NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION, AND REPAIR
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Submitted by

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Volume 6 of 8

Vision, Laser Eye Injury, and Infectious Diseases

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Volume 6 Vision, Laser Eye Injury, and Infectious Diseases

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1. Burgoyne CF, Varma R, Quigley HA, Vitale S, Pease ME, Lenane PL (1994) Global and regional detection of induced optic disc change by digitized image analysis. *Arch Ophthalmol* 112:261-269.

2. Study Number 01

3. Figure: Study Number 01

4. Study Number 04

5. Burgoyne CF, Quigley HA, Varma R (1995) Comparison of clinician judgment with digitized image analysis in the detection of induced optic disk change in monkey eyes. *Am J Ophthalmol* 120:176-183.

6. Burgoyne CF, Quigley HA, Thompson HW, Vitale S, Varma R (1995) Early changes in optic disc compliance and surface position in experimental glaucoma. Submitted.

7. Burgoyne CF, Quigley HA, Thompson HW, Vitale S, Varma R (1995) Measurement of opticdisc compliance by digitized image analysis in the normal monkey eye. Submitted.

I. SPECIFIC AIMS

1. Study a new confocal microscope that can be used in living eyes to understand the earliest stages of trauma, laser injuries and diseases.
2. Evaluate drugs to prevent retinal damage after laser injury.
3. Study traumatic and non-traumatic glaucoma to determine its pathogenesis
4. Study the stress factors involved in the recurrences of ocular herpes
5. Study drugs that will prevent the recurrences of ocular herpes

II. STUDIES AND RESULTS

1. LASER DAMAGE AND TRAUMA

A. Corneal damage after exposure to ultra-violet and short wave length lasers and ultraviolet light

We have been studying the corneal haze that follows corneal exposure to the 193-nm argon-fluoride excimer laser. Twenty two adult New Zealand white rabbits were treated bilaterally with a 6 mm diameter, 146 fm deep keratectomy, and the eyes randomized into three treatment groups: pretreatment for one day with the PAF inhibitor LAU 0603, FML (fluoromethalone, a mild ophthalmic steroid), or BSS (balanced salt solution, an inactive vehicle control) treatment starting immediately after laser application. All drops were administered five times a day in a observer-masked study. Eyes were examined three times a week for eight weeks, at which

time, the code was broken and the results were analyzed. We found that the 0.05% PAF inhibitor did not have any effect on the prevention of corneal haze, but the use of the steroid fluoromethalone prevented the appearance of haze in a significant number of eyes.

The animals with grade 3-4 (moderate to severe) haze were then re-randomized to be treated with either FML or BSS to determine whether starting treatment after the haze was present, a situation that would be more likely to happen in military applications would reduce the haze and allow vision to return to normal. The eyes were treated 5 times a day for seven additional weeks, with evaluations of haze being made two times a week. Althought the number of eyes in this study was small (20 total) a significant difference was found between the FML and the BSS-treated eyes. The delayed FML was effective in resolving the corneal haze.

2. Glaucoma, Traumatic and Non-Traumatic

Part B: Pathogenesis of Optic Nerve damage in glaucoma

Introduction: Glaucoma is a disease which involves two separate pathologic processes. In those forms of the disease in which intraocular pressure is elevated, damage to the trabecular meshwork of the eye has occurred. For active duty military the most important etiologies for this damage are related to ocular trauma and its

primary and secondary effects on the trabecular meshwork. Within the retired military population, the separate etiologies contributing to trabecular meshwork damage in chronic open angle glaucoma, (the most common form of glaucoma, particularly in the elderly) are most important.

Separate from the pathophysiology of elevated intraocular pressure, the progressive loss of vision that occurs in untreated glaucoma results from damage to the retinal ganglion cell axons and eventual ganglion cell death. While it is possible that damage to the retinal ganglion cells occurs primarily, followed by secondary degeneration of the axons, our best evidence to date suggests that Glaucoma or exposure to elevated intraocular pressure causes a progressive optic neuropathy i.e the primary site of damage is the ganglion cell axon as it passes through the connective tissue trabeculae of the lamina cribrosa.

The primary focus of this portion of the grant is to study the mechanism of glaucomatous damage to the axons within the tissues of the optic nervehead. Our goal is to use scanning laser ophthalmoscopy of the optic disc surface as well as confocal microscopy of the lamina cribrosa to study the force distributions, strength and mechanical behavior of the structural tissues. Our underlying hypothesis is that damage to the structural tissues of the optic nerve head occurs early in the pathophysiology of glaucomatous optic disc damage, and may precede damage to neuronal tissues. Early detection of structural damage may allow intervention to

preserve structural tissues, prior to actual loss of axons.

Since the last progress report, my efforts have been concentrated on completing the papers which outline the findings of my research fellowship and beginning two large experiments in which we will use our confocal scanning laser ophthalmoscope to study the onset of glaucomatous damage to the tissues of the optic nervehead. The following is an account of our progress.

1) Publication of Fellowship Data: The second paper based on data from my fellowship research, **Clinician Judgment Compared with Digitized Image Analysis in the Detection of Induced Optic Disc Change,**[1] has been published. It establishes that the small changes in the surface of the optic disc which we detect by digitized image analysis[2], are not consistently seen by experienced clinicians viewing photographs of the same optic discs. This is the first paper to directly compare a system for digitized image analysis of the optic nervehead to experienced clinicians in the detection of small changes in the optic nervehead surface. It provides the first evidence that this technology may be more sensitive than human observers in the detection of small optic nervehead changes.

Two central papers from the fellowship data have been completed and were recently accepted for dual publication in the journal Ophthalmology. **Measurement of optic disc compliance by digitized image analysis in the normal monkey eye,**[3] uses the global, image analysis-based, optic disc parameter mean position of the disc (MPD) to describe the mechanical behavior of the normal monkey optic disc.

Early changes in optic disc compliance and surface position in experimental glaucoma,[4] establishes that for the same eyes that were studied extensively as normals, changes in the compliance of the optic disc surface and the baseline position of the surface occur very early following the onset of the nervehead's exposure to elevated intraocular pressure in experimental glaucoma.

With their publication, these papers establish the validity of the techniques we employ to study the integrity and function of the structural tissues of the optic nervehead. The first of the two,[3] describes the non-human primate optic nervehead as a compliant structure which behaves mechanically when exposed to acute periods of elevated intraocular pressure. It documents our ability to perform mechanical testing of the optic disc, and establishes the range of normal for a single measure of mechanical behavior: global optic disc compliance. The second paper,[4] establishes our ability to detect an early change in the mechanical behavior of the optic nervehead in experimental glaucoma. This too, will be an important paper, in that changes in compliance of the optic nervehead have never before been longitudinally studied following the onset of experimental elevations of IOP. The results provide the first strong evidence for our hypothesis that damage to the structural tissues of the disc may occur early in the pathophysiology of glaucomatous damage to the disc.

2) Study 01 (our laboratory numbering system)**: Characterization of early, middle, and late changes in the surface of the optic nervehead in experimental glaucoma by confocal scanning laser ophthalmoscopy.** The first study our

laboratory has embarked upon is the characterization of the progressive changes in the optic nervehead surface that occur throughout the course of typical "glaucomatous"damage. Our goal in this study is to develop an extensive library of confocal scanning laser ophthalmoscopic images in which normal variation as well as early, middle and late glaucomatous changes are well catalogued. These images will then become the data base, from which statistical strategies for the detection of optic nervehead change will be constructed. Finally, once we have decided on the proper way to detect change, these images will allow us to definitively describe the observed phenomena. That is, we hope to provide the first extensive description of the pattern of optic nervehead surface change that occurs in glaucomatous damage using images acquired with a confocal scanning laser ophthalmoscope.

A detailed outline of this study is contained within the protocol (Study Number 01) included in the addendum. Briefly, a total of 24 eyes of 12 monkeys will be imaged. Twelve eyes (the study eye) of 12 monkeys, will be extensively imaged on 3 different days as normals. Ten will be given experimental glaucoma and 2 will receive optic nerve transections. Each study eye will then be imaged every 2 weeks through endstage changes to the optic nervehead. The monkeys will ultimately be sacrificed, and optic nerve axon counts will be performed.

As noted on page 3 of the experimental protocol (appendix), we are well into the image acquisition stage of this study, with a total of 7 monkeys already extensively imaged following the onset of glaucoma, and several more in the lasering phase. Over 5000 images have been obtained and saved in their entirety to optical

discs (32 sections of data, each 256 x 256 pixels; total of 1.2 mb of data per image). We expect this study to extend through the end of the upcoming year.

Image alignment and data processing has progressed through many preliminary forms. Final registration parameters have been decided, and official data analysis has begun. An example of a plot of our parameter mean position of the disc (MPD) for the 3 normal sessions of one study (and its contralateral normal eye) for one of our monkeys (7407) is included in the appendix. For this monkey, (and most we have studied to date) the interimage-session variability in this parameter is approximately 30 to 40 microns. As can be seen in this representative data, the amount of change in this parameter at just 2 weeks following the onset of elevated IOP in the study eye.

3) Study 04 (our laboratory numbering system)**: Regional Compliance of the Normal and Glaucomatous Monkey Optic Disc.** The second major study we have begun is a direct follow up to the work on compliance testing we have just published.[3,4] Our principle goals are to define the overall of overall tilt that occurs in the posterior pole of the eye following acute pressure elevations, as well as the regional behavior of the surface of the optic nervehead and peri-papillary retina.

This study, (as well as study 01 above), utilizes the system for rapid confocal scanning laser image acquisition and data analysis which was described in our previous progress report and is now fully operational in our laboratory. Our system allows us to acquire 6 to 10 images of the optic nervehead at a given observation point (any point in time at which we wish to characterize the position and/or

configuration of the surface of the nervehead). Each image is then "registered" or "aligned" to a reference image for that eye; and the elevation value for each pixel location is transferred to our silicon graphics INDY, where data is automatically entered into SAS statistical software, and mean data for each pixel value is generated for the 6 to 10 images. At present, the data for the 6 images we routinely require at an observation point is processed, and available to us in 10 minutes, as an "Observation Point summary form" (example in addendum).

As outlined in the protocol for Study 04 (included in the appendix), this study implements a group of protocols in which the optic nervehead is imaged at a series of observation points at low and high intraocular pressure. We have performed preliminary data analysis on a series of the initial studies, and are able to detect a range of 30 to 120 microns of overall posterior deformation of the disc surface with acute pressure elevations from 10 to 30 mm Hg. In some eyes we have completed as many as 5 repetitions of the complete compliance test (obtained on 5 different days), and our data appears reproducible though much additional analysis remains to be performed.

References:

1) Burgoyne CF, Varma R, Quigley HA. Clinician Judgment Compared with Digitized Image Analysis in the Detection of Induced Optic Disc Change. Am J Ophthalmology 120:176-183, 1995.

2) Burgoyne CF, Varma R, Quigley HA, et al. Global and regional detection of induced optic disc change by digitized image analysis. Arch Ophthalmol 1994;112:261-268.

3) Burgoyne CF, Quigley HA, Thompson HW, et al. Measurement of optic disc compliance by digitized image analysis in the normal monkey eye. Ophthalmology, in press.

4) Burgoyne CF, Quigley HA, Thompson HW, et al. Early changes in optic disc compliance and surface position in experimental glaucoma. Ophthalmology, in press.

Appendix:

-Reference 1

-Reference 2

-Reference 3

-Reference 4

-Protocol Study 01

- Representative data from Study 01 for monkey 7407

-Protocol Study 04

-Observation Point Summary Form

-LDT Regional Analysis form

DOD ANNUAL REPORT - VISION LASER AND EYE INJURY SECTION

A. Quantification of Confocal Images of Human Corneal Endothelium

Real-time confocal microscopy was used to obtain images of the endothelial layer of the cornea *in vivo* in human subjects. This monolayer of cells is the most important in maintaining normal corneal thickness and transparency. Confocal examination revealed the endothelial white bodies organized into a generally hexagonal arrangement. Cells formed a continuous mosaic with dark, often unclear borders between cells. The goal of this study was to provide a rapid, quantitative assessment of the cell size and density that could be employed with the images obtained from human subjects. A process was developed using image analysis employing digital filtering techniques and morphological operations to accurately determine the size and location of each cell.

Each image from the confocal microscope was digitized with an 8-bit gray scale frame grabbing board. Initially the image was corrected so that the background illumination level was uniform across the entire image. An additional series of convolutions was applied, as well as histogram statistics for correction of contrast for the image. The effort was made to bring the histogram level into a consistently predictable range across the image luminance that was obtained from different individuals.

The image then underwent a series of manipulations prior to the application of the so-called water shed algorithm which is applied to the blurred image. In the water shed algorithm, a series of local peaks is detected which are actually the cell boundaries of the cells. These points are then grown around the peaks until they begin to overlap into adjacent areas. Application of the water shed segmentation process resulted in a binary image with white bodies representing the cells and black the junctions between. The number of cells,

cell size, and cell position was determined from the binary image. The array of the cell sizes was written into a file allowing cell distribution histograms and cross sample comparisons to be made.

The efficiency of this method was assessed by comparing the number of samples in which cell counts were determined by manually counting cells versus counting by this computer based algorithm. The number of cells counted by this method are consistently within 3% of the number counted manually. The number of cells that were counted in a frame were between 87 and 276. This advantage was noted over many conventional programs that use about 50 cells for automated analysis.

The automatic method counts the number of cells and determines the size and position of each cell. Histograms of cell size which result are readily indicative of cellular density and can be used to illustrate cell loss and deviation from a uniform arrangement. Thus, the real-time clinical confocal microscope coupled with these image analysis and statistical procedures provides an accurate and quantitative approach to monitoring the endothelium under normal pathological and experimental conditions. This process can be used following surgery and trauma and the evaluation of the affects of drugs. This process can be used essentially without operator intervention and it will use a large number of cells in developing the parameters for cell density and cell size which is superior to most of the commonly used *in vivo* specular microscopes. In the coming year this will be used in wound healing analysis of the endothelium after mechanical laser damage.

B. Diagnostic Improvements in Clinical Confocal Microscopy

The confocal microscope provides cellular level resolution for imaging the human

cornea. The instrument optically sections the tissue in the cornea plane and offers an unique method to diagnose and follow corneal disorders. For the first time this device has been used in humans for the purpose of identifying infectious organisms, fungus, bacteria, and *Acanthamoeba*. These studies were carried out in patients and represent a breakthrough in being able to identify specific causes of disease. The value of this is that the identification can be carried out in a period of 3 to 5 minutes and that treatment can be initiated immediately. The particular benefit of this device is that with an improvement in the ruggedness of the design, it would be feasible to take the confocal microscope into positions for immediate determination of treatment for injured military personnel. In the study that was utilized here, a series of six patients with corneal ulcers were examined. The corneas were imaged in the clinic first with the slit lamp with the usual clinical system for examining corneal disease and then secondly with the confocal microscope, and third scrapings and cultures were made for verification of the infectious organism. In addition, in the case of suspected *Acanthamoeba*, a biopsy was performed. The confocal findings and decision were then compared with those from the laboratory.

The confocal microscope diagnosis correlated in each case with the laboratory diagnosis. That suggests that the confocal microscope can rapidly yield information that is clinically significant for the cause and treatment of infectious diseases of the eye. In the case of fungal keratitis, cultures confirmed the causative organism to be *Aspergillus fumigatus* (n=2). The presumptive diagnosis had been made by confocal microscopy (n=1), which showed the fine filaments of the fungus with their typical 45° branching pattern in the stroma. In the bacterial ulcer, small solitary or clustered organisms were seen throughout the epithelium and stroma by confocal microscopy. The dimensions of the organism were

measured and were consistent with those of bacteria. Indeed, the culture grew *Staphylococcus warneri* (n=1). In the case of amoebic keratitis, confocal microscopy revealed *Acanthamoeba* cysts (n=1) and trophozoite (n=1) forms within the stroma and the diagnosis was confirmed by biopsy.

The confocal microscope has proven to be an instrument for rapid diagnosis of eye disease and injury. It allows the decision to be made regarding the type of infection, whether or not the injury is sterile or that an infectious agent is present. Moreover, it is suggested that the instrument should be rugged enough so that this could be transported around and used in various locations for military personnel.

C. Fluorescent Antibody Use with Clinical Confocal Microscope

The confocal microscope provides an *in vivo* real-time high resolution image of cellular detail of the cornea for magnifications of 100 to 500 times. It would be advantageous to also incorporate immunofluorescence capabilities so that chromophore labeled antibodies could be used *in vivo* with the confocal microscope for diagnosis of disease or for basic investigations into wounding in the cornea and other areas of the eye. An internal modification to the confocal microscope allowed the positioning of a dichroic mirror in the light path. Initially this study was carried out using a 75 watt mercury lamp, however, it was realized that the amount of light in the UV region that are used for excitation of fluorescein were not broad in this light source. Therefore, a 300 watt xenon light source was purchased using funds from other sources to provide a more continuous spectrum profile in the UV region. In addition, a number of changes and modifications were made to this light source here using the LSU Eye Center Instrumentation Laboratory.

The results of this work showed in the cornea of New Zealand rabbits that had been previously infected with a McKrae strain of HSV-1 (herpes simplex virus type 1) direct fluorescence observation could be made. The animals were used four days after infection to carry out studies with fluorescein labeled antibodies. The fluorescein labeled antibody was instilled into both the infected corneas, as well as the contralateral uninfected control cornea. The eyes initially were tested for various periods of time and it was found that a 60 minute contact time with the fluorescein labeled antibody was the most successful. In other experiments linear wounds were made in the epithelium and topical fluorescein instillation was used to detail the depth and outline of this wound.

In comparison at the slit lamp, the uninfected eye had an intact epithelium. The infected cornea revealed the disrupted epithelium with conjunctival and iris injection. The confocal view of the infected eye showed areas of epithelial irregularity with cell dropout and a highly reflective disorganized stroma. Exposure to fluorescein labeled antibody for 60 minutes showed localized areas of intense fluorescence in the infected eye. In comparison, the control corneas showed only low background fluorescence with no specific staining. This illustrates that a direct antibody approach could be potentially used for these purposes with real-time confocal microscopy. In the experiments in which a linear epithelial wound was visualized, it was possible to see the cell borders deep within the wound at 20 to 50 microns and to visualize the cell layers in some detail from the basal cell and the wing cell layer, as well as the flattened superficial cells.

This is a very important breakthrough in the use of real-time confocal microscopy. A problem that is foreseen is the ability to construct antibodies that have increased amplification, as with the usual sandwich techniques that employ peroxidase or fluorescein in

an approach so that there is amplification of the epitope site. An additional challenge will be to devise approaches for quantification of the amount of fluorescence seen by different antibodies or different experimental conditions. Both of these will be part of the specific aims for the forthcoming year.

D. Plans for the Coming Year

In addition to the objectives detailed above for these studies, an important new task has been included.

Automatic haze assessment. The microscopic structure of the cornea stroma in humans, as well as in experimental animals changes following excimer laser surgery. It has been shown previously in this laboratory by clinical confocal microscopy that this process begins some hours immediately following surgery and lasts up to at least six days and, in humans, seems to extend to six weeks. During that period of time, the stroma undergoes pronounced microscopic morphological changes. Shortly after the application of the excimer laser process keratocytes, the fibroblasts of the cornea, nuclei were observed to become smaller and more highly reflective. In addition, a fibrous network developed growing increasingly dense in the next several weeks.

Attempting to describe the magnitude of corneal haze using automatic objective assessment to replace the subjective individual ratings that are presently used would lead to a step forward in understanding laser side effects. In addition, this can be potentially used with some modifications to study density changes in the vitreous humor of the posterior chamber of the eye as well.

The procedure that is being developed in this lab will quantify the degree of corneal

haze in an objective manner. A library of precategorized images are being analyzed for specific morphological characteristics such as changes in keratocyte density, keratocyte size, and relative reflectiveness. The extent of fibrosis and collagen structural changes from the confocal images are also being examined. The purpose of this study will be to fit an equation to all of these factors which will be able to be used and applied across both species and time to provide a precise assessment of corneal haze.

3. HERPES

The Prevention of Recurrences of Viral Disease

1. <u>Thymidine kinase inhibitors of recurrent ocular herpes:</u> Last year, we determined that the physicochemical properties of the newly synthetized viral thymidine kinase inhibitor, 9-(4-hydroxybutyl)-N^2-phenylguanine (HBPG), made it suitable for testing in our primate model of cold temperature stress.

Capitalizing upon our recently described primate model of hypothermic induction of reactivation[1], we have tested HBPG in this model and show here that this inhibitor of viral thymidine kinase is effective in reducing the ocular recurrences of herpetic keratitis in a statistically significant manner.

