monkey. Ultrastructural defects suggestive of programmed cell death (apoptosis) were noted by Büch11,21 in nuclei of the outer retina in a rat model of pressure-induced ischemia. In human eyes with secondary angle-closure glaucoma, Panda and Jonas23 counted significantly fewer photoreceptors compared with normal controls. Janssen et al24 found fewer photoreceptors compared with controls as well as pyknotic nuclei in the outer nuclear layer in 2 human eyes with secondary, painful glaucoma (IOPs >40 mm Hg). By contrast, anatomical evidence for outer retinal injury in chronic (moderate pressure) glaucoma has been lacking. Loss of photoreceptors was not found in human eyes with POAG in a study by Kendell et al.25 Nor did Wygnanski et al26 observe cone loss in experimental glaucoma in cynomologus monkeys.

In the present study, we demonstrate that swelling of the red- and green-sensitive cones as well as patchy cone and rod dropout is strongly associated with a diagnosis of glaucoma in a large sample of donated human eyes. We provide confirmation of these correlative findings by demonstrating that, with artificially elevated pressure, rhesus monkeys show a similar pattern of swelling in their red- and green-sensitive cones.

RESULTS
HUMANS

Photoreceptor Swelling

A subset of the eyes with POAG showed marked swelling of the dominant cone population. Cross sections of the cone
an experienced laboratory technician, and the other
(R.W.N.) is a scientist who studies the molecular genetics
of retinal ganglion cells. Segments of the retina were ex-
amined that measured 4 mm vertically and 7.5 mm hori-
zontally, were located just below the horizontal midline,
and extended from 5 to 35 arc degrees temporal to the fo-
vea. Because this portion of the retina corresponds to the
earliest visual field losses (after enlargement of the physi-
ologic blind spot).30,31 we reasoned that the sensitivity of
the study would be greatest if we chose this area.

Scoring was subjective and consisted of “no swell-
ing” (grade 0), “mild swelling” (grade 1), “moderate swell-
ing” (grade 2), and “marked swelling” (grade 3) for both
the swollen perikarya and swollen inner processes. Stan-
dard photographs served as a guide to the observers.

Figure 1, B, and Figure 2, B, represent nearly the maxi-
mum number of swollen perikarya and swollen inner pro-
cesses (grade 3). A section with a grade of 3 was defined as
having at least 75% as many swollen cones (perikarya or
inner processes) as shown in Figure 1, B, and Figure 2, B.
Grade 2 was defined as having 50% to 74% as many swol-
len cells. Grade 1 had 25% to 49% and grade 0 had 0% to
24% of the cones swollen. Due to autolysis, it was not pos-
sible to quantitatively measure these percentages. Because
of the patchy nature of the effects of glaucoma across the
retina, the grade was based on that portion of the retina
that had the most swelling so long as it covered an area of
at least 500 µm², ie, the central field visible with the 40X
objective.

To examine whether loss of photoreceptors was as-
associated with glaucoma, an additional masked analysis was
undertaken by one reviewer (T.M.N.). Such photorecep-
tor dropout could be recognized without using quantita-
tive methods because of the highly regular distribution of
inner segments in some of the eyes revealed 2 populations
based on size, with the smaller one making up 9% of the
total (the same proportion as the blue-sensitive or S cones
in the human retina) (Figure 3, B).27,37 Enzyme histo-
chemical analysis for CA revealed these to be the CA-
negative cones (blue-sensitive cones) (Figure 4, B).27 At
the level of the outer portion of the outer nuclear layer, the
CA-positive (red- and green-sensitive, or L and M) cone
nuclei were enlarged and were surrounded by swollen peri-
karya, giving the cell body a “fried-egg” appearance that was
not evident in the normal retinas (Figure 1, B). Tangential
sections through the outer part of the outer plexiform layer
showed swollen inner processes (Figure 2, B). An additional
feature of a small group of the glaucomatous human eyes
was actual loss of cones (usually the CA-positive cones)
as well as occasional, focal losses of rods (Figure 4).

Masked Study

Agreement among pairs of graders was assessed with the
κ statistic.38 Kappa is a conservative measure of interob-
server reliability that takes into account chance level of
agreement based on the distribution of scores within cat-
egories. Kappa statistics on the agreement between the
trained pathologist and each of the 2 naive graders were
determined separately for the glaucoma and the control
groups. The percentage agreement within each grade
among the trained pathologist and the 2 naive graders was
modest (range, 50.5%-54.1%). However, the value of κ sta-
tistic was significant for each comparison tested (all 8 P
values <.01). Thus, 3 independent observers, 2 with no
formal training in pathology, showed statistically signifi-
cant agreement in independent grading of the extent of
swollen perikarya and inner photoreceptor processes.