Eighteen mixed male and female young adult squirrel monkeys (*Saimiri scuireus*) weighing from 0.46 to 1.08 kg were handled in accordance with the NIH guidelines on the care and use of animals in research, the Institutional Animal Care and Use Committee of the LSU Medical Center in New Orleans, and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. They had been used in breeding research but had not been used in any infectious disease research.

The corneas of the 18 squirrel monkeys were anesthetized with a drop of proparacaine hydrochloride (Alcaine, Alcon, Humacao, PR), the superficial corneal epithelium minimally traumatized with a 27 gauge needle, 25 μl of Rodanus strain HSV-1 dropped into the conjunctival cul-de-sac, and the eyelids gently rubbed over

the cornea for 10 seconds. Three days after infection, the corneas were stained with fluorescein (Fluor-I-Strip, Wyeth-Ayerst, Philadelphia, PA) and the presence of herpetic keratitis was verified in all corneas by examination with the slit lamp biomicroscope. Based on the pharmacokinetic data, we decided to use 150 mg/kg HBPG, every eight hours, and to inject drug or placebo five times before lowering the room temperature. Typically, the room temperature was lowered at 4 pm; treatment was continued two additional times (midnight and 8 am), and the desired low temperature was noted at 6 am. Corneas were examined at 8 am, and the room temperature was raised to the normal level. Animals were exposed to four cycles of temperature stress, with treatments starting on day 21, day 27, day 34, and day 69. The corneal evaluations were done in a masked manner so that the observer had no knowledge of the treatments.

All nine of the HBPG-treated and six of the nine placebo-treated monkeys had at least one incident of recurrent herpetic keratitis during the observation period (figure). For the low temperature periods the difference in the frequency of corneas showing specific herpetic recurrent keratitis was significantly lower in the HBPG treated group (P=.0032, Wilcoxon Rank Sums Test). In the time intervals before the temperature was lowered when no treatment was given, there was no significant difference in corneas showing herpetic keratitis between the two treatment groups (P=.6536). In the time intervals between the periods of lowered temperature there was also no significant difference between the treatment groups (P=.1106).

It is essential to demonstrate the safety and efficacy of drugs which target viral

enzyme systems in primate species in order to speed the testing of these drugs in the clinical setting. In the present study we made use of a model of hypothermic stress in primates. In this model, squirrel monkeys subjected to brief intervals of lowered ambient temperatures (approximately 18°C) were found to exhibit a statistically significantly greater number of corneal herpetic lesions compared to animals maintained in an ambient temperature range of 24 - 27° C. In this investigation we provide evidence that a new drug, HBPG, which inhibits viral thymidine kinase is particularly effective in preventing viral reactivation and recrudescence of ocular viral infection in hypothermically stressed primates.

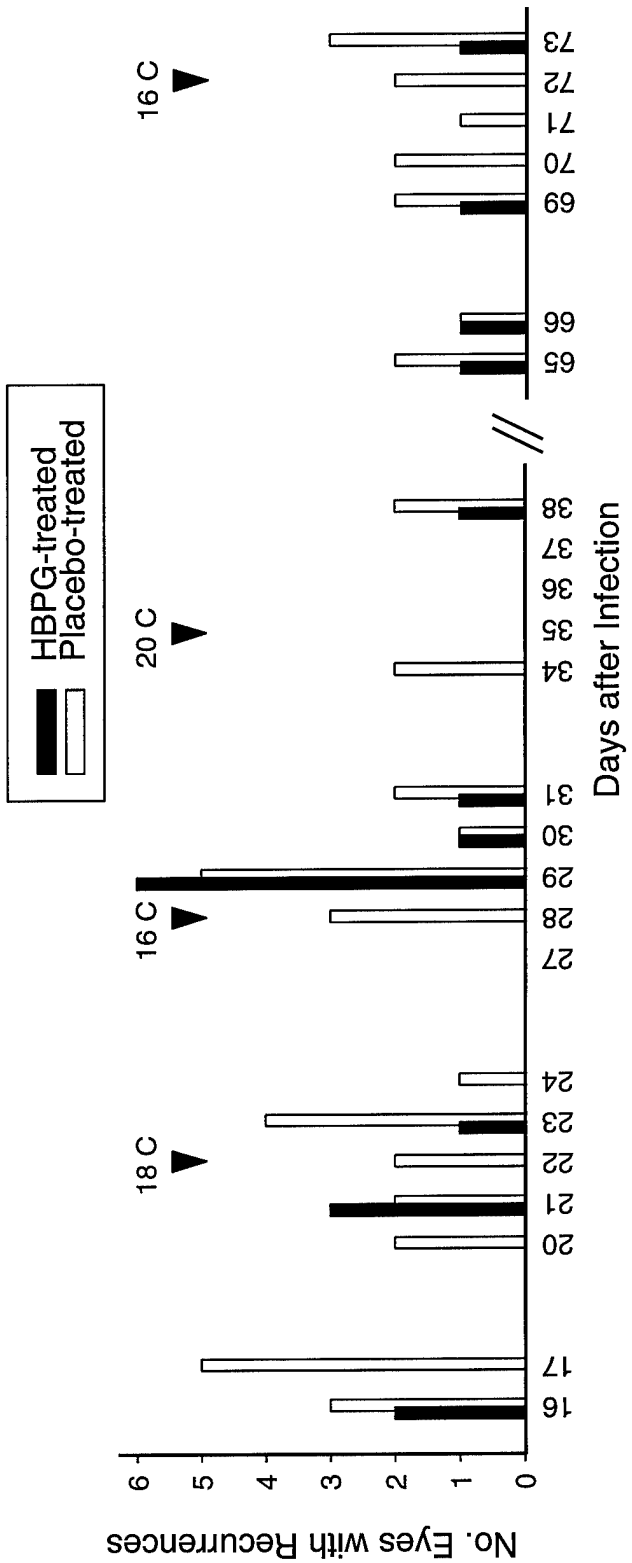

No. Eyes with Recurrences
0
1
2
3
4
5
6
16
17
18 C
20
21
22
23
24
16 C
27
28
29
20 C
30
31
34
35
36
37
38
16 C
65
66
69
70
71
72
73
Days after Infection
HBPG-treated
Placebo-treated

Figure Legend

The animals were examined only on the numbered days after infection; they were not examined on weekends or during the period of PI day 39 through PI day 64.

Placebo-treated group =9 monkeys (18 eyes)

HBPG-treated group = 9 monkeys (18 eyes) thru PI day 28

8 monkeys (16 eyes) thru PI day 34

7 monkeys (14 eyes) thru PI day 69

6 monkeys (12 eyes) to end of study

On PI day 35, the room temperature could not be lowered to the desired range of 16-18°C; a low of 20°C was attained. On PI days 35, 36, and 37, recurrent keratitis was not seen in either group.

2. HBPG in the Mouse Model of Thermal Stress-Induced Reactivation. Mice latently infected with the McKrae strain of HSV-1 were treated with intraperitoneal injections of HBPG in a corn oil vehicle (200 mg/kg every 3 hours for a total of 10 doses) and subjected to hyperthermic stress to stimulate viral reactivation immediately after the third drug treatment. Three hours after the last (tenth) treatment, the mice were sacrificed and the presence of infectious virus was determined by culture of ocular surface swabs and of trigeminal ganglionic homogenates. Additionally, viral DNA and ganglionic extracts were analyzed by quantitative polymerase chain reaction (QC-PCR). Control animals included latently infected, stressed animals receiving injections of the corn oil vehicle only and latently infected, drug and vehicle-treated unstressed animals. We found that HBPG had a statistically significant effect on hyperthermia-induced viral reactivation (Table 6).

TABLE 1: Effect of HBPG on Hyperthermia-Induced Viral Reactivation

Treatment	Number of Mice	Infectious Virus/Number of Samples	
		Ganglion	Ocular Surface
Stressed			
HBPG-treated	10	7/18*	3/20*
Vehicle-treated	10	12/19*	8/17*
Not stressed			
HBPG-treated	5	0/8	0/9
Vehicle-treated	5	0/10	0/10

*The frequencies of infectious virus found in the samples from the HBPG-treated animals and the vehicle-treated animals are significantly different, $P < 0.05$.

We also found that homogenates of trigeminal ganglia, as well as ocular surface swabs from HBPG-treated mice, were less likely to contain infectious virus compared to those of infected, vehicle-treated stressed controls. Animals that were not stressed did not undergo reactivation.

Quantitative analysis of viral DNA in ganglionic extracts revealed that there was a hundredfold reduction in the amount of viral DNA in the ganglia of HBPG-treated animals compared with vehicle-treated controls ($P < 0.05$, Table 2).

TABLE 2: Effect of HBPG on Hyperthermia-Induced Viral DNA Expression

Treatment	Viral DNA*
Stressed	
HBPG-treated	$< 1 \times 10^2 \pm 0.4 \times 10^{1}$**
Vehicle-treated	$1 \times 10^4 \pm 0.7 \times 10^{1}$**

*Ganglionic DNA was amplified in quantitative, competitive polymerase chain reaction (Q-PCR), and the copy number of the viral gene present was determined at the equivalence point.

**These values are significantly different ($P < 0.05$).

These results indicate that the thymidine kinase inhibitor, HBPG, is effective in suppressing viral reactivation and viral DNA synthesis by inhibiting viral thymidine kinase in this mouse model. (Gebhardt BM, Wright GE, Xu H, Focher F, Spadari S, Kaufman HE: 9-(4-hydroxybutyl)-N^2-phenylguanine, HBPG, a thymidine kinase inhibitor, suppresses herpes virus reactivation in vivo. Antiviral Res, submitted.)

3. <u>Beta blocker modulation of viral reactivation:</u> Recurrent ocular herpetic disease is a significant clinical problem. At present, there is no established method for preventing or reducing ocular recurrences. In monkeys harboring latent herpes simplex virus type 1 (HSV-1), we observed that recurrences of ocular lesions increased in frequency shortly after hypothermal stress and we suspected that adrenaline might be the mediator for inducing such recurrences. The idea that adrenaline might induce recurrences is certainly not new; in fact, the first model of recurrent herpes employed by Laibson and Kibrick suggested the possibility that the recurrences they observed in rabbits might be produced by topical epinephrine.

In a hyperthermal stress mouse model of increased reactivation of latent HSV, we observed that injections of propranolol, a beta adrenergic receptor blocker, significantly reduced the frequency and amount of virus shedding in the tears and virus in the trigeminal ganglia. In this study, we evaluated the effect of propranolol on spontaneous recurrences of ocular herpetic disease in the rabbit.

Male and female New Zealand white rabbits were handled in accordance with the NIH guidelines on the care and use of animals in research, Louisiana State University Medical Center Institutional Animal Care and Use Committee approval, and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

The corneas of New Zealand white rabbits were anesthetized with topical proparacaine hydrochloride (Alcaine, Alcon, Humacao, PR) and lightly scratched with a 27-gauge needle. An aliquot (25 μL) of the virus suspension was placed on each cornea, and the eyelids were gently rubbed over the cornea for 10 seconds. Three days after infection, fluorescein (Fluor-I-Strip, Wyeth-Ayerst, Philadelphia, PA) was instilled into the eye, and the presence of dendritic keratitis verified in all corneas by slit lamp biomicroscopy. Beginning 19 days after infection, specimens were obtained from the conjunctival cul-de-sac for virus culture and the corneas were examined for the presence of herpetic keratitis. Examinations were performed on 18 week days from day 19 to day 49 after infection, and culture specimens were obtained on 16 week days from day 24 to day 49 after infection.

On the 21st day after infection, 29 animals were randomized into two coded groups. One group was given intramuscular injections of 1 mg/kg propranolol in water twice a day at 7:30 am and at 5:00 pm for 30 days; the other group was given intramuscular injections of sterile water on the same

schedule. All corneal evaluations and cultures were performed by persons with no knowledge of the treatments given.

Twenty nine rabbits were included at the start of treatment, and 24 were present at completion.There was a dignificant difference in the recurrences of the propranolol-treated animals and the water-treated, control animals.

Beta adrenergic receptor blockers are commonly used to treat hypertension. Systemic beta blockers are well tolerated in general, and would certainly would be an inexpensive and readily available method of reducing recurrences of herpes. Their effect in a thermal stress model of reactivation suggests that they could reduce fever blisters and cold sores in humans, and that they might reduce other stress-related herpetic disease. This study does not answer the question of whether stress is required to show the benefit of propranolol beta blockade since the nature of the experiment requires that the animals be handled and subjected to injections, which could be defined as a form of physical stress. It does suggest, however, that propranolol might be beneficial in humans with stress-related recurrences. How this mechanism might affect nonstress-related recurrences remains to be investigated. Additionally, further studies in progress are designed to determine whether selective beta blockade will be as effective as nonselective blockade, and whether topical beta blockers are as effective as systemic beta blockade.

2. <u>Beta adrenergic receptor blockade in mice:</u>

During the first year of DOD support we initiated a series of studies in mice and rabbits designed to investigate the role of adrenergic receptor blockade in herpes virus reactivation in the nervous system. We were gratified to find that using a stress model of viral reactivation in mice that the nonselective beta adrenergic blocker, propranolol, had a significant and dramatic suppressive affect in terms of the appearance of infectious virus on the ocular surface and the presence of

infectious virus and quantity of viral DNA in the nervous system. These initial studies have now been published and we have expanded our protocols to include stress models in both rabbits and critically important initial studies in primates. In both lagomorphs and in primates we have been able to substantiate and support our studies using propranolol. It appears that stress-induced viral reactivation may be universally inhibited in diverse species of animals by the application of beta adrenergic receptor blockade.

Most recently we have extended these studies to include timolol maleate which is a beta 1 and beta 2, nonselective adrenergic receptor blocking agent commonly used to control intraocular pressure in patients. Timolol is approved for topical application to the human eye for the treatment of glaucoma and is used as one of the primary medications for treating this disease. Since we are particularly interested in entering into clinical studies designed to determine the efficacy of beta adrenergic receptor blocking agents in patients experiencing viral reactivation and ocular disease with approved and tested drugs, we decided to test timolol maleate in our mouse model.

A. Materials and methods

Groups of ten or more mice rendered latent for HSV-1 by primary ocular infection were begun treatment with intraperitoneal injections of timolol maleate (0.6 mg per injection) two days before application of stress. Intraperitoneal injection was repeated at day -1 and on the day of stress. Twenty-four hours after being exposed to 43°C for ten minutes by immersion in water, the animals were sacrificed, their ocular surfaces swabbed for viral culture, and their corneas and trigeminal ganglia collected for the analysis of infectious virus and viral DNA. The studies of the analysis of viral DNA concentration in the trigeminal ganglion are currently in progress.

B. Results

We have observed, as shown in Table 1 below, that pretreatment of the animals with timolol

maleate prior to application of the stress results in the reduction in the frequency that infectious virus is found at the ocular surface of treated animals as compared to placebo-treated latent and stressed animals. Investigations of the trigeminal ganglion for the presence of infectious virus has similarly revealed that the presence of infectious virus was less likely to be found in the ganglia of timolol-treated animals as compared to control placebo-treated animals (Table 2).

C. Discussion

These studies with a clinically tested and proven nonselective beta 1 and beta 2 receptor blocking agent expand the potential armamentarium of drugs which can be used to prevent viral reactivation in humans. The implications of these findings are considerable and have provided stimulus for us to press on with further investigations of both the mechanisms of action of the beta blocking agents and to begin to design clinical trials for testing such drugs in patients prone to viral reactivation.

TABLE 1: INFECTIOUS VIRUS ON THE OCULAR SURFACE*

	Experiment	
Treatment	1	2
Timolol	3/16‡	4/18
No Timolol	11/16	13/18

*Fifty days after infection mice were treated with timolol maleate (0.6 mg/0.1 ml) daily for three days. On the third day the animals were heat stressed and their corneas swabbed for infectious virus 24 hr later.

‡The numbers represent the swabs which exhibited cytopathic effect (CPE) over the total number of eye swabs performed. There is a statistically significant difference between the frequency of infectious virus between the treated and untreated animals, $p < 0.005$.

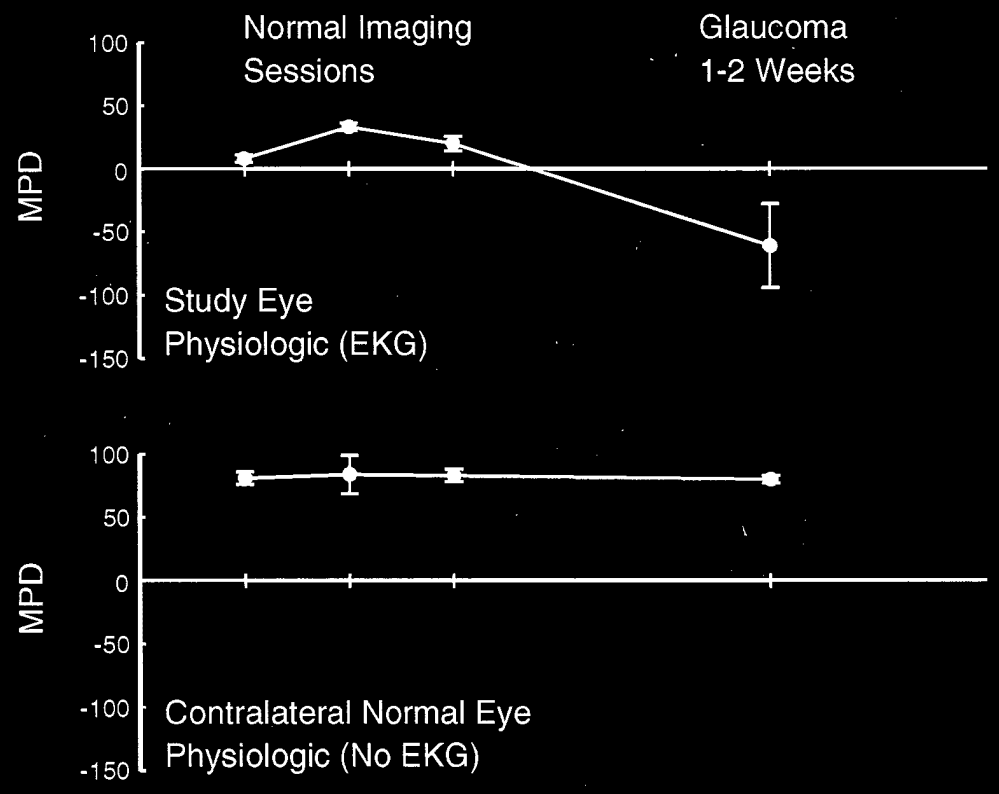
TABLE 2: INFECTIOUS VIRUS IN THE TRIGEMINAL GANGLION*		
	Experiment	
Treatment	1	2
Timolol	6/19‡	5/14
No Timolol	16/19	13/15

*Fifty days after infection mice were treated with timolol maleate (0.6 mg/0.1 ml) daily for three days. On the third day the animals were heat stressed and sacrificed 24 hr later. Ganglia were homogenized and plated onto CV1 cells for analysis of infectious virus.

‡The numbers represent the homogenates which exhibited cytopathic effect (CPE) over the total number of homogenates tested. There is a statistically significant difference between the frequency of infectious virus between the treated and untreated animals, $p < 0.005$.

III. VERTEBRATE ANIMALS

SPECIES	NUMBER ALLOWED	NUMBER USED	LSU IACUC #
Macaca fascicularis	16 monkeys	13	1015
Saimiri sciureus	20 monkeys	18	1218
Mus musculus	270 mice	180	1019
Oryctolagus cuniculus	498 rabbits	31	1049
Oryctolagus cuniculus	842 rabbits	50	1218



10° Images – MPD ± 95% Confidence Interval
Monkey 7407

Study Number 01

Long Study Name: Characterization of Early, middle and late changes in the optic disc in Experimental Glaucoma by Confocal Scanning Laser Ophthalmoscope

Short Study Name: Longitudinal Glaucoma Study

Overall Study Design

24 eyes, 12 monkeys
3 separate imaging sessions while normal both eyes
10 eyes, 10 monkeys experimental glaucoma
2 eyes , 2 monkeys optic nerve transection ***(abandoned, 4 August, 95 see note)***
imaging sessions every 2 weeks post-intervention

Imaging sesssion design

(6 images obtained at each of the following Observation Points):

Study Eye
Physiologic 10 degree with EKG
Physiologic 15 degree with EKG

IOP 10, 60 minutes, 10 Degree, without EKG
IOP 10, 60 minutes, 15 degree, without EKG

IOP 10, 90 minutes, 10 degree, with EKG
IOP 10, 90 minutes, 15 degree, with EKG

Contralateral Normal Eye
Physiologic, 10 degree, without EKG
Physiologic, 15 degree, without EKG

Ongoing list of animals used in this study

Monkey Number	File Naming Code	Dates between which onset of elevated IOP occurred in study eye
7350	*1C*	(dropped from study01 072695, switched to 03; see notes)
7364	1D	
7367	1E	
7373	*1F*	(prob dropped from study01 09_ _95, switched to 03; see notes)
7397	1G	
7407	1I	
7416	1J	
7432	*1K*	(prob dropped from study01 09_ _95, switched to 03; see notes)
7442	*1L*	(dropped from study01 072695; switched to 03, see notes)
9142	*1m*	(lost due to Pento barb overdose 080495 see notes)
8940	1n	
9154	1o	
2452	1p	

Ongoing List of Official Imaging sessions for each study monkey (updated 072695)

Monkey	Date Normal #1	Date Normal #2	Date Normal #3	Post-Intervention #1	Post-Intervention #2	Post-Intervention #3	Post-Intervention #4	Post-Intervention #5
7350 (1C)	020195	021495	030195	061495 *(7)* (hypotonous, disc damaged)	062895 *(13)*	071295 (07)	071995 (08)	**Prob dropped** from study 072595
7364 (1D)	121694	012495	020895	052695 *(31)*	060795 *(11)*	062195 *(21)*	070595 (07)	072795(-)$_{L7/27?}$
7367 (1E)	012595	020895	022495	052495 *(28)* (7367a)	060695 *(15)*	062095 *(14)*	070595 (20)	071895 (23)
7373 (1F)	011895	020795	022495	053195 *(18)* (? IOP)	061395 *(16)*	062795 *(15)* (disc damaged ?)	072095(08)$_{L7/27}$	
7397 (1G)	012795	021095	032895	053095 *(20)*	061495 *(28)*	062895 *(21)*	071195 (11)	072597( )$_{L4/5}$
7407 (1I)	012595	021095	032895	050395 *(42)*	052495 *(25)* (7407a)	060795 *(33)*	062195 *(19)*	070695 *(32)*
7416 (1J)	020395	021595	033195	052695 *(26)* (7416a)	060995 *(27)*	062395 *(22)*	070795 (12)	072195(10)$_{L7/27}$
7432 (1K)	013195	021495	030195	060995 *(4)* (7432a)	062395 *(9)*	071195 (05)	072595( )$_{L4/5}$	
7442 (1L)	020395	030795	040495	061695 *(10)* (hypotonous - poss cleft)	063095 (06)	071295 (15)	071995 (12)	**Prob dropped from study 072695**
9142 (1m)	061695	063095	071395	died 080495				
8940 (1n)	062095	070795	072695					
9154 (1o)	072695	080195						
2452 (1p)	062795	072895						

Ongoing List of Official Imaging sessions for each study monkey (continued)

Monkey Intervention	Post-Intervention #6	Post-Intervention #7	Post-Intervention #8	Post-Intervention #9	Post-Intervention #10	Post-Intervention #11	Post-#12	Post-#13	Intevention
7350 (1C)									
7364 (1D)	072895(22)								
7367 (1E)									
7373 (1F)									
7397 (1G)									
7407 (1I)	072195 (30)	080295(26)							
7416 (1J)	080195(23)								
7432 (1K)									
7442 (1L)									
9142 (1m)									
8940 (1n)									
9154 (1o)									
2452 (1p)									

Reference images for study 01,version a (from WP file refimg01.va)

BASELINE IMAGES LIST

ID	EYE	DEGREE	LDT DATE (M/D/Y)	TIME (M/D/Y)	SOURCE	
7350	OS	10	030395	1106	021495	
		15	030395	1116	030195	
	OD	10	030395	1127	030195	
		15	030395	1149	030195	
7364	OS	10	030395	0954	012495	
		15	030295	1728	012495	
	OD	10	030395	1003	012495	*(deleted)
		15	030295	1737	012495	*(deleted)
7367	OS	10	030395	1020	012595	
		15	030295	1746	012595	
	OD	10	030395	1035	020895	
		15	030395	1052	020895	
7373	OS	10	030395	1212	011895	
		15	030395	1233	020795	
	OD	10	030395	1241	011895	
		15	030395	1248	020795	
7397	OS	10	030395	1317	021095	
		15	030395	1328	021095	
	OD	10	030395	1333	012795	
		15	030395	1349	012795	
7407	OS	10	030395	1400	012595	#no access
		15	030395	1409	012595	#no access
	OD	10	030395	1417	021095	#no access
		15	030395	1457	021095	#no access
7416	OS	10	030395	1531	030895	*(deleted)
		15	030395	1600	030895	*(deleted)
	OD*(study eye)*	10	030395	1511	020395	
		15	030395	1521	020395	
7432	OS	10	030395	1608	021495	
		15	030395	1619	021495	
	OD	10	030395	1628	013195	
		15	030395	1645	013195	
7442	OS	10	030795	1646	030795	
		15	030995	1635	030795	
	OD	10	030395	1717	021595	*(deleted)
		15	030395	1724	021595	*(deleted)

*(deleted) This version of reference image inadvertently deleted when Liqian created new AOI for version b (as of creation of version b images)

#no access These images not accessible since database update using version 2.2.04, somewhere around Saturday, 0722995). New reference images created for 7407 using same constituient images, but a newly assinged AOI for version b.

Study Number 04

Long Study Name: Regional Compliance of the Normal and Glaucomatous Monkey Optic Disc #3

Short Study Name: Regional Compliance #3

Overall Study Design

Study 02 (regional compliance #1) was a ball park study to work out parameters
Study 03 (regional compliance #2) was first attempt at rigous testing;
Study 04 is an adjustment to Study 03, to account for having lost use of the 6 new monkeys we had planned on using for that study; we will use some of the images acquired and labeled as being part of Study 03

3 eyes, 3 monkeys (Monkeys 6938, 7350, 7415)**:**

1) go through imaging protocol 03, repeated 5 times

(protocol 03 is essentially equivalent to protocol 04a)

(images from these sessions will be labeled as being part of study 03)

3 eyes, 3 monkeys (Monkeys 7442 +7397? and 7432?)**:**

1) go through imaging protocol 04b, repeated 5 times
2) go through imaging protocol 04c, repeated 5 times
3) go through imaging protocol 04a, repeated 5 times

8 normal eyes, 8 monkeys (all 8 normal eyes, study 01)**:**

1) go thorugh imaging protocol 04a, just 1 time

8 glaucomatous eyes, 8 monkeys (all 8 glaucomatous eyes, study 01)**:**

1) go through imaging protocol 04a, just 1 time

Imaging Protocol 03 (see file proto03.agh)

The original protocol for Study 03, now: the first imaging protocol in Study 04

(6 images obtained at each of the following Observation Points in the study eye):	Observation Point	Naming Code	Study Days
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	101A	01	All
IOP 10 mm Hg, 20 degree, 20 minutes, EKG	101B	02	"
IOP 10 mm Hg, 15 degree ,30 minutes, EKG	102A	03	"
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	102B	04	"
IOP 10 mm Hg, 10 degree, 50 minutes, EKG	*102C*	*05**	*1,2,3*
IOP 30 mm Hg, 15 degree, 10 minutes, EKG	301A	06	All
IOP 30 mm Hg, 20 degree, 20 minutes, EKG	301B	07	"
IOP 30 mm Hg, 15 degree, 30 minutes, EKG	302A	08	"
IOP 30 mm Hg, 20 degree, 40 minutes, EKG	302B	09	"
IOP 30 mm Hg, 10 degree, 50 minutes, EKG	*302C*	*10 **	*1,2,3*
IOP 45 mm Hg, 15 degree, 10 minutes, EKG	451A	11	All
IOP 45 mm Hg, 20 degree, 20 minutes, EKG	451B	12	"
IOP 45 mm Hg, 15degree, 30 minutes, EKG	452A	13	"
IOP 45 mm Hg, 20 degree, 40 minutes, EKG	452B	14	"
IOP 45 mm Hg, 10 degree, 50 minutes, EKG	*452C*	*15 **	*1,2,3*
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	103A	16	All
IOP 10 mm Hg, 20 degree, 20 minutes, EKG	103B	17	"
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	104A	18	"
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	104B	19	"
Physiologic Images contralateral eye, 15 degree _ _ ph		20	All
Physiologic Images contralateral eye, 20 degree _ _ ph		21	"

Imaging Protocol 04a (see file prot04a.agh)

The second imaging protocol in Study 04

(same as protocol 03 except a few observation points have been dropped to economize and make it faster to do)

(6 images obtained at each of the following Observation Points in the study eye):	Observation Point Naming Code	Repetition Naming Code
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	01	N1,N2.....N5
IOP 10 mm Hg, 15 degree ,30 minutes, EKG	03	
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	04	
IOP 30 mm Hg, 15 degree, 10 minutes, EKG	06	
IOP 30 mm Hg, 15 degree, 30 minutes, EKG	08	
IOP 30 mm Hg, 20 degree, 40 minutes, EKG	09	
IOP 45 mm Hg, 15 degree, 10 minutes, EKG	11	
IOP 45 mm Hg, 15degree, 30 minutes, EKG	13	
IOP 45 mm Hg, 20 degree, 40 minutes, EKG	14	
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	16	
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	18	
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	19	
Physiologic, contralateral eye, 15 degree, EKG	20	
Physiologic, contralateral eye, 10 degree, EKG	22	

Imaging Protocol 04b (see file prot04b.agh)

The third imaging protocol in Study 04:

(6 images obtained at each of the following Observation Points in the study eye):	Observation Point Naming Code	Repetition Naming Code
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	31	N1,N2.....N5
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	33	
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	34	
IOP 30 mm Hg, 15 degree, 10 minutes, EKG	36	
IOP 30 mm Hg, 15 degree, 30 minutes, EKG	38	
IOP 30 mm Hg, 20 degree, 40 minutes, EKG	39	
IOP 30 mm Hg, 15 degree, 60 minutes, EKG	53	
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	46	
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	48	
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	49	
Physiologic, contralateral eye, 15 degree, EKG	50	
Physiologic, contralateral eye, 10 degree, EKG	52	

Imaging Protocol 04c (see file prot04c.agh)

The fourth imaging protocol in Study 04:

This is a repeat of Protocol 04b, only its done with a suction cup on the eye instead of a needle in the anterior chamber

(6 images obtained at each of the following Observation Points in the study eye):	Observation Point Naming Code	Repetition Naming Code
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	61	N1,N2....N5
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	63	"
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	64	"
IOP 30 mm Hg, 15 degree, 10 minutes, EKG	66	
IOP 30 mm Hg, 15 degree, 30 minutes, EKG	68	
IOP 30 mm Hg, 20 degree, 40 minutes, EKG	69	
IOP 30 mm Hg, 15 degree, 60 minutes, EKG	83	
IOP 10 mm Hg, 15 degree, 10 minutes, EKG	76	
IOP 10 mm Hg, 15 degree, 30 minutes, EKG	78	
IOP 10 mm Hg, 20 degree, 40 minutes, EKG	79	
Physiologic, contralateral eye, 15 degree, EKG	80	
Physiologic, contralateral eye, 10 degree, EKG	82	

Ongoing Notes on Study Design

Central Issues:

1) scan angle

-document stability of zero-reference plane within acute compliance tests at multiple distances from the peri-papillary retina; also over time, since there will be 5 different tests; i.e how far out do you need to get to have the most stable zero-reference plane and can you still align there

-include minimum 10 degree images to demonstrate that nothing is gained from their use, ie no more detail is obtained and 15 degree images are adquate

- include 10 degree images so that a "video" at 20 and 15 degree could "zoom" down to the disc (10 degree images to show finer detail

-include enough repetition to demonstrate whatever efffect we decide is present, i.e 10 degree images do not, make a difference or 10 degree images do make a difference

2) Compliance test IOP

-can we see enough change from IOP elevation of 30 mm Hg either at 10 minutes or at 30 minutes to use this as maximum IOP elevation for subsequent tests and human compliance tests

-are the trends apparent at 45 mm Hg, detectable at 30 mm Hg.

-is there evidence in the 4th and 5th tests that the disc has changed from the tests of compliance alone (IOP to 45 mm Hg) if there is, then a followup study must be done on elevations to 30 mm Hg

-is a 10 minute period of time adequate to detect changes? this is not ideally being evaluated, in that time and scan angle both change at the same observation points

3) Effect of length of exposure

- is 10 minutes enough to detect a change (regional compliance 1 would suggest it is) is the amount of change that occurs within the next 20 minutes worth waiting for

-just in terms of the phenomena, even if it turns out that waiting an additional 20 minutes at each observation point doesn't make a lot of difference, it is good to have a second observation point that confirms the changes present in the first

3) How do the disc and peri-papillary retina move?

-this is really the central point of interest, to define the behavior of the surface, and infer behavior of the lamina that follows

-how reproducible is the behavior in multiple testing sessions within a single eye?

-how reproducible is it between the 6 study eyes

-how are we going to describe the movement:

a) regional means

b) individ data point mapping or some derivation thereof

c) volumetric parameters

d) develop the ability to show video of the images with magnitude and statistical significance of the changes depicted

e) Hilary's parrallel wavelet study on a subgroup of selected images to introduce this as a change detection strategy

4) Any evidence for change in compliance after 5 compliance testing sessions that may suggest damage to structural tissues as part of test.

-we will first do 3 of the planned 5 repetitions and look at the data, if there is the suggestion of damage, we may not need to do 5 repetitions to demonstrate that the test is harmful.

- there might be evidence in the repetitions of protocol 03 and 04a for damage, since these have observation points at 45 mm Hg. If this is present after 3 reps in 6938,7415 and 7350 then:

1) may not need to do 5, if the trend is consistent in all three.

2) will need to 5 reps in each of 04b and 04c to state confidently that you do not see same trend when you do compliance test up to 30 mm Hg.

-this will in effect allow you to state that in the three eyes in which this was done a total of 10 compliance tests had no net effect

3) should do protocol 04b or 04c in the study 01 monkeys to avoid inducing damage from compliance testing in these eyes.

5) Will allow us to verify in 3 eyes that needle in AC compliance testing is not significantly different than external suction cup testing (protocol 04b versus protocol 04c)

6) will give you the following numbers:

14 normal eyes, 14 monkeys at least one protocol 04a *(including 03)* **compliance test**

subset of 6 eyes of 6 monkeys, in which test repeated 5 times

subset of 3 eyes, 3 monkeys in which IOP elevation to 30 only done 5 times first; then IOP eleveation to 30 and 45 done 5 times. If, there is no evidence of damage from IOP elevation to 30, can look for it following IOP elevation to 45.

subset of 3 eyes, 3 monkeys in which needle in AC compliance test compared to suction cup compliance test.

7) what to do with histologic data and axon counts post sacrafice

Running List of Ideas for follow up studies

1) if there is no evidence for changes overall, then do a new form of testing in these same eyes to compare 2 different forms of compliance testing in the same eyes
   - how high do you go before inducing damage
   - how long does pressure stay there, before damage occurs

2) if evidence for alterations from IOP elevations to 45 mm Hg, then next study might be in new batch of monkeys in which one eye gets simplified compliance test to 45 mm Hg and the other gets simplified compliance test to 30 mm Hg. Repeated 5 times in each eye, and look for evidence of change in mech behavior in 45 IOP eye not present in 30 IOP eye.

3) next study carried out with suction cup apparatus, perhaps in these same monkey eyes after five times imaged with needle, to show that the suction cup does influence behavior of the disc

not influence behavior

Ongoing list of animals used in study 04 (as of 081395)

Protocol	Repetitions	Monkey (Study eye) (see monklist.cfb for monkey ID)
03	5	6938R, 7415L, 7350R
04b	5	7442R, 7373R?, 7432R?
04c	5	
04a	5	
04a	1	Normal eye: 7364 7367 7397 7407 7416 *7373 or 9154* *7432 or 2452* 8940
04a	1	Glaucoma eyes: 7364 7367 7397 7407 7416 *9154* or *7373* *2452* or *7432* 8940

Ongoing List of Official Imaging sessions for each study monkey

Protocol	Monkey	First Imaging Session Date	Second Imaging Session Date	Third Imaging Session Date	Fourth Imaging Session Date (if necessary)	Fifth Imaging Session Date (if necessary)
03	6938	062695(6938b)	070395(6938b)	071095(6938c)	071795(6938c)	072495(6938d)
	7415	062095	070695	072095	wait	wait
	7350	073195(7350b)	080795(7350b)		wait	wait
04b	7442	081095(7442b)			wait	wait
	7373?				"	"
	7432?				"	"
04c	7442				"	"
	7373?				"	"
	7432?				"	"
04a	7442				"	"
	7373?				"	"
	7432?				"	"

	Normal Eye Imaging Session	Glaucoma Eye Imaging Session
7364		
7367		
7397		
7407		
7416		
9154?		
2452?		
8940		

OBSERVATION POINT SUMMARY PAGE

7350B RIGHT EYE

Study Date: 08/07/95
Field: 15 degree Report Date: 08/11/95 15:54
Clinic ID #: Program: obspt1_3
SSN : Directory: /usr/people/ldt/primate/7350br/080795/b0808951.623
Date of Birth: 00/00/00
Date of Last Visit: 00/00/00 Output File Name: 031c1101.s2m

IMAGES IN OBSERVATION POINT: 08/07/95-10:14; 08/07/95-10:12; 08/07/95-10:14;
08/07/95-10:14; 08/07/95-10:15; 08/07/95-10:21;

REFERENCE IMAGE INFO:
LDT date/time: 08/08/95 16:23
SGI file name: b0808951.623
Focal Correction: Ref. image, 031c1403.1-6
Images used to make reference: AL 19.94, SE 1.75

OBSERV PT FOCAL CORRECTION DATA:
Spherical equivalent: 2.00 diopters
Axial Length: 19.91 mm
Effective Length: 13.71 mm

Study	03
Animal ID	1c
Observation Point	01
Actual IOP	11
Registration Version	03m
# images analyzed	6
EKG Aquisition	yes

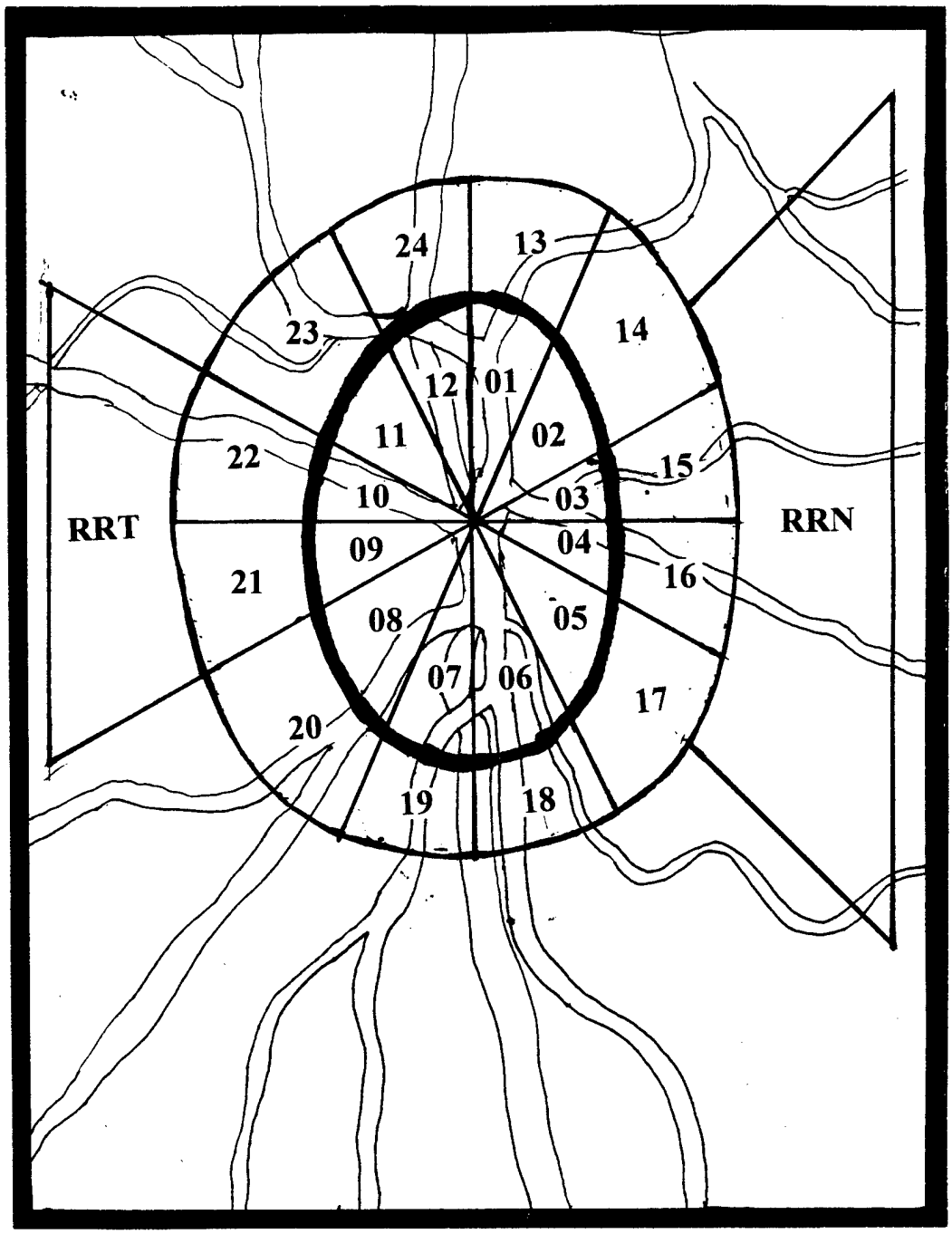
Total Scan Depth: 1536 microns

INDIVIDUAL REGIONAL MEANS (n=6):