There was a difference in mean autolysis times (in-
terval between death and placement of the eye into fixa-
tive) as well as age between the glaucoma and control
groups (Figure 5 and Figure 6). To examine whether swel-
ling of perikarya or inner processes was associated
with level of autolysis or age of the patient, correlations
between autolysis scores, grade, and age were con-
ducted on the data from one of the reviewers (T.M.N.).
Nonparametric ρ correlations, adjusted for the large num-
ber of ties in the grades, revealed no statistical associa-
tion (P>.1) between level of autolysis and grading of peri-
karya or inner processes. Nor was there an association
between level of autolysis and age in this analysis.

Group Differences

Nonparametric analyses indicated that the distribution
of grades of the slides from subjects with glaucoma was
significantly different from that of the control subjects.
Using one grader’s (T.M.N.) data, the distribution of

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All animal procedures adhered to the Association for Re-
search in Vision and Ophthalmology statement on the use
of animals in vision research.

To investigate directly whether the photoreceptors are
involved in the disease process, we first looked at experi-
mental glaucoma in monkeys. Chronically elevated IOP was
created in the right eyes of 9 rhesus monkeys using argon
laser trabecular destruction.34,35 The untreated left eyes
created in the right eyes of 9 rhesus monkeys using argon
laser trabecular destruction.34,35 The untreated left eyes
were used for other studies. The IOP was measured with a
handheld electric tonometer (Tono-Pen XL; Mentor O &
O, Norwell, Mass). The average pressures and durations are
shown in Table 2. The Tono-Pen has been found to un-
derestimate the true IOP in cynomolgus monkeys36; how-
ever, a similar study has not been done in rhesus mon-
keys, in which the correlation is likely to be better because
the eyes are larger and the sclera and cornea thicker than
in the cynomolgus monkey.

Following enucleation, the retinas were fixed in 4% parafo-
ormaldehyde, segments of the retinas were taken from
the midperipheral macula (about 5-30 arc degrees of ec-
centricity), embedded in glycol methacrylate, cut 1 to 2 µm
thick, and stained with toluidine blue. The retinas were sec-
tioned in planes parallel to a tangent of the globe at vari-
ous levels though the photoreceptors.13 Tangential sec-
tions allowed for a much greater number of photoreceptors
to be examined on each slide than radial sections, so that
disruption in the normal 2-dimensional matrix of rods and
cones could be readily identified.
grades differed significantly between groups for both perikaryal swelling and swollen inner photoreceptor processes (likelihood ratio = 14.34, df = 3, \( P < .01 \), and 11.57, df = 3, \( P < .01 \), respectively) (Figure 7). Examination of the standardized residuals of the likelihood analysis indicates that the largest deviation from expected levels occurred in the 3+ grade in the glaucoma group. These analyses were performed for the other 2 graders with similar results. The only exception was for a grader (G.L.P.) whose gradings of swollen inner retinal processes did not distinguish groups. Thus, with one exception, 3 independent gradings of the 218 slides for the 2 types of abnormalities resulted in significantly greater numbers of 3+ scores in the glaucoma group than in the control group.

### Photoreceptor Loss

Thirteen eyes had evidence of cone loss, an example of which is shown in Figure 4, C and E. Fifteen eyes had focal rod loss (Figure 4, D and F), 7 eyes had both cone and rod loss, and 21 eyes had either cone or rod loss. Photoreceptor loss (ie, missing cells) was confirmed by examining the outer nuclear layer in regions of missing inner segments to rule out the possibility that the inner and outer segments had merely contracted.

All 13 eyes with cone loss had a clinical diagnosis of glaucoma, for a total of about 10% (13 of 128) of the eyes with glaucoma. Of the 15 eyes with some rod loss, only 1 did not have a clinical diagnosis of glaucoma. Twenty eyes (16%) with glaucoma had either cone or rod loss. These findings probably underestimate photoreceptor dropout because the loss is patchy and the sampling area was limited.