	(Depths) Mean	SD	LowCI	HighCI	Total	(% Bad Points) Mean	SD	LowCI	HighCI
1	5	42	-28	39	510	0.0	0.0	0.0	0.0
2	-58	44	-93	-22	511	0.0	0.0	0.0	0.0
3	-122	36	-150	-93	507	0.0	0.0	0.0	0.0
4	-121	14	-133	-110	509	0.0	0.0	0.0	0.0
5	-26	76	-86	35	511	0.0	0.0	0.0	0.0
6	48	83	-19	115	509	0.0	0.0	0.0	0.0
7	30	155	-94	155	508	0.0	0.0	0.0	0.0
8	-97	67	-151	-44	511	0.0	0.0	0.0	0.0
9	-165	31	-190	-140	510	0.0	0.0	0.0	0.0
10	-106	37	-136	-77	508	0.0	0.0	0.0	0.0
11	8	22	-10	25	511	0.0	0.0	0.0	0.0
12	79	28	57	101	508	0.0	0.0	0.0	0.0
13	32	21	15	48	1093	0.0	0.0	0.0	0.0
14	-118	38	-148	-88	1134	0.0	0.0	0.0	0.0
15	-190	60	-238	-141	1180	0.0	0.0	0.0	0.0
16	-159	37	-188	-130	1179	0.0	0.0	0.0	0.0
17	-8	19	-23	8	1134	0.0	0.0	0.0	0.0
18	106	10	98	114	1092	0.0	0.0	0.0	0.0
19	193	78	130	255	1093	0.0	0.0	0.0	0.0
20	128	93	54	203	1134	0.0	0.0	0.0	0.0
21	64	42	31	98	1180	0.0	0.0	0.0	0.0
22	122	27	100	144	1179	0.0	0.0	0.0	0.0
23	189	34	162	216	1134	0.0	0.0	0.0	0.0
24	203	10	195	211	1092	0.0	0.0	0.0	0.0

POOLED REGIONAL MEANS

	(Depths) Mean	SD	LowCI	HighCI	Total	(% Bad Points) Mean	SD	LowCI	HighCI
MPD (1-12)	-44	18	-58	-29	6113	0.0	0.0	0.0	0.0
DiscS (12,1)	42	34	14	69	1018	0.0	0.0	0.0	0.0
ppRetS (24,13)	118	11	109	126	2185	0.0	0.0	0.0	0.0
RRS 500- 800	122	12	113	132	2002	0.0	0.0	0.0	0.0
RRS 800-1100	113	30	89	137	2379	10.1	24.8	-9.7	30.0
RRS 1100-1400	102	73	43	160	1041	18.5	37.6	-11.6	48.6
DiscI (6,7)	39	119	-56	134	1017	0.0	0.0	0.0	0.0
ppRetI (18,19)	149	41	117	182	2185	0.0	0.0	0.0	0.0
RRI 500- 800	121	8	114	127	2002	0.0	0.0	0.0	0.0
RRI 800-1100	97	8	90	104	2350	19.0	10.9	10.3	27.7
RRI 1100-1400	22	39	-9	53	662	69.3	32.2	43.6	95.1
DiscN (3,4)	-122	18	-136	-107	1016	0.0	0.0	0.0	0.0
ppRetN (15,16)	-175	48	-213	-136	2359	0.0	0.0	0.0	0.0
RRN 500- 800	-222	68	-276	-167	2168	0.0	0.0	0.0	0.0
RRN 800-1100	-274	86	-343	-205	2545	0.0	0.0	0.0	0.0
RRN 1100-1400	-288	96	-365	-212	1848	4.4	2.5	2.4	6.4
DiscT (9,10)	-136	33	-162	-110	1018	0.0	0.0	0.0	0.0
ppRetT (21,22)	93	33	67	119	2359	0.0	0.0	0.0	0.0
RRT 500- 800	223	28	201	245	2168	0.0	0.0	0.0	0.0
RRT 800-1100	334	31	309	359	2545	0.0	0.0	0.0	0.0
RRT 1100-1400	420	79	357	483	2620	31.8	18.0	17.4	46.2
RRT/N 500- 800	1	21	-16	18	4336	0.0	0.0	0.0	0.0
RRT/N 800-1100	30	32	4	56	5090	0.0	0.0	0.0	0.0
RRT/N 1100-1400	66	84	-1	133	4468	20.5	10.6	12.0	28.9
RRS/I 500- 800	121	5	118	125	4004	0.0	0.0	0.0	0.0
RRS/I 800-1100	103	13	93	114	4729	14.6	8.3	7.9	21.2
RRS/I 1100-1400	72	22	55	90	1703	38.3	11.2	29.3	47.2
RR 500- 800	59	12	49	68	8340	0.0	0.0	0.0	0.0
RR 800-1100	63	22	45	80	9819	7.0	4.0	3.8	10.2
RR 1100-1400	69	65	17	120	6171	25.4	8.9	18.3	32.5



15 DEGREE REGIONAL ANALYSIS

RIGHT EYE

Comparison of Clinician Judgment With Digitized Image Analysis in the Detection of Induced Optic Disk Change in Monkey Eyes

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• PURPOSE: To compare the ability of clinicians to detect change in the photographic appearance of the optic disk with the performance of a system for digitized image analysis.

• METHODS: In 11 monkey eyes, a Topcon Imagenet System was used to acquire eight digitized image pairs and four stereoscopic photographs at an intraocular pressure of 10 mm Hg, and then, again, 45 minutes after intraocular pressure was increased to 45 mm Hg. We recently reported detection of global (ten of 11 eyes) and regional (11 of 11 eyes) change in the digitized images of these eyes by using two new statistical strategies for optic disk analysis. For the current study, we evaluated the ability of three clinicians (the authors) to detect a change within the stereoscopic photographs of these 11 optic disks. For each eye, the eight stereoscopic photographs (four at intraocular pressure of 10 mm Hg and four at intraocular pressure of 45 mm Hg) were developed as stereoscopic slides and arranged into four pairs (10/10, 45/45, 10/45, and 45/10 mm Hg). Thus, two pairs represented no change in intraocular pressure (10/10 and 45/45 mm Hg) and the other two pairs represented either an increase or a decrease in intraocular pressure (10/45 and 45/10 mm Hg). The 44 pairs of stereoscopic slides (four pairs for each of 11 eyes) were masked then randomly mixed. On two separate occasions, each clinician evaluated each pair of stereoscopic slides for the presence or absence of optic disk change.

• RESULTS: Reproducibility between the two readings of each clinician ranged from .50 to .64 (kappa statistic). Clinicians correctly detected change (as detected by image analysis) within 45% to 64% of the 10/45 and 45/10 pairs of stereoscopic slides. Clinicians correctly identified no change within 86% to 100% of the 10/10- and 45/45-mm Hg pairs of stereoscopic slides. Clinicians correctly identified no change significantly more often than change ($P < .01$, χ^2 test). Change was not detected consistently by all three clinicians in any of the 11 eyes.

• CONCLUSION: In a controlled experimental setting, digitized image acquisition with extensive secondary statistical analysis more sensitively detected small short-term changes in the surface of the optic disks of monkeys than did three masked clinicians.

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THE GOAL OF LONGITUDINAL OPTIC DISK ASSESSment in glaucoma is to detect optic disk change in an eye at risk for glaucomatous damage. To accomplish this goal, both digitized systems of image analysis and human observers must

characterize the disk at two observation points and detect a difference in the two characterizations that exceeds the variability of each.

The reproducibility of clinician evaluation of the optic disk at a single observation point has been the subject of previous studies.[1-5] Additionally, the ability of clinicians to detect the onset and progression of optic disk change by using cup/disk ratio, rim area, optic disk pallor, and nerve fiber layer examination has been extensively assessed.[6-9] Systems for analysis of digitized images of the optic disk have been tested to determine the reproducibility of individual data point and pooled parameter characterizations of the optic disk surface at a single observation point.[10-19] To date, however, few studies have used digitized image analysis to detect change in the optic disk.[20-23] No studies directly compare the ability of clinicians and the ability of a system for digitized image analysis to detect optic disk change.

To induce small changes in the surface of the optic disk, we increased intraocular pressure from 10 to 45 mm Hg in 11 eyes of anesthetized monkeys and used a Topcon Imagenet System (Topcon Instrument Corporation of America, Paramus, New Jersey) to acquire digitized stereoscopic image pairs and simultaneous stereoscopic photographs of the disk at both intraocular pressure levels. In a previous study, we described two statistical strategies for image analysis of the optic disk that detected global and regional change in the digitized images of each disk.[23] In the present study, we assess the ability of three clinicians to detect changes in the stereoscopic photographs of these same 11 optic disks.

MATERIAL AND METHODS

DETAILS OF OUR EXPERIMENTAL PROTOCOL HAVE BEEN outlined elsewhere[23]; all of the procedures involving animals were performed in compliance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. Briefly, 11 adult cynomolgus monkeys (*Macaca fascicularis*) with normal eyes were sedated and anesthetized, and a plano contact lens was placed on one cornea of each animal. Intraocular pressure was lowered from the normal value of approximately 15 mm Hg to 10 mm Hg by insertion of a 25-gauge needle (connected to a saline manometer) into the anterior chamber. After 45 minutes, eight stereoscopic digitized image pairs and four simultaneous stereoscopic photographs were obtained with the Topcon Imagenet Digital Imaging System. Intraocular pressure was then increased to 45 mm Hg over one minute. After 45 minutes, a second set of eight image pairs and four photographs were obtained.

The eight stereoscopic photographs for each disk (four at intraocular pressure of 10 mm Hg and four at intraocular pressure of 45 mm Hg) were developed as color stereoscopic slides and divided into four ordered pairs (slide 1/slide 2) as follows: 10/10, slide 1 obtained at intraocular pressure of 10 mm Hg/slide 2 obtained at intraocular pressure of 10 mm Hg; 10/45, slide 1 obtained at intraocular pressure of 10 mm Hg/slide 2 obtained at intraocular pressure of 45 mm Hg; 45/10, slide 1 obtained at intraocular pressure of 45 mm Hg/slide 2 obtained at intraocular pressure of 10 mm Hg; and 45/45, slide 1 obtained at intraocular pressure of 45 mm Hg/slide 2 obtained at intraocular pressure of 45 mm Hg. With each pair of stereoscopic slides labeled only "slide 1" and "slide 2," the 44 randomly mixed pairs were viewed by three clinicians (the authors) on two occasions separated by at least two weeks. For each pair of stereoscopic slides, each individual stereoscopic slide was placed into its own stereoscopic slide viewer. The two stereoscopic slide viewers were then held together over a light box to allow comparative viewing of the two stereoscopic slides. For each pair of stereoscopic slides, the clinician was instructed to choose one of the following: (1) no change, no change between slide 1 and slide 2 (either 10/10 or 45/45 mm Hg); (2) change 10/45, slide 1 obtained at intraocular pressure of 10 mm Hg/slide 2 obtained at intraocular pressure of 45 mm Hg; or (3) change 45/10, slide 1 obtained at intraocular pressure of 45 mm Hg/slide 2 obtained at intraocular pressure of 10 mm Hg. The observer could also declare the stereoscopic slides to be too blurred to evaluate.

Since the image analysis system had detected statistically significant change in each disk,[23] we assumed that change occurred in each optic disk after increase of intraocular pressure. The correct answer

for pairs of slides obtained at the same intraocular pressure (10/10 and 45/45 mm Hg) was no change. The correct answer for pairs of slides obtained at different intraocular pressures required the correct identification of which slide represented the higher intraocular pressure and which the lower intraocular pressure. By convention, within the two stereoscopic slides of a slide pair that appeared to display change, the clinicians assigned the higher intraocular pressure to the slide in which the optic disk appeared to be displaced posteriorly. Thus, the correct answer for the pair of slides in which slide 1 was obtained at an intraocular pressure of 10 mm Hg and slide 2 at an intraocular pressure of 45 mm Hg was "change 10/45." The correct answer for a pair of slides in which slide 1 was taken at an intraocular pressure of 45 mm Hg and slide 2 at 10 mm Hg was "change 45/10." We compared the reproducibility of each clinician's judgment by determining the kappa statistic for agreement between their readings for each pair of stereoscopic slides on examination days 1 and 2.

The ability of each clinician to detect change correctly was calculated as the percentage of correct answers for the pairs of slides obtained at different intraocular pressures (10/45 or 45/10 mm Hg). The ability of each clinician to detect no change correctly was determined as the percentage of correct answers for the pairs of slides obtained at the same intraocular pressure (10/10 or 45/45 mm Hg). A χ^2 test was used to compare the ability of each clinician to detect change correctly with the ability to detect no change.

We established two levels of stringency to assess the consistency of a clinician's ability to detect change within the four pairs of stereoscopic slides of an individual optic disk: (1) most stringent, the clinician correctly identified no change in the two pairs of stereoscopic slides depicting no change and correctly identified change 10/45 and change 45/10 in the two pairs of stereoscopic slides depicting change; and (2) less stringent, the clinician correctly identified no change in the two pairs of stereoscopic slides depicting no change, and correctly identified change 10/45 or change 45/10 in at least one of the two pairs of stereoscopic slides depicting change.

We studied the ability of the clinicians as a group to detect change, for a given optic disk, as the number of clinicians who detected change by each of the above criteria.

Topographic mapping of the optic nerve head with the Topcon Imagenet System has been described elsewhere.[14,23] To analyze a single stereoscopic image pair, the analyst chose four reference points at the disk margin in the left and right images. From these points, image alignment and elevation values for a grid of data points were derived relative to a zero-reference plane based on the disk margin. All 16 image pairs for a given monkey optic disk (eight at intraocular pressure of 10 mm Hg and eight at intraocular pressure of 45 mm Hg) were analyzed in a single session by a single analyst using the same four reference points.

For each image pair, elevation data for all data points within the optic disk margin were downloaded from the Topcon Imagenet System to a SAS statistical program (SAS Institute, Inc., Cary, North Carolina) within a UNIX-based Sparc Station for extensive secondary data analysis. The average value of all data points considered to be within the disk margin was calculated. For a given disk, mean position of the disk at intraocular pressure of 10 mm Hg was calculated as the mean and standard deviation of the eight average elevations for the image pairs acquired at intraocular pressure of 10 mm Hg. Mean position of the disk at intraocular pressure of 45 mm Hg was calculated as the mean and standard deviation of the eight average elevations for the eight image pairs acquired at intraocular pressure of 45 mm Hg. Change in the overall (global) position of the disk surface after a short-term increase in intraocular pressure was determined by comparing the mean position of the disk at intraocular pressure of 10 mm Hg with the mean position of the disk at intraocular pressure of 45 mm Hg by two-sample *t*-test. Posterior deformation of the disk surface was defined as a decrease in mean position of the disk for which the probability of the null hypothesis was less than .05 (two-sample *t*-test).[23]

Regional optic disk change was detected by first calculating the 95% confidence interval for change[23] at each individual data point and then mapping this probability at each data point considered to be within the margin of the disk. For an individual data point,

the 95% confidence interval for change generates a 95% confidence interval for the change in the mean elevation on the basis of the mean elevation (± S.D.) of that data point at each intraocular pressure. Probable posterior deformation of an individual data point was defined as a change in the mean elevation that gave a 95% confidence interval for change that was entirely negative. Such a definition excluded points (with 95% certainty) where anterior change (positive change in the mean elevation) or no change (zero change in the mean elevation) may have occurred. Regions of probable posterior deformation of the optic disk surface were then defined as localized areas of confluent data points in which, for each point, the full extent of the 95% confidence interval for change was negative.

RESULTS

OF THE INITIAL 44 PAIRS OF STEREOSCOPIC SLIDES, TWO slides making up one pair were unreadable by all observers on both days and were eliminated from the study. Our analysis was therefore based on two separate readings of 43 pairs of stereoscopic slides: 22 pairs of slides depicting change and 21 pairs of slides depicting no change. Clinician agreement with the known composition of the pairs of stereoscopic slides was moderate (Table 1). Intraobserver agreement for each clinician's two readings of the 43 pairs of slides was also moderate, with kappa values ranging from .50 to .64 (Table 2) (agreement was considered to be moderate for .41 < kappa < .60 and substantial for .61 < kappa < .80).[24] The ability of the clinicians to detect change (sensitivity) and no change (specificity) for the 43 pairs of stereoscopic slides is shown in Table 3. Clinicians were able to identify no change correctly in a significantly higher percentage of the pairs of slides taken at the same intraocular pressure, compared with the percentage of correct identifications of change 10/45 or change 45/10 in pairs of slides acquired at different intraocular pressures ($P < .01$, χ^2 test) (Table 3).

Individual clinicians detected change correctly in up to four of the 11 optic disks by the most stringent criteria and in as many as eight of the 11 disks by the less-strict criteria (Table 4). However, all three clinicians did not agree unanimously on the presence of change in any of the 11 disks by our most stringent criteria (Table 5). The clinicians did agree on the presence of change in four of the 11 disks by the criteria that were less strict (Table 5).

As reported elsewhere,[23] we detected statistically significant optic disk change in all 11 eyes by digitized image analysis; global change in the position of the disk surface was detected in ten of 11 eyes by the parameter mean position of the disk, and regional change was detected in 11 of 11 eyes by 95% confidence interval for change mapping.

TABLE 1

CLINICIAN AGREEMENT WITH KNOWN COMPOSITION OF PAIRS OF STEREOSCOPIC SLIDES

	KAPPA STATISTIC	
CLINICIAN NO.	DAY 1	DAY 2
1	.60	.54
2	.56	.58
3	.48	.53

TABLE 2

AGREEMENT BETWEEN THE CLINICIAN'S TWO READINGS OF THE 43 PAIRS OF STEREOSCOPIC SLIDES

CLINICIAN NO.	KAPPA STATISTIC
1	.57
2	.50
3	.64

DISCUSSION

OBJECTIVE CRITERIA FOR DETECTING A CHANGE IN THE position and configuration of the optic disk surface are important in the treatment of glaucoma. Subtle changes in optic disk topography may indicate the onset of damage to underlying structural tissues or frank loss of retinal ganglion cell axons.[25] Detection

TABLE 3

ABILITY OF THE CLINICIAN TO DETECT CHANGE OR NO CHANGE IN THE 43 PAIRS OF STEREOSCOPIC SLIDES

	ABILITY TO DETECT CHANGE CORRECTLY		ABILITY TO DETECT NO CHANGE CORRECTLY	
CLINICIAN NO.	DAY 1	DAY 2	DAY 1	DAY 2
1	0.45*	0.45*	1.00	0.90
2	0.41*	0.36*	0.86	1.00
3	0.55*	0.55*	1.00	0.90

*Ability to detect change in the optic disk is significantly different from ability to detect no change ($P < .01$, χ^2 test).

of such a change may indicate the onset of glaucomatous damage or may represent progression in an eye in which the process of glaucomatous damage has already begun. In this study, we used short-term increases in intraocular pressure in 11 monkey eyes to model the clinical problem of detecting small changes in the surface of the optic disk. Image analysis detected a statistically significant change in the surface of the disk in ten of the 11 eyes by characterization of mean position of the disk and in 11 of the 11 eyes by 95% confidence interval for change mapping. Individual clinicians detected change in only two to four of the same 11 eyes by our most stringent criteria. The three clinicians, as a group, failed to reach a consensus on the presence of optic disk change in even one of the 11 monkey eyes.

One of the strengths inherent in digitized image analysis is the ability to synthesize information from multiple images, thereby generating the ability to use statistical techniques to assess change. To attain a high level of detection, eight digitized image pairs were analyzed at each of two observation points. The performance of digitized image analysis declined when a smaller number of images was used to characterize the disk at each intraocular pressure.[23] Statistically significant optic disk change was detected in only three of the 11 eyes by characterization of mean position of the disk when two image pairs were analyzed at each intraocular pressure. This result was similar to the performance of two of the three

TABLE 4

NUMBER OF EYES (N = 11) IN WHICH THE CLINICIANS CORRECTLY DETECTED CHANGE IN THE OPTIC DISK

	MORE-STRINGENT CRITERIA*		LESS-STRINGENT CRITERIA*	
CLINICIAN NO.	DAY 1	DAY 2	DAY 1	DAY 2
1	3	3	8	7
2	4	4	8	7
3	2	1	7	6

*More-stringent criteria for the detection of change within the four pairs of stereoscopic slides of an individual eye required the correct identification of no change in the two pairs of slides obtained at the same intraocular pressure and the correct identification of change 10/45 and change 45/10 in both pairs of slides obtained at different intraocular pressures. Less-stringent criteria required the correct identification of change 10/45 or change 45/10 in only one of the two pairs of slides depicting change.

clinicians in the present study (Table 5). Although it could be argued that clinician performance might have improved if they had been given multiple sets of stereoscopic slides of each disk to assess, we believe that such a situation is both impractical and unlikely.

TABLE 5

NUMBER OF CLINICIANS (N = 3) WHO CORRECTLY DETECTED CHANGE IN THE OPTIC DISK OF EACH MONKEY

	MORE-STRINGENT CRITERIA		LESS-STRINGENT CRITERIA	
MONKEY NO.	DAY 1	DAY 2	DAY 1	DAY 2
1	2	2	3	2
2	1	0	2	2
3	1	0	2	3
4	0	0	2	0
5	1	1	2	3
6	1	1	3	3
7	0	0	1	1
8	1	2	3	2
9	1	1	3	3
10	0	0	1	2
11	1	0	1	0

There are no studies that suggest that clinicians are more sensitive to optic disk change if multiple slides of the disk at each observation point are viewed simultaneously.

Computer-based image analysis allows for the rapid acquisition of many images at a given observation point. To date, the proper number of images and the efficacy of multiple image acquisition are not known.[19,23] This study provides the first direct evidence that statistical analyses of data derived from multiple digitized images of the optic nerve head may be more sensitive than clinical examination of stereoscopic slides in the detection of small changes in the surface of the optic disk.

Changes in the monkey optic disk induced by short-term increases in intraocular pressure may or may not resemble those that follow the onset and progression of glaucomatous damage to the human optic disk. We believe that this study reasonably compares clinicians' judgment with digitized image analysis in the detection of small changes in the surface of the optic disk. However, clinicians may be better at detecting optic disk change in pictures of human optic nerves that have been damaged by long-term increases in intraocular pressure. As such, our study provides valuable information about detection of optic disk change, although our model may be less than perfect as a model of early optic disk change in human glaucoma.

In this study, the clinicians were better at determining that the two stereoscopic slides of a pair showed no change than they were at detecting a change in the appearance of the optic disk as defined by image analysis. One explanation for this finding might be that the clinicians were biased against detecting change in the stereoscopic slides. We believe that the clinicians were trying to detect change but found it difficult to do so. The clinicians did identify optic disk change in from one third to one half of the pairs of stereoscopic slides taken at different intraocular pressures (Table 4). Additionally, intraobserver reproducibility was only moderate, chiefly because of inconsistent interpretation (on the two examination days) of the pairs of stereoscopic slides depicting change. This implies that on each day the clinicians were making an effort to detect change but did so inconsistently, rather than that they were making a more consistent attempt to detect no change.

We did not attempt to evaluate how the clinicians assessed optic disk change or how their method differed from the statistical strategies used by the computers. Future studies will compare clinical judgment with systems for digitized image analysis in the detection of both the onset and progression of optic disk change in experimental glaucoma. From these studies, the strategies for detecting early, middle, and late optic disk change by digitized image analysis may direct clinicians to new techniques for the clinical detection of optic disk change.

The following considerations limit interpretation of our study. First, both photographic and digital imaging were performed in an idealized research setting, in which anesthetized monkeys did not move, their pupils were well dilated, and no cataractous changes were present in the lens. Second, we assumed that small changes occurred in the surface of all 11 optic disks after a short-term increase in intraocular pressure. Although we cannot be certain that the optic disk changed in every eye, substantial evidence indicates that the tissues of the optic nerve head undergo rapid displacement under the influence of changes in intraocular pressure.[20-23,26-29] Recently, ten of the 11 eyes in this study were included in a larger study of 29 eyes from 18 monkeys, in which characterization of mean position of the disk was used to describe reversible changes in the surface of the optic disk during extensive compliance testing of the optic nerve head.[30] In that study, we reported additional values for mean position of the disk generated from eight images acquired two, 17, and 32 minutes after intraocular pressure increase to 45 mm Hg, as well as the values for 45 minutes (actually 47 minutes) reported in our original study.[23] The data show a progressive posterior shift in mean position of the disk over the course of these additional observation points that is consistent with a progressive posterior deformation of the optic disk surface.[30]

Third, the clinicians and the image analysis system did not perform identical tasks. The system for digitized image analysis was asked to perform a single evaluation of two sets of eight images for each eye and

to determine the presence or absence of statistically significant change. In the current study, the clinicians not only were required to detect change and no change, but were required to do so reproducibly to meet our most stringent criteria for the detection of optic disk change. The reproducibility of the ability of the system for digitized image analysis to detect change by both characterization of mean position of the disk and 95% confidence interval for change mapping was extensively studied previously.[23] For three of these eyes (representing a range of minimal, moderate, and maximal change in mean position of the disk), a single person (analyst 1) analyzed all 16 image pairs (eight at intraocular pressure of 10 mm Hg and eight at intraocular pressure of 45 mm Hg) in three single analysis sessions performed on three separate days. Three assessments of mean position of the disk at intraocular pressure of 10 mm Hg and three assessments of mean position of the disk at intraocular pressure of 45 mm Hg were thus obtained. A second person (analyst 2) then performed a similar set of three analyses on the same images. By analysis of variance testing, characterization of mean position of the disk showed statistically significant change for all three eyes when the variability in mean position of the disk at each intraocular pressure for an individual analyst and the variability in mean position of the disk for different analysts were taken into account.[23]

Although clinician performance in this study cannot be compared directly with that of the two statistical strategies for digitized image analysis, we believe that the present study fairly evaluates clinician ability to detect change within the stereoscopic slides of these disks. This study suggests that digitized image analysis-based statistical strategies may be more sensitive than clinicians in the detection of small, short-term changes in the surface of the optic disk. Further studies comparing clinical judgment with systems for digitized image analysis in the detection of glaucomatous damage to the human optic disk are required.

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Early Changes in Optic Disc Compliance and Surface Position in Experimental Glaucoma

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ABSTRACT

Purpose: To detect changes in the compliance (elasticity) and baseline position (position at the baseline time point of a compliance test) of the monkey optic disc following the onset of chronic experimental glaucoma.

Methods: This report includes 66 compliance tests performed on 26 eyes of 13 monkeys. *Longitudinal Study*. In seven normal monkeys, compliance tests were performed three times in one eye (study eye) and once in the contralateral eye. In the study eye of five of these monkeys, chronic experimental glaucoma was then induced and compliance tests were performed at some or all of the following post-glaucoma testing intervals: *1-2 weeks*, *3-4 weeks*, *5-8 weeks*, *9-12 weeks*, *13-18 weeks*, and *greater than 18 weeks* after the onset of elevated IOP. In the study eye of the remaining two monkeys, the optic nerve was transected, and compliance was tested at *5*, *9*, and *13 weeks* after transection. An analysis of variance (ANOVA) was performed to detect an increase (hyperelasticity) or decrease (rigidity) in the compliance of the glaucomatous eyes at each testing interval. A second ANOVA was performed to detect the onset of chronic posterior deformation of the baseline position of each disc. *Cross-Sectional Study*. In six additional monkeys with pre-existing experimental glaucoma, the glaucomatous study eye was compliance tested at one of the post-glaucoma testing intervals used in the longitudinal study. The contralateral normal eye was compliance tested once. These data were then added to the data from the five longitudinally studied monkeys at the appropriate pre- and post-glaucoma testing intervals. A third ANOVA compared the compliance of the expanded group of glaucomatous eyes at each post-intervention testing interval with the compliance of the 13 normal contralateral eyes.

Results: *Compliance.* In both the longitudinally ($Pr > F = .0005$) and cross-sectionally ($Pr > F = .0001$) studied glaucomatous eyes, optic disc compliance (elasticity) increased significantly by *1-2 weeks*, then returned to a level statistically indistinguishable from normal by *13-18 weeks* post-glaucoma. In the transection eyes, the optic discs were significantly less compliant (more rigid) at *5* and *9 weeks* post-transection, compared with the discs in either the normal or the glaucomatous eyes ($Pr > F < .05$). *Baseline Optic Disc Position.* Chronic posterior deformation of the disc was detected in one of three eyes tested *1-2 weeks* and three of four eyes tested *3-4 weeks* following the onset of glaucoma ($Pr > F < .05$). Chronic posterior deformation was not detected in the discs of either of the transection eyes at any of the post-transection testing intervals.

Conclusion: Changes in optic disc elasticity and surface position were detected by digitized image analysis within 2 to 4 weeks of the onset of experimental glaucoma in the monkey eye. These findings are unlikely to be due to axon loss alone, since they did not occur in optic nerve transection eyes (which constitute a model of axon loss in which intraocular pressures remain normal). Our results suggest that IOP-induced damage to the load-bearing tissues of the optic nerve head may occur early in experimental glaucoma.

In the companion paper,[1] we described our technique for compliance testing of the monkey optic disc and proposed that measuring optic disc compliance (elasticity) is one form of mechanically testing the structural tissues of the optic nerve head. In that study, we detected reversible, posterior deformations of the optic disc surface in normal monkey eyes subjected to acute fluctuations in intraocular pressure (IOP) and found those deformations to be reproducible in a given eye and statistically similar in the two eyes of an individual monkey. We proposed that the connective tissues of the normal lamina cribrosa and peripapillary sclera experience a predictable, but as yet undefined, distribution of force and suggested that these tissue are elastic in their response to acute pressure elevations from 10 to 45 mm Hg.

In the present paper, we hypothesize that the lamina cribrosa and peripapillary sclera are damaged early in the pathophysiology of glaucomatous damage to the optic disc. We propose that a change in mechanical behavior would be evidence of such damage and seek to detect such change in monkey discs in which the normal compliance and baseline position (position at the baseline time point of a compliance test) are well characterized. This study concentrates on detecting change within two aspects of our compliance testing sessions. First, we attempted to detect an increase (hyperelasticity) or decrease (rigidity) in the amount the disc moves during the 47-minute period of elevated IOP *within* a compliance test. Second, we attempted to determine whether an irreversible posterior shift of the baseline position of the disc occurs during the period of chronically elevated IOP *between* compliance tests (chronic posterior deformation of the disc or yield).

Materials and Methods

A total of 66 compliance testing sessions performed on 26 eyes of 13 monkeys are included in this report. The animals in these studies were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Longitudinal Study

Seven juvenile (exact age unknown), male cynomolgus monkeys (*Macaca fascicularis*) underwent three separate compliance testing sessions in one eye (study eye), followed by a separate session in the contralateral eye. In five of the seven monkeys, multiple laser treatments to the trabecular meshwork of the study eye were performed to induce chronic elevation of IOP (22 to 60 mm Hg).[2] In the remaining two monkeys, surgical optic nerve transection was carried out in the study eye[3]; two weeks later, fluorescein angiography was performed to establish that no surgically related arterial or venous occlusion had occurred. Compliance testing was performed in the five glaucomatous eyes at some or all of the following post-intervention testing intervals: *1-2 weeks*, *3-4 weeks*, *5-8 weeks*, *9-12 weeks*, *13-18 weeks*, and $>$ *18 weeks*; and in the two transection eyes at *5, 9,* and *13 weeks* (Table 1).

Cross-Sectional Study

In an additional six monkeys with previously induced chronic experimental glaucoma, the glaucomatous study eyes were compliance tested once at one of the testing intervals given above (Table 1), and the normal contralateral eyes were tested once in separate sessions (Table 1).

Compliance Testing Session

All compliance testing sessions were performed exactly as previously described.[1] Briefly, during a compliance test, the optic disc was imaged with a Topcon Imagenet System (Imagenet Ophthalmic Digital Imaging System, Topcon America Corporation, Paramus NJ) 2 (time point 10-1A) and 47 minutes (baseline time point of the compliance test; 10-1$D_{Baseline}$) after IOP was lowered to 10 mm Hg with a saline manometer; then at 2, 17, 32, and 47 minutes after IOP was elevated to 45 mm Hg (time points 45-1A, B, C, and D); then at 2, 47, and in some cases 92 minutes after IOP was lowered back to 10 mm Hg (time points 10-2A, D, and E).

Analysis of Images

Topographic mapping of the optic nerve head with the Topcon Imagenet System has previously been described.[1,4] To analyze a single stereoscopic image-pair, the analyst entered spherical equivalent refractive error, corneal base curve, and axial length, and then attempted to choose the same four points (indicating the disc margin) in the left and right images. From these reference points, image alignment and elevation values for a grid of data points were derived. The average height of the four disc-margin-based reference points established the zero-reference height. All image-pairs of all compliance testing sessions for a given monkey disc were analyzed by a single analyst. Before the image-pairs were analyzed, a photographic record of the four reference points for that disc was consulted. The analyst then attempted to choose these same four reference points in all analyses.

Mean Position of the Disc

For each image-pair, elevation data for all disc data points were downloaded from the Topcon Imagenet System to a SAS statistical program (SAS Institute Inc., Cary, NC) running on a UNIX-based work station, and the average value was calculated.[1,5] For a given disc, mean position of the disc (MPD) ± 95% confidence interval (CI) at a given time point was calculated as the mean and 95%CI of the eight average elevations acquired at that time point. For each compliance test, the *Change from* $MPD_{Baseline}$ (in microns) for each time point was calculated by subtracting the value of MPD at time point 10-1$D_{Baseline}$ ($MPD_{Baseline}$) from the value for MPD at each of the other time points. The *Change from* $MPD_{Baseline}$ was considered to be statistically significant for those time points at which MPD ± 95%CI showed no overlap with $MPD_{Baseline}$ ± 95%CI.[1]

Study Group Compliance

To characterize the overall compliance of a study group (the group of eyes compliance tested at one testing interval, Tables 1 and 2 and Fig 1), data from all compliance tests conducted during a testing interval were pooled and the mean *Change from* $MPD_{Baseline}$ with an analysis of variance (ANOVA)-generated 95%CI was calculated at each compliance test time point.

Statistical Methods

Changes in Optic Disc Compliance. *Longitudinal Study*. To detect changes in optic disc compliance, a repeated measures ANOVA was performed on the dependent variable *Change from* $MPD_{Baseline}$. This analysis included multivariate tests for the significance of

overall compliance differences among study groups as well as univariate tests to determine the significance of differences in the *Change from* $MPD_{Baseline}$ at each time point within the longitudinal data for each study eye. We defined a significant change in the compliance of an individual glaucomatous or transection eye to have occurred at a particular post-intervention testing interval when the *Change from* $MPD_{Baseline}$ at one of the 45-mm-Hg time points (45-1A, B, C or D) was significantly different ($p < .05$) from the mean *Change from* $MPD_{Baseline}$ at that time point in the compliance testing performed before intervention, when the eye was normal.

Cross-Sectional Study. A repeated measures ANOVA identical to that described for the longitudinal study was carried out using the 46 compliance testing sessions performed on the expanded group of 11 glaucomatous eyes, the two optic nerve transection eyes, and the 13 normal contralateral eyes (Table 1).

Chronic Posterior Deformation of the Disc. We defined chronic posterior deformation of the disc to be a chronic or irreversible deformation of the position of an optic disc at the baseline time point of a compliance test. Analysis of baseline time point data allowed us to study deformation that was "chronic" or still present after 47 minutes of low IOP (between initiation of IOP lowering and time point 10-$1D_{Baseline}$). Thus, chronic posterior deformation is deformation that persisted after the disc was subjected to 47 minutes of low IOP to allow it to return to its most "normal" or anterior position.

To detect change within the $MPD_{Baseline}$ data for each study eye, a separate ANOVA was performed on the dependent variable $MPD_{Baseline}$. This analysis compared the $MPD_{Baseline}$ value at each post-intervention testing interval with the three normal values for $MPD_{Baseline}$ for each disc. Chronic posterior deformation of the disc was defined as present

at those post-intervention testing intervals for which $MPD_{Baseline}$ was significantly ($p < .05$, ANOVA) posterior to the three values for $MPD_{Baseline}$ when normal.

Optic Nerve Axon Estimation

The seven monkeys of the longitudinal study were sacrificed by exsanguination under deep intravenous pentobarbital anesthesia within 1 week of their final compliance testing session. Optic nerve cross-sections taken 1-3 mm behind the globe were postfixed in 1% osmium tetroxide in 0.1 mol/L phosphate buffer, dehydrated, embedded in epoxy resin, cut in 1-μm sections, and stained with toluidine blue. The number of remaining nerve fibers was estimated by a reported method in which at least 5% of the axons were counted by image analysis.[6] Mild, moderate, and severe axon loss were defined as 25-50% loss, 50-75% loss, and greater than 75% loss of axons, respectively, as estimated from neural area measurements of the optic nerve cross-sections, comparing each study eye with its normal contralateral control.

Results

Changes in Optic Disc Compliance

Longitudinal Study. In the glaucomatous eyes studied as a group, a significant increase in optic disc compliance was seen *1-2 weeks* after the induction of chronic experimental glaucoma (*1-2 weeks, Glaucoma*, Fig 1). At this testing interval, hyperelasticity (a statistically significant increase in the amount of posterior *Change from* $MPD_{Baseline}$) was detected as early as 2 minutes (compliance test time point 45-1A) after elevation of IOP from 10 mm Hg to 45 mm Hg (Fig 1) and persisted through all of the time points at 45 mm Hg

(compliance test time points 45-1B, 45-1C, and 45-1D). This increase in compliance continued to be seen through the *9-12 week* testing interval. By *13-18 weeks*, however, compliance returned to values that were statistically indistinguishable from normal (Fig 1).

In striking contrast, the post-transection eyes showed no increase in compliance (Fig 1). The only statistically significant change in compliance demonstrated after transection was a decrease in compliance at time point 45-1C at the *5-8 week* testing interval, and at time points 45-1D and 10-2A at *9-12 weeks* (Fig 1). At both *5-8 weeks* and at *9-12 weeks* post-intervention, the optic discs in the transection eyes were significantly less compliant than the discs in the glaucomatous eyes (Fig 1). However, by *13-18 weeks*, the compliances of the discs in the glaucomatous and transection eyes were statistically indistinguishable.

Overall, compliance differences among the individual study groups (*Study eyes as normals, Glaucoma 1-2 weeks, 3-4 weeks, 5-8 weeks, 9-12 weeks, 13-18 weeks*, and *> 18 weeks*; and *Transection 5, 9,* and *13 weeks*) achieved statistical significance ($Pr < .0015$, MANOVA). More specifically, overall compliance differences among the study groups achieved statistical significance at the following compliance test time points: 10-1A, 45-1A, 45-1B, 45-1C, and 45-1D ($Pr > F < .05$, ANOVA,).

Finally, when the seven study eyes were evaluated individually, a significant increase in compliance was detected in two of the three glaucomatous eyes tested at *1-2 weeks* and in all four glaucomatous eyes tested at *3-4 weeks* following the onset of elevated IOP (Table 2). In the one glaucomatous eye that was compliance tested only at the *5-8 week* and *9-12 week* testing intervals (Monkey 17, Tables 1 and 2), a significant increase in compliance was not present at *5-8 weeks* but was present by *9-12 weeks*. No statistically significant change in optic disc compliance occurred in the two transection eyes considered individually at *5, 9,* or

13 weeks post-transection.

Cross-Sectional Study. Expansion of the original group of seven eyes to include a group of cross-sectionally imaged eyes and comparison with the contralateral normal eyes did not change our results. Neither the mean *Change from* $MPD_{Baseline}$ data for the expanded groups (data not shown), nor the results of an identical repeated measures ANOVA were qualitatively different. The Pr > F values for groups overall, groups at each compliance test time point, and individual comparisons of study groups at each compliance test time point (data not shown) were only marginally different (no overall differences in statistical significance).

Chronic Posterior Deformation of the Disc

Chronic posterior deformation of the disc was present in one of the three glaucomatous study eyes tested at *1-2 weeks* and three of the four glaucomatous eyes tested at *3-4 weeks* (Table 3). At *1-2 weeks*, the deformation in monkey 15 (-87 microns) was significant ($Pr > |T| = .0042$), whereas deformations of -42 microns in monkey 14 ($Pr > |T| = .1228$) and -19 microns in monkey 16 ($Pr > |T| = .4894$) did not achieve significance. At *3-4 weeks*, the deformation decreased slightly to -63 microns in monkey 15, which was not significantly different from the value at *1-2 weeks* ($Pr > |T| = .42$), but increased to -195 microns in monkey 14 ($Pr > |T| = .0001$) and -108 microns in monkey 16 ($Pr > |T| = .0009$). Progressive posterior deformation of the disc continued at the later testing intervals in four of the five glaucomatous study eyes (Table 3). In one of the glaucomatous study eyes (monkey 13), chronic posterior deformation of the disc was not detected at any of the three post-intervention testing intervals (Table 3). No significant

change in optic disc position was detected in the two transection eyes at *5, 9*, or *13 weeks* after transection (Table 3).