### Relationship Between Photoreceptor Swelling and Ganglion Cell Loss

To test whether the photoreceptor swelling was a response to degenerating ganglion cells, tangential sections

![Figure 1](#)
at the level of the ganglion cells were examined in all 128 eyes with a diagnosis of glaucoma. Twenty of these had severe (>90%) loss of the ganglion cells (i.e., end-stage disease). Five (25%) of the 20 had 3+ swelling of both the cone perikarya and inner processes. However, 7 of the eyes with severe ganglion cell dropout (35%) had no sign of photoreceptor swelling.

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In the area sampled, 8 of the 9 eyes with experimental glaucoma contained regions of swollen cones compared with the controls (Table 2). At the level of the inner segments, 2 populations of cones could be identified (Figure 3). The majority of cones had enlarged inner segments with irregular-appearing borders, while an evenly distributed minority population (10.3% of the cones) was not swollen. This is the same proportion and distribution found for the red- and green-sensitive cones and the blue-sensitive cones, respectively, in the midperipheral retina of the rhesus monkey. Sections through the outer part of the outer nuclear layer (where the cone nuclei are found) showed increased size of the nuclei in the treated compared with the control eyes (Figure 1). Marked swelling of the inner photoreceptor processes was evident at the level of the outer part of the outer plexiform layer (the location of the cone and rod inner processes just distal to the pedicles and spherules, respectively) (Figure 2).

![Figure 2. Outer part of outer plexiform layer, tangential sections. A, Control human eye; B, another human eye (same location in retina) with primary open-angle glaucoma; C, control monkey, left eye; and D, left eye with experimental glaucoma of same animal and location as in panel C. For both the human glaucomatous eyes (B) and experimental glaucomatous eyes (D), there is dramatic swelling of at least some photoreceptor inner processes. Staining with toluidine blue. Bar = 10 µm.](https://www.archophthalmol.com/article-pdf/118/2/238/1201933/archophthalmol_2000_118_2_238.pdf)

**Table 2. Intraocular Pressures and Presence of Cone Swelling for Treated and Control Eyes of 9 Monkeys With Experimental Glaucoma**

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Duration, wk</th>
<th>Treated Eye IOP, mm Hg</th>
<th>Cone Swelling</th>
<th>Control Eye IOP, mm Hg</th>
<th>Cone Swelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>32.0 ± 13.3</td>
<td>+</td>
<td>16.8 ± 3.6</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>51.7 ± 9.1</td>
<td>+</td>
<td>16.0 ± 2.9</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>26.6 ± 11.6</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>41.1 ± 12.4</td>
<td>+</td>
<td>16.0 ± 1.9</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>45.1 ± 15.4</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>39.0 ± 10.7</td>
<td>−</td>
<td>17.2 ± 3.0</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>27.3 ± 12.4</td>
<td>−</td>
<td>14.7 ± 2.2</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>45.1 ± 12.1</td>
<td>+</td>
<td>18.8 ± 3.2</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>53.6 ± 10.4</td>
<td>+</td>
<td>19.4 ± 2.3</td>
<td>−</td>
</tr>
</tbody>
</table>

*IOP indicates intraocular pressure (given as mean ± SD); plus sign, present; minus sign, absent; and NA, not available for examination.*

Quantitative measurements of all of the cone inner segment cross sections in the vicinity of areas shown in Figure 3, C and D (the control and treated eye from the same monkey), were made using image analysis software (Optimas Corporation, Bothell, Wash). The mean cone area for the control retina (47.52 µm², n = 41, SE = 0.56) was intermediate in size between the majority population of cones of the fellow eye (57.04 µm², n = 36, SE = 0.56) and the minority cones (25.74 µm², n = 11, SE = 0.88).
Quantitative measurements of all of the clearly identifiable cone nuclei in the vicinity of the areas shown in Figure 1, C and D, were made. The cone nuclei were significantly larger in the glaucomatous eye (mean area = 57.04 µm², SE = 0.56) than corresponding values from the control eye (mean area = 47.52 µm², SE = 0.56) (t_{14} = 12.05, P < .001).

COMMENT

CHRONIC HUMAN GLAUCOMA

These data indicate that cone swelling, especially of the red and green cones, is statistically associated with a diagnosis of glaucoma in humans. Due to a number of factors, such as accuracy of the diagnosis, the limited sampling area, the effects (if any) of IOP control with treatment, and possible masking of changes by autolysis in this postmortem tissue, we cannot be certain of the true incidence of swelling in this disease process. Likewise, there is no way to know from these data whether photoreceptor swelling contributes to visual field loss in glaucoma.