Discussion

This study reports a change in the compliance and surface position of individual monkey optic discs following the onset of experimental glaucoma. We detected an increase in optic disc compliance as early as 2 weeks and a chronic posterior shift in the baseline position of the disc as early as 1-2 weeks following the onset of elevated IOP. Later, compliance returned to normal levels in the majority of the glaucomatous eyes. Optic nerve transection eyes failed to show similar changes in the compliance and baseline position of the disc. We propose that early changes in the mechanical behavior of the disc surface in experimental glaucoma may be a manifestation of IOP-induced damage to the deeper, load-bearing tissues of the optic nerve head.

Optic disc compliance changes in glaucoma have been studied previously.[7-9] Zeimer et al[7] used laser Doppler velocimetry to measure instantaneous movements of the optic disc surface following acute elevations of IOP in ocular-hypertensive beagle eyes. They reported instantaneous posterior displacements of the disc surface ranging from 16 to 53 microns in four hypertensive eyes following IOP elevations of approximately 32 mm Hg. In glaucomatous human postmortem eyes, Zeimer and Ogura[8] reported an overall reduction in compliance and a weak correlation of extent of visual field loss with decreased compliance in those eyes with concomitant visual field data. None of the postmortem human glaucoma eyes displayed an increase in compliance.

Coleman et al[9] measured in vivo changes in the optic disc surface of hypertensive

monkey eyes using a Humphrey Retinal Analyzer. Hypertensive optic nerve heads moved anteriorly a mean of 47.4 ± 74.2 microns with lowering of IOP to 15 mm Hg. However, the anterior movement of the hypertensive eyes failed to achieve statistical significance ($p < .02$).

Shirakashi, Nanba, and Iwata[10] studied differences in the amount of cupping-reversal that occurred in five glaucomatous monkey eyes following spontaneous reductions in IOP. The amount of optic disc change following IOP reductions during the first four months of laser-induced glaucoma were compared with the amount of optic disc change following similar magnitudes of IOP reduction during months 9 to 12. Four months following the onset of experimental glaucoma, IOP reductions of 22.6 ± 2.7 mm Hg resulted in statistically significant reductions of 20.8% in vertical cup-to-disc ratio, 20.2% in horizontal cup-to-disc ratio, and 57.6% in cup volume/disc area ratio, as well as a 47.2% increase in rim area/disc area ratio. However, at 9-12 months a similar level of IOP reduction (22.4 ± 2.2 mm Hg) resulted in significantly less change in each parameter.

In contrast to the results of previous studies,[8,10] we were not able to detect optic disc rigidity in glaucomatous study eyes considered individually or as a group. While a less compliant phase followed the early period of hyperelasticity, a statistically significant decrease in compliance was not detected. However, our study design may have limited our ability to detect such change. Within the longitudinal study, in which the power to detect a statistical decrease from normal compliance was greatest (due to the 21 compliance testing sessions performed in the seven study eyes when they were normal), we followed only one eye to the > *18 weeks* testing interval. Within the cross-sectional study, although the number of eyes within the > *18 weeks* testing interval was increased (n=4), the diminished number

of normal contralateral eyes (n=13) reduced our ability to detect significant change.

At least one of the study eyes, however, displayed a qualitative reduction in compliance, compared with its normal contralateral eye (Monkey 1, Fig 2). While this monkey was exposed to only 13 weeks of elevated IOP (followed by a long period of normal pressures), it was judged to have severe axon loss at the time of compliance testing and sacrifice. Future studies, which will include a large number of eyes studied longitudinally from normal to endstage glaucomatous damage, may demonstrate a late period of optic disc rigidity in glaucoma.

Apart from changes in optic disc compliance (the acute movement of the disc within a compliance testing session), our study provides evidence that the tissues of the optic nerve head undergo a chronic deformation early in response to chronic elevation of IOP, which is not reversed with subsequent lowering of IOP. We refer to this phenomenon as chronic posterior deformation of the disc, and we suggest that it represents the onset of permanent strain or yield within the load-bearing tissues of the lamina cribrosa and peripapillary sclera. We use the term "chronic" posterior deformation to separate this irreversible component of posterior displacement from any reversible deformation caused by the elevated level of IOP at the time of the compliance test. Each compliance testing session began with a 47-minute period of low IOP (the period at 10 mm Hg between compliance test time points 10-1A and the baseline time point, 10-1D; Fig 1, companion report[1]). Although the position of the disc changed little during this period in normal eyes (normal eyes, Fig 1), once the discs were chronically exposed to elevated IOP (*1-2*, *3-4*, *5-8,* and *9-12 week* testing intervals, Fig 1), statistically significant anterior movement occurred (*3-4 weeks*, -31 microns, $Pr > |T| = .009$; *9-12 weeks*, -31 microns, $Pr > |T| = .009$; Fig 1) during this period of the test.

Thus, the chronic posterior deformation that we report is deformation that persisted after the disc was subjected to a low IOP for 47 minutes to permit the return to its most "normal" or anterior position.

Our data suggest the following overall sequence of events (Fig 1). During a compliance testing session, the normal monkey optic disc moved little when IOP was lowered from its ambient level (10 to 20 mm Hg) to 10 mm Hg (time points 10-1A and 10-$1D_{Baseline}$, normal eyes, Fig 1). During the period of acute elevation of IOP to 45 mm Hg, there was progressive posterior deformation of the disc surface that reached approximately 30 microns over the course of 47 minutes (time points 45-1A, B, C, and D, normal eyes, Fig 1). When IOP was returned to 10 mm Hg, the discs returned to their baseline positions, presumably because movements of 30 microns are within the elastic limits of the structural tissues of the normal monkey disc.

With exposure of the discs to chronic elevations of IOP (22 to 60 mm Hg) for as little as 2 weeks, the principal change in compliance was a marked increase in the amount of posterior deformation (hyperelasticity) that occurred within the first 2 minutes of IOP elevation from 10 to 45 mm Hg (time point 45-1A, *1-2 weeks, Glaucoma*, Fig 1). Separate from compliance changes, the baseline position of the disc shifted posteriorly in all three eyes at the *1-2 week* testing interval, but this deformation achieved significance in only one of the three eyes (Table 3).

By week *3-4* however, the discs showed evidence of posterior deformation in response to elevated IOP. At this point, there were two components to this deformation. First, overall (Fig 1) and in each eye individually (data not shown), the discs moved anteriorly during the initial period of IOP lowering between time points 10-1A and 10-$1D_{Baseline}$. This

demonstrates a reversible component to the posterior deformation which is related to the elasticity of each individual disc and the ambient level of elevated IOP within the eye at the time of the test. In addition, at this testing interval, three of the four eyes demonstrated chronic or irreversible posterior deformation of the disc (Table 3). This is an irreversible posterior deformation (compared with the position at time point $10\text{-}1D_{Baseline}$ in the three normal compliance tests), which may be early evidence for damage to the structural tissues of the disc (yield). By this testing interval, the hyperelasticity observed at time point 45-1A had increased to a mean deformation of approximately -90 microns (Fig 1).

At *9-12 weeks*, progressive chronic posterior deformation of the baseline position of the disc had occurred in four of the five eyes (Table 3), but because significant axon loss, with resultant thinning of the prelaminar tissues, may have occurred by this testing interval, the structural implications of posterior deformation of the baseline disc position at this and later testing intervals are less clear. Hyperelasticity during the period between time points 45-1A and 45-1D was still present; although the discs were less elastic than at *5-8 weeks*, the difference did not achieve statistical significance (data not shown).

By *13 weeks* and beyond, the discs were markedly less elastic than they had been earlier, but not significantly less elastic than normal. Only 21 microns of posterior displacement occurred over the first 2 minutes of elevated IOP, which is significantly less than the amount of movement observed at *3-4, 5-8,* and *9-12 weeks* ($Pr > |T| = .0001$, 0012, and .0040, respectively).

In marked contrast to the series of findings outlined above, optic nerve transection eyes showed no early period of hyperelasticity. Instead, transection eyes progressed to qualitatively less movement of the disc *5, 9,* and *13 weeks* after transection (Fig 1). We did

not image optic nerve transection eyes earlier than 5 weeks and may, therefore, have missed a short period of early hyperelasticity in these eyes. We chose to image these eyes first at the *5-week* observation point because previous studies suggested that the majority of axons are absent by 6 to 8 weeks post-transection.[3,11] If the hyperelasticity of the glaucomatous discs was caused by loss of axons alone, we would expect to see it in transection eyes at the *9-* and *13-week* testing intervals.

In addition, optic nerve transection eyes demonstrated no statistically significant change in the baseline position of the optic disc *5*, *9*, or *13 weeks* post-transection (Table 3). This was surprising because, in the setting of total axon loss (Table 2), one might expect the optic disc surface to move posteriorly following thinning of the prelaminar tissues. Quigley and Pease, using a Glaucoma-Scope with a retinal-based, zero-reference plane, recently reported posterior shifts in MPD of only 20 to 40 microns 4 months post-transection in two monkey eyes (personal communication; Harry A. Quigley, MD, Baltimore, Maryland).

Thinning of the prelaminar tissues (with subsequent posterior shift of the optic disc surface) may have occurred and been undetected in this study due to a progressive posterior shift in the Topcon's disc margin-based, zero-reference height. In addition, because the Topcon system seeks contrast differences within a stereo image-pair, the instrument may have been more variable in its mapping of the pale discs in the transection eyes. If this were the case, the images from the transection eyes would be more variable at 9 and 13 weeks post-transection (due to pallor), than when the eyes were normal. However, when we reviewed the arithmetic 95% confidence intervals for $MPD_{Baseline}$ in the transection eyes, we found them qualitatively similar throughout the entire period of testing. The widths of the 95%CI for $MPD_{Baseline}$ in the three normal tests were 12, 17, and 30 microns, respectively,

for monkey 18 and 18, 21, and 25 microns, respectively, for monkey 19. The widths in the tests post-transection were 25, 17, and 11 microns for monkey 18 and 11, 5, and 18 microns for monkey 19.

The absence of optic disc hyperelasticity and our inability to detect chronic posterior deformation in transection eyes suggest that these phenomena in the glaucomatous eyes are not the result of axon loss alone. While our data do not exclude the possibility that IOP-damaged axons could affect the mechanical behavior of the disc, we propose that optic disc hyperelasticity and chronic posterior deformation of the disc are manifestations of IOP-induced alterations to the connective tissues of the lamina cribrosa and peripapillary sclera. These alterations could include new synthesis of extracellular matrix components,[12,13] physical disruption of the connective tissue components[14-18] with a concomitant change in their mechanical behavior, or some combination of the two processes.

Our study is limited by the small number of eyes that were compliance tested as normals before the induction of experimental glaucoma (n = 5) or transection of the optic nerve (n = 2). However, the results of the group of five glaucomatous eyes studied longitudinally were confirmed in the expanded group of 11 glaucomatous eyes studied cross-sectionally. Furthermore, the progression from optic disc hyperelasticity to a normal level of compliance was remarkably similar in the five longitudinally studied glaucomatous eyes. While small differences existed, in all five eyes optic disc hyperelasticity occurred first (in all cases by the *9-12 week* testing interval), followed by the slow return to normal compliance no later than the *13-18 week* testing interval in the four eyes tested beyond 12 weeks (Table 2).

While the optic disc compliance changes in glaucoma described here occurred early

and appear to be qualitatively related to the length of exposure to elevated IOP, it is not clear from our data whether changes in compliance bear a relationship to other estimates of optic nerve damage, such as the extent of axon loss. Data from monkeys 13 and 15 (Table 2) suggest that both the onset of hyperelasticity and the progression to rigidity can occur prior to or coincident with mild loss of axons. However, the data for monkey 17 (Table 2) suggests that a moderate loss of axons may have already occurred in an optic disc that is hyperelastic but not yet rigid.

Finally, our report documents the ability of image analysis-based optic disc compliance testing to detect early changes in the mechanical behavior of the optic disc in experimental glaucoma. Characterization of the histologic correlates to these changes may provide new insight into the early stages of glaucomatous damage to the optic disc. While our present protocol is too rigorous for clinical use, efforts to develop a safe form of compliance testing in humans are ongoing.

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Table 1. Schedule of Compliance Testing Sessions

	Normal Eye Testing Sessions				Post-intervention Testing Sessions					
	Contralateral	Study Eye			1-2	3-4	5-8	9-12	13-18	> 18
Monkeys	Normal Eye	1st	2nd	3rd	Weeks	Weeks	Weeks	Weeks	Weeks	Weeks
Glaucoma Eyes										
Studied as Normals										
Monkey No. 13	●	●	●	●		■	■		■	
Monkey No. 14	●	●	●	●	■	■	■	■	■	
Monkey No. 15	●	●	●	●	■	■	■	■	■	■
Monkey No. 16	●	●	●	●	■	■	■	■	■	
Monkey No. 17	●	●	●	●			■	■		
Not Studied as Normals										
Monkey No. 1	●								■	
Monkey No. 2	●									■
Monkey No. 5	●									■
Monkey No. 7	●							■		
Monkey No. 8	●							■		
Monkey No. 9	●									■
Optic Nerve Transection Eyes										
Monkey No. 18	●	●	●	●			▲	▲	▲	
Monkey No. 19	●	●	●	●			▲	▲	▲	

● = Normal eye; ■ = Glaucomatous eye; ▲ = Transection eye.

Table 2. Magnitude and Statistical Significance of Optic Disc Compliance Change in Individual Study Eyes

Monkey Eyes Studied as Normals	Mean of Three Normal Tests (ANOVA)	Posterior Deformation* at Compliance Test Time point 45-1D in Microns at Given Post-intervention Testing Interval						Axon Loss at Final Testing Session
		1-2 Weeks	3-4 Weeks	5-8 Weeks	9-12 Weeks	13-18 Weeks	> 18 Weeks	
Glaucoma Eyes								
Monkey No. 13	-23		-100†	-87†		-63		Mild
Monkey No. 14	-23	-113†	-93†	-48	-44	-29		Moderate
Monkey No. 15	-45	-60	-162†	-113†	-93†	-30	-25	Mild
Monkey No. 16	-28	-74†	-82†	-58†	-38	-6		Severe
Monkey No. 17	-43			-53	-112†			Moderate
Transection Eyes								
Monkey No. 18	-30			+7	+3	-13		Total atrophy
Monkey No. 19	-37			-14	+1	+3		Total atrophy

*Change from $MPD_{Baseline}$. Negative values denote posterior displacement and positive values denote anterior displacement.

† Increase or decrease from mean normal value is statistically significant ($Pr > |T| < .05$, ANOVA).

___ Testing interval during which study eye was compliance tested.

Table 3. Magnitude and Statistical Significance of Chronic Posterior Deformation of the Disc in Individual Study Eyes

Monkey Eyes Studied as Normals	Change from Normal $MPD_{Baseline}$ in Microns at Given Post-intervention Testing Interval					
	1-2 Weeks	3-4 Weeks	5-8 Weeks	9-12 Weeks	13-18 Weeks	> 18 Weeks
Glaucoma Eyes						
Monkey No. 13		+9	-10		-21	
Monkey No. 14	-42	-195*	-126*	-81*	-182*	
Monkey No. 15	-87*	-62*	-133*	-162*	-153*	-87*
Monkey No. 16	-19	-108*	-183*	-212*	-193*	
Monkey No. 17			-110*	-172*		
Transection Eyes						
Monkey No. 18			-15	-41	-2	
Monkey No. 19			-37	-28	-27	

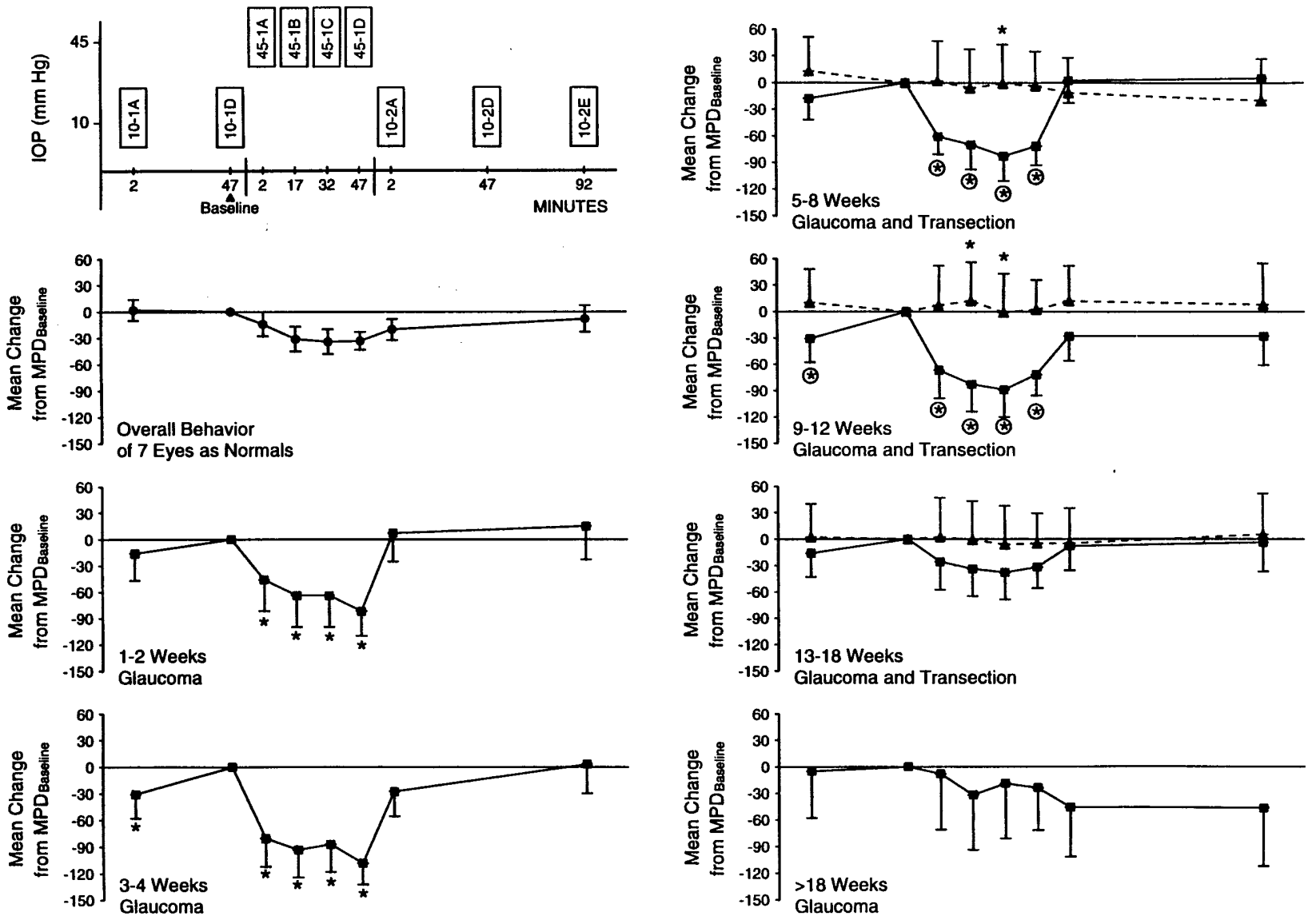
____ Testing interval during which study eye was compliance tested.

*$P < 0.05$, ANOVA, see methods.

FIGURE LEGENDS

Figure 1. Optic disc compliance changes within the longitudinally study eyes. Mean *Change from* $MPD_{Baseline}$ and ANOVA-generated 95% confidence interval at each compliance test time point for glaucomatous eyes (■), transection eyes (▲), and the study eyes as normals (●). An asterisk denotes a compliance test time point at which the mean *Change from* $MPD_{Baseline}$ was significantly different ($P < .05$, ANOVA) from the mean *Change from* $MPD_{Baseline}$ for the study eyes tested as normals at the same time point. A circle around the asterisk denotes time points at which the mean *Change from* $MPD_{Baseline}$ for the glaucomatous eyes was significantly greater ($P < .05$, ANOVA) than the corresponding mean *Change from* $MPD_{Baseline}$ for the transection eyes.

Figure 2. Apparent optic disc rigidity in a glaucomatous study eye. MPD with arithmetic 95% confidence interval at each time point for the glaucomatous study eye of Monkey 1 tested after a total of 13 weeks of exposure to elevated IOP (severe axon loss, data not shown) compared with the contralateral normal eye. While there is a qualitative decrease in the amount of movement of the glaucomatous disc, there is no statistically significant difference, compared with the movement of the disc in the normal eye, by our ANOVA testing (data not shown).