AGING AND PHOTORECEPTOR LOSS IN HUMAN GLAUCOMA

The mean age of the subjects with glaucoma was older than that of the controls. Other investigators have observed an association between photoreceptor loss and aging. However, the most obvious effect of aging is a loss of rods rather than cones. Furthermore, the pattern of loss is much different than that found in our study. Extensive cell counting showed generalized losses. In eyes with glaucoma in this study, the losses were highly localized and very obvious subjectively (Figure 4, C-F). The cone dropout that we observed occurred exclusively in subjects with glaucoma. Only 1 of the 15 eyes with focal rod loss was in the control group. Considering the overlap in ages, and the fact that this pattern of loss was not described in either of the 2 extensive studies cited, we believe that the photoreceptor losses are an effect of POAG. Indeed, it may be a more specific indicator than cone swelling in postmortem eyes, perhaps because events occurring just prior to death (such as vascular insufficiency, drug toxicity, electrolyte imbalances, etc) are less likely to cause cell death, which may take several days.

Although Kendell et al found no photoreceptor losses in their study of eyes with chronic open-angle glaucoma, they had a 5% experimental error. Considering that the cones constitute only 5% of all photoreceptors, their negative results do not contradict our findings of rare, patchy cone and rod losses.

EXPERIMENTAL GLAUCOMA

The animal data provided a correlative association between IOP and photoreceptor swelling. There was a re-
Markable similarity in the swelling pattern of the rhesus monkey eyes with experimental glaucoma as compared with the human specimens (Figures 1-3). Specifically, the red- and green-sensitive cones showed swelling, while the blue-sensitive cones were somewhat reduced in size. Unlike the human eyes, the amount and duration of IOP elevation was precisely known. Furthermore, autolysis, age, sex, and other patient demographics were not potential sources of variance. Thus, 8 of the 9 glaucomatous monkey eyes had photoreceptor swelling (89%) vs none of the controls, as contrasted with the human eyes that had a 22% to 26% prevalence of swelling compared with 7% to 8% for the controls.

The primary disadvantage of this experimental model is that the pressures were often higher than with the most common form of moderate-pressure human glaucoma. However, we did observe the swelling even in the 3 eyes with the lowest average pressures. The similarity of the mor-
phological changes compared with the human disease also suggests that the mechanism of injury may be similar.

Work by many other investigators supports trabecular laser destruction in monkey as an appropriate model for chronic human glaucoma. For example, the pattern of ganglion cell loss is much like that seen in humans.46,47 Recently, Harwerth et al48 performed an elegant behavioral study with this model of experimental glaucoma showing that the visual field defects are similar to the human disease.

OTHER REGIONS

Because the method of embedding, sectioning, and analysis that we used was rather time-consuming, we have not yet been able to formally analyze areas of the retina other than the inferotemporal midperiphery. However, brief inspection of other regions suggests that cone swelling is not limited to the study area.

POSSIBLE MECHANISMS

Swelling of the cones, as with other neurons, may indicate ischemic injury, which could arise from a decrease in choroidal circulation. Only a moderate increase in the IOP in cats leads to a reduction in choroidal blood flow and a precipitous fall in oxygen saturation at the vortex veins.99,100 Consistent with this finding, functional changes specific to the outer retina—decreased electroretinographic c-wave and the dark-adapted standing potential—have been shown to be reduced in cats and monkeys with experimentally induced IOP elevations as low as 15 to 20 mm Hg above baseline.31 In humans with POAG, several studies using Doppler ultrasonography have shown that blood flow is significantly decreased in the ophthalmic artery, most of whose circulation supplies the choroid.52-57 Finally, histopathologic evidence suggests that there is loss of the innermost choroidal vessels in POAG.58

COLOR VISION

As discussed in the Introduction, Kollner's rule seems to suggest that the blue-yellow color confusion seen in glaucoma is more consistent with a retinal degenerative process than one involving only the optic nerve. Our study shows that glaucoma is associated with anatomical changes to the photoreceptors. However, the situation is distinct from our earlier work with retinal detachment and diabetic retinopathy where we found selective loss of the blue cones.13,59 The human and monkey eyes with glaucoma showed mostly swelling and loss of the red- and green-sensitive cones. However, we also measured an apparent shrinkage of the blue cones in some of the monkey eyes (Figure 3, C and D).