MS # 95179
Burgoyne et al
Figure 1
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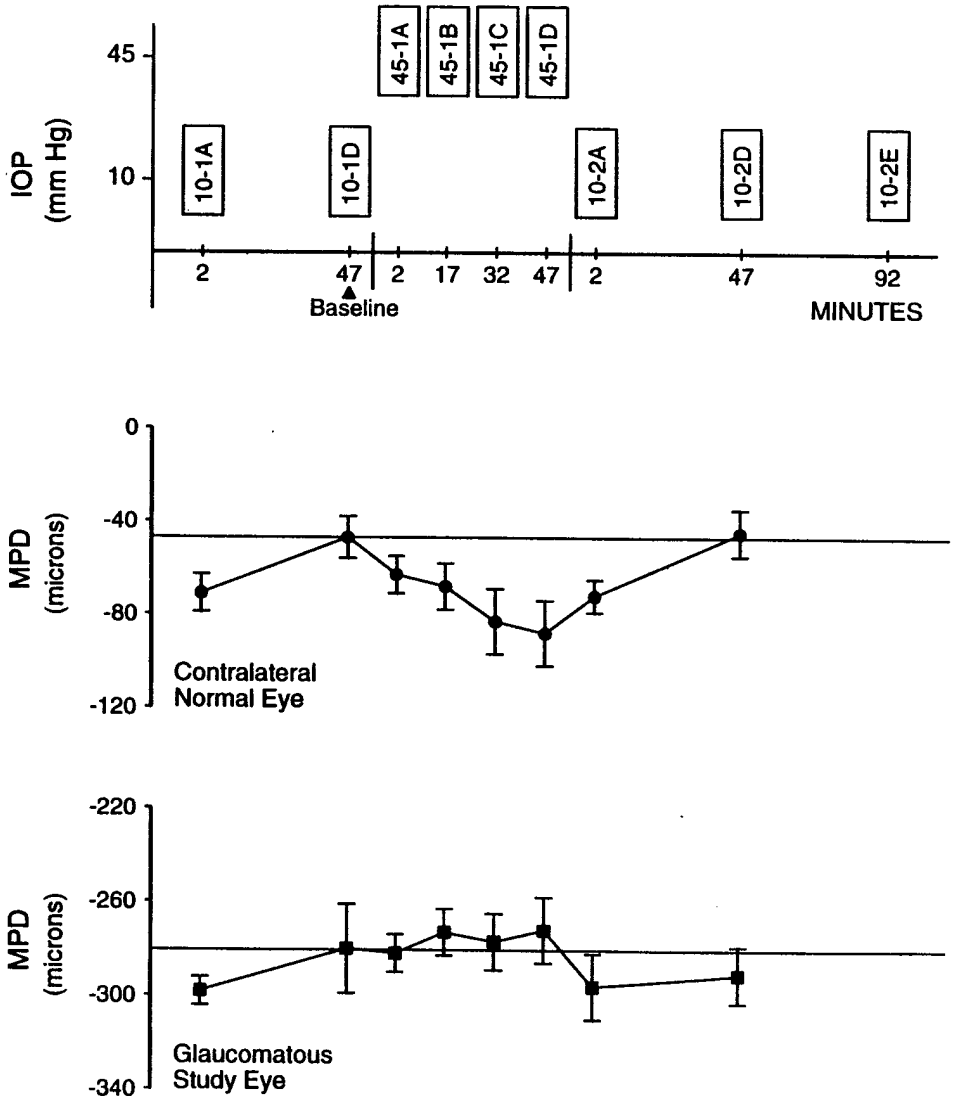

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Figure 2
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Measurement of Optic Disc Compliance by Digitized Image Analysis in the Normal Monkey Eye

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Each author states that s/he has no proprietary interest in the development or marketing of the Topcon Imagenet System, nor do they receive payment as consultants.

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ABSTRACT

Purpose: To characterize the compliance (elasticity) of the normal monkey optic disc under conditions of induced short-term fluctuations in intraocular pressure (IOP).

Methods: In 10 monkeys, one eye was compliance tested on three separate days followed by a single test of the contralateral eye (40 compliance tests). In a testing session, the optic disc was imaged 2 and 47 minutes (baseline time point) after IOP was lowered to 10 mm Hg; then 2, 17, 32, and 47 minutes after IOP was elevated to 45 mm Hg; then 2, 47, and in some cases 92 minutes after IOP was lowered back to 10 mm Hg. Eight digitized images were analyzed at each time point, yielding two parameters to characterize the position of the disc: the *Mean Position of the Disc* (MPD) and the *Change from* $MPD_{Baseline}$ (the value of MPD at a given time point minus the value for MPD at the baseline time point of that testing session). Analysis of variance (ANOVA) testing evaluated the overall effect of IOP on both parameters while taking into account the effects of variability due to different monkeys and repetitions of the test, as well as differences between the two eyes of an individual monkey. With the addition of data from 11 compliance tests performed on eight additional monkeys, the overall results were calculated in terms of the mean *Change from* $MPD_{Baseline}$ at each time point for a total of 51 compliance testing sessions.

Results: The mean *Change from* $MPD_{Baseline}$ was -28 microns (95% confidence interval, -23 to -33 microns) 47 minutes after elevation of IOP. The disc surface returned to its baseline position 92 minutes after IOP was lowered back to 10 mm Hg. Elevation of IOP within a compliance test had a significant effect on the position of the optic disc surface (P=.0002, ANOVA), as characterized by the parameter *Change from* $MPD_{Baseline}$. Neither differences in the amount of movement between the two eyes of an individual monkey nor

variability within the three repetitions of the test in a given eye were statistically significant.

Conclusion: Small, reversible, posterior deformations of the optic disc surface follow acute elevations of IOP in the normal monkey eye. Detection of acute, IOP-induced deformations of the optic disc surface may represent a means to mechanically test the deeper structural tissues of the optic nerve head.

The connective tissues of the lamina cribrosa and peripapillary sclera are presumed to resist the principal forces that act upon the optic nerve head. These load-bearing, or structural tissues may suffer damage early in the pathophysiology of glaucomatous damage to the optic disc. Alteration of these tissues may precede or coincide with early axonal damage in glaucoma.[1,2] Clinical detection of such alterations, as manifested by a change in their mechanical behavior, would allow for intraocular pressure (IOP) lowering interventions aimed at preserving laminar trabeculae before physiologic support to axons was compromised.

As a first step toward detecting damage to the structural tissues of the nerve head, we have developed methods to characterize the position of the optic disc surface by digitized image analysis.[3] In this report, we use the optic disc parameter *Mean Position of the Disc* (MPD)[3] to detect changes in the position of the optic disc surface during acute alterations of IOP in normal monkey eyes. We believe that changes in the position of the surface of the optic disc that follow acute elevation and lowering of IOP are a manifestation of optic disc compliance (elasticity). We propose that measuring the compliance of the optic disc surface is one means of testing the mechanical properties of the deeper, structural tissues of the optic disc.

Our method of compliance testing uses a Topcon Imagenet System[4] for digitized image analysis to characterize optic disc surface position at a series of eight time points during successive periods of low (10 mm Hg), high (45 mm Hg), and low (10 mm Hg) IOP. In this article, we report the results of compliance testing sessions performed in normal monkey eyes prior to the onset of chronic experimental glaucoma. In our companion report,[5] we describe early and late changes in optic disc compliance that follow the onset of

experimental glaucoma.

Materials and Methods

Compliance Testing Sessions

Animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. Eighteen juvenile (exact age unknown), male cynomolgus monkeys *(Macaca fascicularis)* were used. Ten of the 18 monkeys underwent a total of 40 compliance testing sessions, three sessions in one eye on three separate days and one session in the contralateral eye. Eight additional monkeys underwent a total of 11 compliance tests performed only in one eye. Six eyes of six monkeys were compliance tested once, one eye of the seventh monkey was tested twice, and one eye of the eighth monkey was tested three times. While all hypothesis testing was carried out on the data from the 10 monkeys tested in both eyes, we report our overall results in terms of the 51 compliance tests we performed.

The animals were sedated with 7 mg/kg intramuscular ketamine hydrochloride, after which 1% tropicamide, 2.5% phenylephrine hydrochloride, and 2% cyclopentolate hydrochloride were instilled in the eyes to be tested. The animals were then anesthetized with 12 mg/kg intravenous sodium pentobarbital. For each testing session, a rigid, plano contact lens (6.38 mm base curve, 0.24 mm central thickness, 8 mm diameter) was placed on the cornea of one eye. Intraocular pressure was lowered from the normal value of approximately 15 mm Hg to 10 mm Hg by insertion of a 25-gauge needle connected to a saline manometer into the anterior chamber. An estimate of refractive error was made by retinoscopy. Four simultaneous stereoscopic photographs (Topcon Simultaneous Stereoscopic Fundus Camera TRC-SS II, Topcon America Corporation, Paramus, NJ) and eight stereoscopic videoimage-

pairs were obtained (Imagenet Ophthalmic Digital Imaging System, Topcon America Corporation, Paramus, NJ).

The first set of images, obtained 2 minutes after IOP was lowered to 10 mm Hg, was termed *time-point 10-1A* (see schematic diagram of a single compliance test, Fig 1). The next set of images, obtained 45 minutes later, was termed *time point 10-1D*$_{Baseline}$; the images obtained at this time point provided the baseline values with which the values at the other time points were compared.

Intraocular pressure was then raised to 45 mm Hg over 1 minute. An estimate of refractive error was again made by retinoscopy and IOP was checked in the peripheral cornea by Tono-Pen™ (Bausch & Lomb, Tampa, FL). Images were acquired at 2 (*time point 45-1A*), 17 (*time point 45-1B*), 32 (*time point 45-1C*), and 47 (*time point 45-1D*) minutes after elevation of IOP. Finally, IOP was again lowered to 10 mm Hg over 1 minute and images were acquired 2 (*time point 10-2A*) and 47 minutes (*time point 10-2D*) thereafter. In later sessions, an additional time point (92 minutes after IOP was lowered to 10 mm Hg; *time point 10-2E*) was added.

After the complete series of images was acquired, the contact lens was removed, and IOP was measured by Goldmann applanation tonometry. With the manometer apparatus still in place, IOP was raised to 20 mm Hg, and three separate sets of axial length measurements were performed (Canon KU/1 IOL Estimator, Canon USA Inc., Lake Success, NY). Each set consisted of three readings at different axes, with the longest of the three means chosen as the best estimate of axial length. Preliminary experiments had shown no statistically significant change in axial length with acute elevation of IOP (data not shown).

Our analysis is based on the 10-1A, 10-1D$_{Baseline}$, 45-1A, 45-1B, 45-1C, 45-1D,

10-2A, and either the 10-2D or 10-2E time points from each compliance testing session. For the 19 initial sessions in which images were not obtained at time point 10-2E, the final data point used was time point 10-2D. Where both 10-2D and 10-2E images were acquired (32 sessions), only the data from time point 10-2E were used.

Analysis of Images

Topographic mapping of the optic nerve head with the Topcon Imagenet System has previously been described.[4] To analyze a single stereoscopic image-pair, the analyst entered spherical equivalent refractive error, corneal base curve, and axial length, and then attempted to choose the same four points (indicating the disc margin) in the left and right images. From these reference points, image alignment and elevation values for a grid of data points were derived. The average height of the four disc-margin-based reference points established the zero-reference height. All image-pairs of all compliance testing sessions for a given monkey disc were analyzed by a single analyst. Before the image-pairs were analyzed, a photographic record of the four reference points for that disc was consulted. The analyst then attempted to choose these same four reference points in all analyses.

Mean Position of the Disc (MPD)

For each image-pair, elevation data for all disc data points were downloaded from the Topcon Imagenet System to a SAS statistical program (SAS Institute Inc., Cary, NC) running on a UNIX-based work station, and the average value was calculated.[3] For a given disc, MPD ± 95% confidence interval (CI) at a given time point was calculated as the mean and 95%CI of the eight average elevations acquired at that time point. For each compliance test, the

Change from $MPD_{Baseline}$ (in microns) for each time point was calculated by subtracting the value of MPD at time point 10-1$D_{Baseline}$ ($MPD_{Baseline}$) from the value for MPD at each of the other time points. The *Change from* $MPD_{Baseline}$ was considered to be statistically significant for those time points at which MPD ± 95%CI showed no overlap with $MPD_{Baseline}$ ± 95%CI (Fig 1).

Repetitive Imaging for Analysis of Variance (ANOVA) Testing

All hypothesis testing concerning the effect of acute elevation of IOP on the parameters MPD and *Change from* $MPD_{Baseline}$ was performed by ANOVA testing of data derived from a subset of 10 repetitively tested monkeys. In these monkeys, one eye underwent three complete compliance testing sessions on three separate days. The contralateral eye was tested once on another day (Fig 2).

Relationships between the independent variables (differences between monkeys, differences between the two eyes of an individual monkey, differences between successive testing sessions for a single eye of an individual monkey, and intraocular pressure) and the two dependent variables (MPD and *Change from* $MPD_{Baseline}$) were evaluated by separate repeated measures ANOVAs, using the data from the 40 testing sessions described above. The first ANOVA evaluated the first of the three testing sessions from one eye and the single session from the contralateral eye to determine whether statistically significant differences in compliance between monkeys and between the two eyes of an individual monkey could be detected. The second ANOVA compared the three testing sessions for each repetitively tested eye to determine whether statistically significant differences in compliance occurred when the test was repeated on the same eye. Within each ANOVA, both the overall effect of elevated

IOP, as well as the significance and magnitude of the IOP effect at each time point of the test, was determined. Each ANOVA was run twice, once to evaluate the effects of these independent variables on the dependent variable MPD, and the second time to evaluate the effects on the dependent variable *Change from* $MPD_{Baseline}$

Parameters Used to Characterize Optic Disc Compliance

Amount and statistical significance of deformation. The amount and statistical significance of deformation at each time point of an individual compliance testing session was assessed by the parameter *Change from* $MPD_{Baseline}$ (Fig 1). The amount and statistical significance of the overall IOP effect on the parameter *Change from* $MPD_{Baseline}$ was assessed by ANOVA testing as outlined above.

To depict the overall trend in the data for all compliance testing sessions, the mean *Change from* $MPD_{Baseline}$ with its 95%CI at each time point was calculated using the *Change from* $MPD_{Baseline}$ data from all 51 compliance testing sessions. For depiction of overall trends, we did not arbitrarily select one compliance test from each monkey (18 compliance testing sessions, 18 monkeys) for two reasons. First, all hypothesis testing regarding the effect of IOP elevation was separately performed within an ANOVA that took into account the effect of variabilities due to the two eyes of a monkey, as well as multiple repetitions of the test within one eye (and the effect of IOP was still found to be statistically significant). Second, selection of one test per monkey would, itself, have required statistical justification that the selected test adequately represented the monkey's other tests.

Reversibility of deformation. We defined reversibility of deformation or resilience to be the ability of the optic disc surface to return to its baseline position (time point

10-1D$_{Baseline}$) after IOP was lowered back to 10 mm Hg (time points 10-2A and 10-2D or E). We thus assessed reversibility only for the compliance testing sessions in which we detected a statistically significant change in MPD at time points 45-1A, B, C, or D.

Additional Analyses

Evaluation of conventional parameters. For each image-pair, the following conventional optic disc parameters were obtained from Topcon Imagenet software as previously described[3,4]: disc area, cup/disc ratio-vertical, cup/disc ratio-horizontal, cup volume, and rim area/disc area ratio. At each time point the mean ± 95%CI was calculated for each conventional parameter based on the values from the eight acquired image-pairs. The change from baseline was calculated for each conventional parameter by subtracting the value for that parameter at time point 10-1D$_{Baseline}$ from the value for the parameter at each of the other time points. The change from baseline was considered to be significant for a given parameter when the mean ± 95%CI for that parameter at that time point showed no overlap with the mean ± 95%CI for that parameter at baseline.

Results

Amount, Reproducibility and Statistical Significance of Deformation

The overall mean *Change from MPD$_{Baseline}$* for all 51 compliance testing sessions was -11 microns (95%CI, -6 to -15 microns) 2 minutes after elevation of IOP to 45 mm Hg and increased to a maximum of -28 microns (95%CI, -23 to -33 microns) 45 minutes later (Fig 3; MPD). As characterized by MPD, the disc had returned to a position indistinguishable from the baseline position at both 47 minutes (95% CI, -9 to +3 microns) and at 92 minutes

(95%CI, -10 to +4 microns) after IOP was lowered back to 10 mm Hg. These values, which were derived from all 51 testing sessions, are qualitatively similar to the mean *Change from* $MPD_{Baseline}$ values for a subset of 18 testing sessions, one randomly selected session from each monkey: -12 microns (95%CI, -6 to -17 microns) 2 minutes after IOP elevation; -28 microns (95%CI, -22 to -35 microns) 45 minutes later; and -1 micron (95%CI, -10 to +8 microns) 92 minutes after IOP was lowered back to 10 mm Hg.

We detected a statistically significant posterior *Change from* $MPD_{Baseline}$ in 15 of the 51 sessions 2 minutes after IOP was elevated to 45 mm Hg and in 39 of the 51 sessions after 47 minutes of exposure to an IOP of 45 mm Hg. Overall, in 17 of the 18 monkeys and 47 of the 51 compliance testing sessions, a statistically significant posterior change in MPD occurred in at least one compliance test time point at elevated IOP (45-1A, B, C, or D).

Overall, the effect of IOP was significant for both MPD ($Pr > F = .0001$) and *Change from* $MPD_{Baseline}$ ($Pr > F = .0001$) by ANOVA testing, even when the variability due to differences between monkeys and between the two eyes of a monkey (first ANOVA) and the variability due to repetitions of the test (second ANOVA) were taken into account. No significant effect on MPD was detected at the time point (10-1A) for which IOP remained at 10 mm Hg by either the first ANOVA ($Pr >F = .5413$) or the second ANOVA ($Pr > F = .6314$), as determined by a sub-analysis performed within each ANOVA to evaluate the significance and magnitude of the effect of IOP on the parameter MPD at each time point. However, the effect of IOP was significant at all time points following elevation of IOP by both ANOVAs. As estimated by the first ANOVA, the effect of IOP at time point 45-1A was -9.6 microns ($Pr > F =.0226$), increasing to -25.8 microns ($Pr > F = .0003$) at time point 45-1D. By the second ANOVA, the corresponding values were -12.2 microns (time point

45-1A; Pr > F = .0004) and -29.8 microns (time point 45-1D; Pr > F = .0001).

Finally, when we considered the mechanical behavior of these discs, as characterized by the parameter *Change from* $MPD_{Baseline}$, only the variability due to inherent differences in mechanical behavior between monkeys was significant (Pr > F = .0001, both ANOVAs). Neither the variability due to differences between the two eyes of a monkey (Pr > F = .2940, first ANOVA) nor the variability due to repetitions of the test in a given eye (Pr > F = .4246, second ANOVA) was significant. Qualitatively, the maximum *Change from* $MPD_{Baseline}$ was similar for the three compliance tests in the majority of the repetitively tested eyes (Table 1).

Reversibility of Deformation

The mean *Change from* $MPD_{Baseline}$ returned to zero at both 47 minutes and 92 minutes following return of the IOP to 10 mm Hg (Fig 3). However, in seven eyes, the disc remained posterior to its baseline position at these time points (10-2D or 10-2E, Table 1) in at least one test. Six of the seven eyes had been tested three times. In these six eyes, the nonreversibility occurred in a variable manner (Table 1); incomplete return to baseline position occurred in all three compliance tests in two eyes, in two of three tests in one eye, and in one of three tests in the remaining three eyes.

Conventional Parameters

The behavior of the conventional optic disc parameters *cup/disc ratio-vertical*, *cup/disc ratio-horizontal*, and *cup volume* also indicated a reversible posterior deformation of the optic disc surface following IOP elevation and reduction (Fig 3). The parameters *rim area/disc area ratio* and *disc area* displayed no significant change from baseline (Fig 3).

Discussion

We have previously shown the image-analysis based parameter *MPD* to be more sensitive than conventional optic disc parameters[3] and clinicians[6] in the detection of acute, IOP-induced changes in the surface position of the monkey optic disc. In this report, we used MPD to detect alterations in the position of the optic disc surface through a series of time points at low, high, and then low IOP. Our results suggest that the normal monkey optic disc is a compliant structure, and exhibits reversible, posterior deformation in response to acute elevation of IOP. Our findings support clinical reports of optic disc change following IOP lowering in humans[7-12] and suggest that anterior movement of the disc tissue may be a principal mechanism underlying this change.

We believe that the IOP-induced alterations of the optic disc surface we measured are a manifestation of the response of the lamina cribrosa to IOP-induced force. Posterior bowing of the laminar beams following acute elevation of IOP has been described in published reports[13,14] and in a presentation at the 1994 ARVO annual meeting (Albon J, May 3, 1994, Sarasota, Florida, unpublished data). It is possible, however, that the posterior deformation we measured at the optic disc surface was due to compression of the prelaminar tissues rather than posterior bowing at the level of the lamina. While our data do not exclude compression of the prelaminar tissues, several points suggest that it would be insignificant.