Although we can only speculate about function based on morphological findings, recent careful analysis of the blue-yellow deficit in glaucoma reveals that unlike other retinal disorders, in which there is relatively more selective suppression of the blue mechanism,50-52 in glaucoma there is significant decrease in the sensitivity of the red and green systems as well as the blue.53 Likewise, a behavioral study in an experimental model of POAG in monkeys has shown that early glaucomatous changes affect mostly the blue pathways but that the red and green systems are involved in more advanced disease.54 Perhaps we found changes in the red and green cones to be so common because our subjects had advanced disease or it may be because the red/green cone swelling is easier to detect than more subtle biochemical changes that might be occurring in the blue cones. Due to the opponency aspect of color
vision, decreased sensitivity (or actual loss) of the blue cones alone or a process that equally affects both the red and green cones could result in blue-yellow confusion.

Indeed, all 3 cone types could be injured and the result would still be blue-yellow confusion so long as the red and green cones respond equally and the blue cones are affected to a relatively greater or lesser extent. This is not an unreasonable scenario considering the 96% identity of the amino acid sequences of the red and green cone opsins as well as many other biochemical similarities between the red and green cones, such as the CA27 and calbindin66 distributions, the immunochemical similarity of S antigen isoforms,67 and similar lectin-binding characteristics.68 By contrast, the blue cones share only a 43% identity in the amino acid opsin sequences compared with red or green. Blue cones even differ morphologically from the longer wavelength cones.37

PHOTORECEPTOR CHANGES AND GANGLION CELLS

There are 3 possibilities regarding photoreceptor pathology and ganglion cell death in glaucoma. First, swelling of the photoreceptors could be an effect of the disease that is independent of ganglion cell injury. Second, the photoreceptor changes might be a retrograde response to dead or dying ganglion cells. If the latter possibility were true, then we would expect to see no photoreceptor swelling in eyes with end-stage disease, i.e., those retinas with no or few surviving ganglion cells. However, when we looked at the subset of 20 eyes with more than 90% ganglion cell loss, we found 5 eyes with marked cone swelling and 7 with no detectable swelling (the remainder had various grades of swelling). In other words, the photoreceptor changes cannot be the result of degenerating or missing ganglion cells, since there were almost no ganglion cells remaining in this subset of subjects; yet, there is no consistent pattern of photoreceptor swelling. Indeed, photoreceptor swelling may well be a transient response to decreased choroidal blood flow—resolving once the blood flow has been restored to normal levels, such as by adequate IOP management.

A third possibility is that the ischemic photoreceptors may contribute to ganglion cell death. Ganglion cells are known to survive in the absence of photoreceptors in such conditions as retinal laser photocoagulation,69,70 retinitis pigmentosa,71 and the RCS (Royal College of Surgeons) rat.72 Thus, cell death due to a lack of trophic factors seems unlikely. However, ganglion cells are known to die via apoptosis in glaucoma,3 and they contain N-methyl-D-aspartate (NMDA) receptors,73,74 which, when acutely overstimulated by glutamate, can lead to programmed cell death.75 Elevated vitreous levels of glutamate have been found in POAG76 and it has recently been shown that chronically elevated levels of glutamate can trigger ganglion cell death as well.77 Since glutamate is the major excitatory neurotransmitter of the photoreceptors,78 it seems plausible that in POAG, the ischemia that may result from lowered choroidal blood flow could cause a decreased reuptake of glutamate. Higher levels of extracellular glutamate might chronically overstimulate the bipolar cells, which would then overexcite the ganglion cells. Although the present study does not directly test this hypothesis, it does show that photoreceptor swelling (especially of the red- and green-sensitive cones) is a widespread phenomenon in POAG, which suggests that alternative or additional pathological mechanisms are at play in this disease.

Accepted for publication September 1, 1999.

This work was supported by grants EY08724 and EY01917 from the National Eye Institute, Bethesda, Md; an unrestricted gift from Research to Prevent Blindness, Inc, New York, NY; the Wisconsin Lions Eye Research Fund, Stevens Point, and the Steve J. Miller Foundation, Marshfield, Wis.

We thank the Glaucoma Research Foundation, San Francisco, Calif, and the Wisconsin Eye Bank, Madison, for providing the human specimens. We also thank Paul L. Kaufman, MD, for his critical review of the manuscript.

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