First, while compression of the major optic disc vessels may have accounted for a portion of the movement, individual data point analysis of the elevation data in a subset of these eyes revealed movement in regions of the disc that did not contain major blood vessels.[3] Second, while compression of the prelaminar capillaries may have occurred, no obvious optic disc pallor was detected in stereophotographs obtained in the same subset of eyes 47 minutes

after IOP had been elevated to 45 mm Hg.[6] In addition, filling of prelaminar capillaries has previously been shown to be normal in experimental models with IOP elevations to 45 mm Hg.[15,16] Third, while a decrease in the prelaminar extracellular fluid may have occurred, theoretical calculations suggest that the potential volume of such a decrease could not account for the shifts in surface position that we report. While there may be more extracellular space in vivo, the extracellular space in optic nerve heads prepared for electron microscopy occupies less than 1% of the tissue.[17] If the prelaminar tissues are modeled as a cylinder with a radius of 1 mm and a height of 500 microns, a 1% volume loss would manifest as a 5-micron posterior shift in the surface of the disc. This is considerably less than the 28-micron movement that we report.

The compliance of the normal optic disc has been studied by a series of investigators.[13,14,18,19] Levy et al[13] placed fine platinum wires strung in the peripapillary sclera and optic disc in nonhuman primate autopsy eyes and observed their movement before and after acute elevations of IOP with x-ray photography. They reported 22 microns of retrodisplacement of nerve head tissues relative to the peripapillary sclera after a 6-minute elevation of IOP from 10 mm Hg to 40 mm Hg. There was a progressive increase in this deformation as IOP was raised to 70 mm Hg. Yan and co-workers[14] fixed 10 pairs of normal human cadaver eyes at 5 and 50 mm Hg and measured posterior deformation of the lamina without a change in its thickness in stained histologic sections. In a study that used laser Doppler velocimetry to measure movement of the disc tissues in normal human autopsy eyes, Zeimer and Ogura[18] estimated that 23 microns of posterior deformation occurred following momentary elevations of IOP from 12 to 72 mm Hg. Coleman et al[19] used a Humphrey Retinal Analyzer to detect an overall mean posterior deformation of 17.8 ± 21.1 microns for

12 normal monkey optic discs following acute elevation of IOP to 45 mm Hg. Recently, Quigley and Pease used a Glaucoma-Scope to evaluate changes in the parameter MPD in seven eyes of seven monkeys following 45-minute elevations of IOP from 10 to 45 mm Hg; their findings (mean change 47 microns, range 20-60 microns) were similar to the changes detected in this report (personal communication; Harry A. Quigley, MD, Baltimore, Maryland).

Within the 35 mm Hg increase in IOP used in our study, the optic disc surface behaved reversibly, displaying progressive posterior deformation over the 45-minute period of elevated IOP and a return to baseline position with lowered IOP. The exact distribution of force within the load-bearing tissues of the optic nerve head and, therefore, the changes in that distribution following acute IOP elevation are not known. Because Yan and co-authors[14] detected more posterior deformation of the lamina peripherally (compared with the central region of the disc) in fixed human cadaver eyes, they modelled the optic disc as a flat rigid plate supported at its periphery, and suggested shear forces to be the principal forces present within the peripheral laminar beams.

Unlike the model of a fixed plate in which shear stresses predominate, posterior deformation of the disc surface following IOP elevation may be principally resisted by tensile stress within the laminar beams and scleral anchoring ring. In this model (similar to a trampoline), intraocular pressure directly influences the posteriorly directed force at the disc surface, and indirectly influences tensile stress within the laminar beams through the relationship of IOP to scleral wall tension[20]:

$$s = (p_i - p_e)R/2h$$

where: s = stress (force / cross-sectional area of tissue resisting that force)

$(p_i - p_e)$ = difference between internal and external pressure

R = radius of sphere

h = thickness of the sclera (where this thickness is assumed to be small relative to R).

The connective tissue constituents and architecture of the lamina cribrosa and peripapillary sclera are similar to those of other biological tissues that resist force. Compliance testing of skin and aorta[21,22] has demonstrated that both collagen and elastin resist tensile forces applied along their principal axes. Within the laminar beams, fibrils of collagen types 1 and 3, as well as elastin, are embedded within a dense proteoglycan matrix, with their long axes parallel to the plane of the sclera (longitudinally within a beam).[23-29] At the insertion of the peripheral laminar beams into the peripapillary sclera, elastin fibers extend and insert into a circle of elastin that rings the scleral canal.[30-32]

Regardless of whether tensile or shear stresses predominate, we propose that the amount of deformation that occurs during a compliance test and its reversibility are separate indications of the strength and integrity of the connective tissues of the lamina cribrosa and peripapillary sclera. Our results confirm the feasibility of studying such variables in a research setting and establish baseline values for the normal monkey eye. Because there was no significant difference in the compliance of the two eyes of an individual monkey by ANOVA testing, our results support the use of the contralateral eye as a control for studies of optic disc compliance in which one eye has received a laser-induced, chronic elevation of IOP (experimental glaucoma).[5]

Interestingly, for the 51 compliance testing sessions considered as a group, there appeared to be no significant increase or decrease in the diameter of the anterior scleral

anchoring ring during the 45-minute period of elevated IOP as measured by the optic disc parameter *disc area* (Fig 3). The fact that disc area did not change while other parameters did is of interest for two reasons. First, it argues against a nonmechanical explanation for the changes in the parameters we report. For example, posterior displacement of the lens by our IOP-elevating canula system would have caused magnification changes at high IOP that would have affected all parameters including disc area. The absence of statistically significant change in optic disc area serves as an internal control for artifacts related to changes in lens position or length of the eye. Second, it implies that the extracellular matrix of the anterior scleral ring capably prevents a measurable enlargement of the anterior-most scleral canal within the conditions of our test. Our data, however, offer no information on the behavior of the deeper scleral canal, where the peripheral laminar beams insert into the peripapillary sclera. Future studies will attempt to detect a measurable increase in the size of the posterior scleral canal during similar compliance testing conditions.

A potential limitation of our study is the fact that IOP-induced movement of the optic disc surface was determined relative to a zero-reference plane situated at the disc margin. This reference plane itself may have moved during the period of elevated IOP. Our study thus estimates only movement of the disc surface relative to the disc margin and does not address the behavior of the peripapillary retina. Future studies will employ a retina-based zero-reference plane[33] and will include elevation values from the peripapillary retina in our analyses.

Finally, our study design assesses only global deformation of the disc surface at a pressure of 45 mm Hg over a 47-minute period. As such, our protocol evaluates certain, but not all aspects of mechanical behavior. We designed our study to incorporate a change in IOP

that approximates the upper end of intraocular pressure elevations seen clinically in human glaucoma patients. We avoided elevating the IOP beyond 45 mm Hg in case such elevations would, themselves, damage the structural tissues of the disc.

Despite this caution, it is possible that the 47-minute period of IOP elevated to 45 mm Hg damaged the structural tissues of some discs. Within the group of 10 eyes tested three times, the repetitions of the test were not a significant source of the variability in the parameter *Change from* $MPD_{Baseline}$. Qualitatively, the maximum *Change from* $MPD_{Baseline}$ was similar for the three compliance tests in the majority of the repetitively tested eyes (Table 1). However, in two eyes (monkey 15 and monkey 16, Table 1), a qualitative increase in compliance occurred in the third compliance testing session. In both eyes, this was accompanied by a progressive loss of resilience through the three testing sessions, as demonstrated by an inability to return to baseline position during the second period of low IOP within a test (Table 1). While these findings may be due to variability in our method, they suggest that a minority of monkey optic discs may manifest alterations to their structural tissues from periods of IOP elevation to 45 mm Hg of as little as 47 minutes.

Our study employed a rigorous experimental protocol and an enhanced system for digitized image acquisition and statistical analysis to confirm previous characterizations of optic disc compliance. The strength of our study lies in the rigor of the compliance testing sessions; the large numbers of monkeys, eyes, and compliance tests; and a study design that allowed for analysis of variance testing to estimate the principal causes of variability in our MPD characterizations of the position of the optic disc. Our study not only confirms that the disc is compliant, but suggests that optic disc compliance measurements, as a form of mechanical testing, are reproducible for a given eye, similar within the two eyes of an

individual monkey, and capable of detecting differences in the amount of compliance demonstrated by different monkey optic discs. In the article that follows,[5] we use this technique to describe early and late changes in the compliance of the monkey optic disc following the onset of experimental glaucoma.

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Table 1. Reproducibility of Maximum Change from $MPD_{Baseline}$ and Return to $MPD_{Baseline}$ (Reversibility) in the Ten Repetitively Tested Eyes

			Change from $MPD_{Baseline}$ (microns)			
Monkey No.	Eye	Testing Session	Maximum Value during Elevated IOP	Mean ± 95%CI	Value 47 or 92 Minutes after IOP Lowered to 10 mm Hg	Mean ± 95%CI
4	OD	1	-37*		-10	
		2	-34*	-34.7 ± 4.7	+5	+0.7 ± 21.0
		3	-33*		+7	
11	OD	1	-16*		-8 †	
		2	-14*	-16.3 ± 5.7	-15 †	-14.3 ± 13.6
		3	-19*		-20 †	
12	OD	1	-39*		-23	
		2	-30*	-33.3 ± 11.2	+18	-6.7 ± 49.2
		3	-31*		-15	
13	OD	1	-31*		+9	
		2	-31*	-36.0 ± 19.6	-11	-6.3 ± 30.8
		3	-46*		-17 †	
14	OD	1	-12*		-6	
		2	-48*	-31.3 ± 41.1	-50 †	-20.7 ± 57.5
		3	-34*		-6	
15	OD	1	-46*		-27 †	
		2	-32*	-49.0 ± 42.3	-18 †	-25.7 ± 16.1
		3	-69*		-32 †	

Table 1 continues....

Table 1. continued.

			Change from $MPD_{Baseline}$ (microns)			
Monkey No.	Eye	Testing Session	Maximum Value during Elevated IOP	Mean ± 95%CI	Value 47 or 92 Minutes after IOP Lowered to 10 mm Hg	Mean ± 95%CI
16	OD	1	-18*		+17 ‡	
		2	-34*	-38.0 ± 50.4	-15 †	-14.3 ± 70.2
		3	-62*		-45 †	
17	OS	1	-88*		+26 ‡	
		2	-34*	-50.3 ± 74.0	+20	+20.0 ± 13.6
		3	-29*		+14	
18	OD	1	-41*		-13 †	
		2	-26*	-40.7 ± 32.8	+1	-11.0 ± 25.2
		3	-55*		-21	
19	OD	1	-33*		+13	
		2	-45*	-40.7 ± 15.1	+13	+4.3 ± 34.0
		3	-44*		-13	

*Statistically significant posterior deformation compared with baseline time point of compliance test (see methods).

†Statistically significant posterior deformation persisting at time point 10-2D (47 minutes) or 10-2E (92 minutes).

‡Statistically significant anterior displacement at time point 10-2D (47 minutes) or 10-2E (92 minutes).

FIGURE LEGENDS

Figure 1. Representative data for a single compliance test.

Top: Schematic diagram of the eight time points of an individual compliance testing session. (Images were acquired at either the 10-2D or 10-2E time point for a given compliance testing session.) Eight digitized image-pairs were acquired and analyzed at each time point to characterize the global position of the optic disc surface (MPD ± 95%CI) (see Materials and Methods, Compliance Testing Sessions).

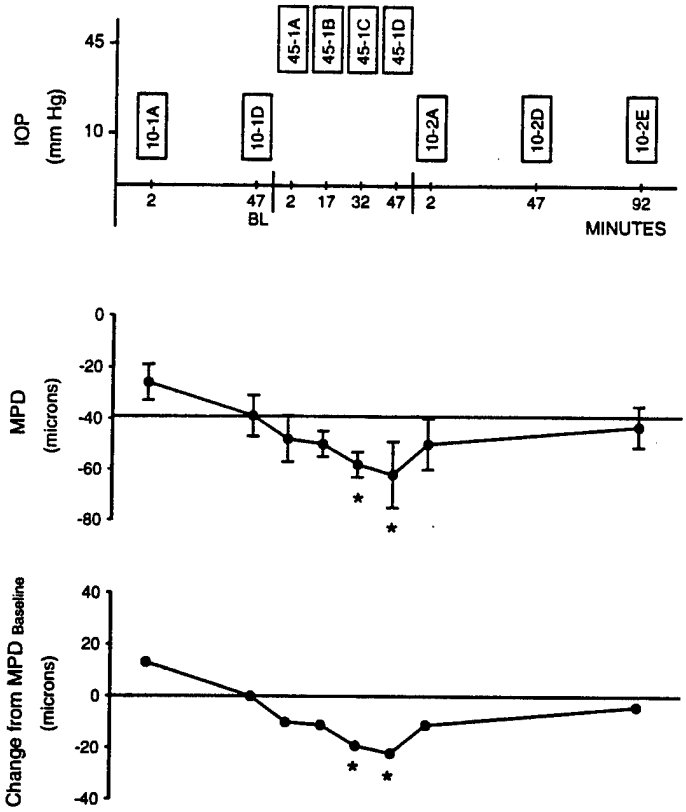
Middle: Raw MPD ± 95%CI data for a representative compliance testing session (Monkey No. 8).

Bottom: Same data plotted as *Change from* $MPD_{Baseline}$. By convention, time point 10-1D was considered to be the baseline time point. *Change from* $MPD_{Baseline}$ was calculated by subtracting $MPD_{10\text{-}1D}$ ($MPD_{Baseline}$) from the MPD at each of the other time points.

* Time points at which change in MPD met our criteria for statistically significant change (MPD ± 95%CI at that time point shows no overlap with $MPD_{Baseline}$ ± 95%CI).

Figure 2. **Raw MPD ± 95%CI data for the subset of 10 monkeys that underwent repetitive testing in one eye and a single session in the contralateral control eye.** (●———● first testing session) (▲·····▲ second testing session) (■ · – · ■ third testing session).

Figure 3. **Mean Change from Baseline for 51 compliance testing sessions by time point and optic disc parameter.** The means ± 95%CI for the 51 Change from Baseline values at each time point were calculated for MPD and each conventional parameter.



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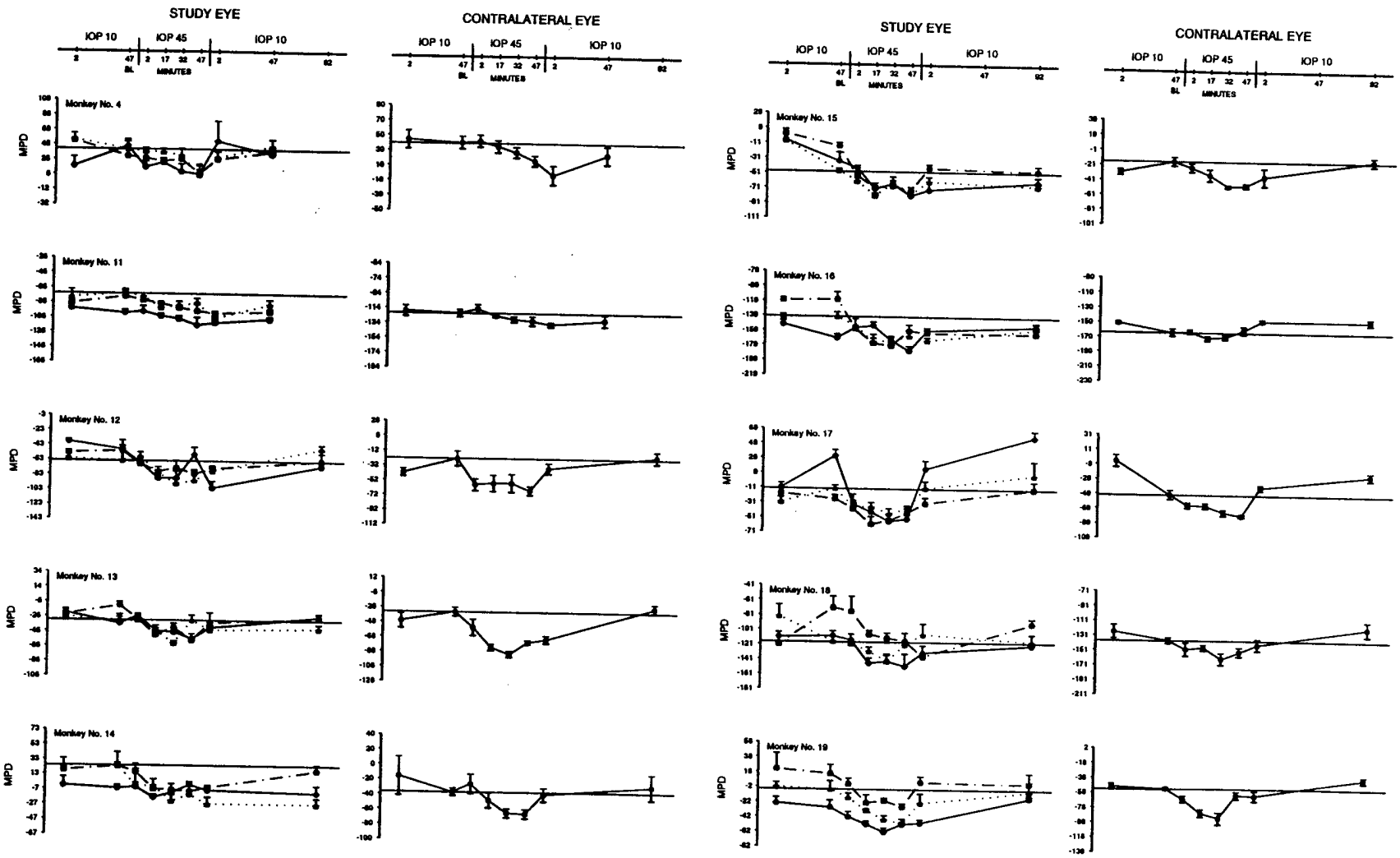

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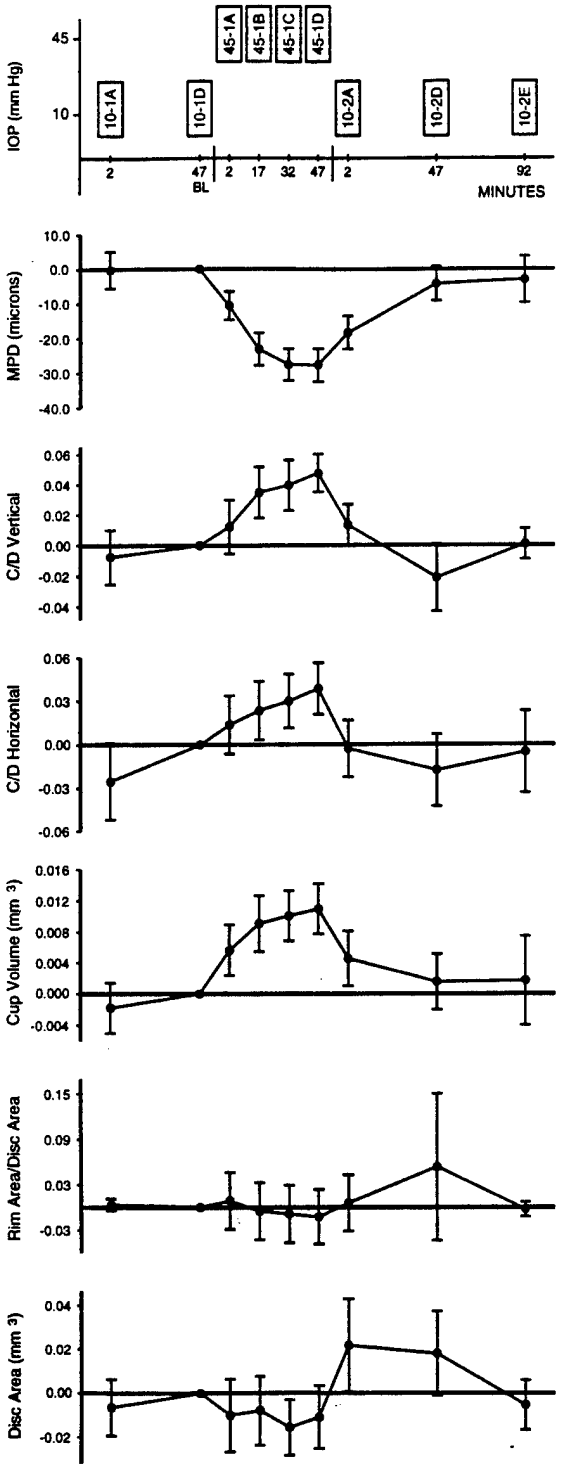

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