Comparison of a Noninvasive Measurement of Optic Nervehead Mechanical Compliance With an Invasive Method

Ron C. Zeimer and Ke Chen

We have postulated that abnormal mechanical support of the optic nervehead at the level of the lamina cribrosa could be the precursor of glaucomatous damage. Recent studies have shown deformations of the lamina cribrosa to be among the earliest changes in glaucoma. To evaluate the support of the nervehead, we have developed a noninvasive optical method to measure the optic nervehead compliance, namely, the displacement of the optic nervehead induced by a range of intraocular pressure. To test the validity of the method, we have compared noninvasive measurements obtained in post-mortem enucleated human eyes with those recorded using an invasive technique. Both methods had a reproducibility better than 6 μm and induced no damage capable of interfering with the results. The displacements measured by both methods were similar, thus indicating that our optical method is capable of measuring bulk motion of the optic nervehead. Our results were identical with those obtained by other authors using a third method. The data obtained also established the normal range of optic nervehead displacements induced by a range of intraocular pressure increments. Invest Ophthalmol Vis Sci 28:1735-1739, 1987

Histologic studies of post-mortem human eyes with glaucoma have shown that damage to the axonal fibers is located at the lamina cribrosa. 1 Maumenee suggested in 1973 that the damage may be caused by a misalignment of holes within the lamellar layers through which the bundles of axons pass. 1 The important role played by the lamina cribrosa was further documented by electron microscopic studies that showed the following: (1) The normal lamina cribrosa is less dense at the superior and inferior poles, suggesting a sensitivity compatible with the location of arcuate scotomas; (2) at an early stage of glaucoma, when the visual field (by Goldmann perimetry) is still intact, the lamellar sheets are abnormally compressed and little or no posterior bowing can be documented; and (3) significant bowing and distortion of the lamina cribrosa are present in later stages of glaucoma. 2

The above findings, especially those related to early stages of glaucoma, support our 1980 hypothesis. 2 We suggested that an altered elastic strength of the lamina cribrosa caused the susceptibility to nerve fiber damage in eyes that develop glaucoma. The findings of Quigley and associates 5-8 may be interpreted to indicate either an increased weakness or an increased stiffness of the lamina cribrosa: if the lamellar sheets are weak, they would become stressed, ultimately reaching their nonelastic limit. If this limit is surpassed, permanent deformation would result, expressed as the observed compression of the anterior sheets that increases with the severity and duration of the process. The reversible bowing present in the elastic mode of lamellar stress would not be documented histologically, because the tissues are fixed at zero intraocular pressure (IOP). On the other hand, if the lamellar sheets are too stiff, they cannot stretch and accommodate the strain produced by the pressure gradient. Consequently, they would tear and collapse. This would be observed as a collapse of the sheets.

The potential role that the elastic compliance of the lamina cribrosa may play in the pathogenesis of glaucomatous damage has prompted us to develop a method to measure the compliance noninvasively. 10,11 We compare herein, in post-mortem human eyes, the results from our noninvasive method with those of an invasive technique that measures elastic compliance directly. Levy and co-workers have developed an invasive method, 12 but we chose to design a new procedure that may be less susceptible to artifacts and would allow an independent comparison with their results. Finally, the values obtained in normal human eyes should provide a baseline to which glaucomatous eyes can be compared.

Materials and Methods. Preparation of the globe: Human eyes were obtained from the Illinois Eye...
Bank and were refrigerated on wet gauze in a sealed container until the experiment. The exterior tissues such as muscles and fat were removed, the nasal orientation was marked with a suture, and the globe was bisected coronally along the equator. After the vitreous was removed, the retina was cut carefully around the optic nervehead, leaving a rim of retina that is \( \frac{1}{2} \) to 1 disc diameter in width. The remaining retina was removed, because it was detached and folded above the optic nervehead, precluding a view of the nervehead and preventing laser Doppler velocimetry.

The prepared tissue was placed in a specially designed holder (Figure 1), which supported the half-globe at its rim and clamped it in place by a flange with an O-ring. The cavity behind the globe was filled with saline solution, and the air was vented out. The holder was connected to a reservoir and a pressure gauge. By lowering the reservoir below the level of the cavity a pressure gradient was applied to the half-globe, causing stresses similar to those produced by a positive IOP in an intact eye. A baseline under pressure of —10 mm Hg was maintained during the experiment. The surface of the half-globe that was exposed to air was wetted periodically to keep it moist at all times.

**Noninvasive measurement of elastic compliance by laser Doppler velocimetry:** The method we used to measure elastic compliance has been described in detail previously. Briefly, a momentary pressure gradient is applied to the globe, and the motion of the tissue is measured by laser Doppler velocimetry. This method involves splitting a laser beam into two: one beam is sent to the tissue (optic nervehead or retina) and the other to a reference on the globe holder. The radiation emitted by the moving tissue and that released by the reference area are collected and mixed on the cathode of a photomultiplier. The motion of the tissue causes the emitted light to shift in frequency (Doppler shift) by an amount proportional to the velocity. The shift is detected by the photomultiplier and recorded on magnetic tape along with the pressure pulse.

The data were processed by a waveform analyzer (Data Precision #6000; Danvers, MA), which was controlled by a microcomputer (IBM PC; Boca Raton, FL). Segments of 5.1 msec of the Doppler signal were analyzed by Fast Fourier Transform. The frequency of the peak was plotted as a function of time and translated into velocity units. The total displacement was obtained by integrating the velocity over time. The cavity behind the half-globe was connected to a pressure pulse generator, which has been described elsewhere. Pressure pulses of 150 msec in duration were generated with amplitudes of —35, —50, and —65 mm Hg below the baseline under pressure of —10 mm Hg.

The laser beam, which had a 100 µm focal diameter, was placed at the center of the disc and a measurement was performed. The beam was then moved to a location on the nasal sclera, \( \frac{1}{2} \) disc diameter from the rim of the disc, and a second measurement was performed. The amplitude of the pressure pulse was then changed, and the procedure was repeated.

The reproducibility of the measurement was assessed by measuring the standard deviation of the measurements at the same pressure amplitude. To assess possible eye damage caused by the measurement, we calculated the reversibility defined as the difference between the last and first readings at the —35 mm Hg amplitude after each cycle of —50 and —65 mm Hg.

**Invasive measurement of elastic compliance by monitoring implanted probes:** Our noninvasive method relies on radiation scatter by the tissue. To evaluate if the information is related to bulk tissue motion, and therefore directly related to the compliance of the lamina cribrosa, we need a method that directly measures displacement of the bulk tissue and not merely its surface. Moreover, the noninvasive method is dynamic and it would thus be instructive to determine if the tissue responds fast enough to follow the change in pressure; therefore the independent method must measure displacements at equilibrium.

Levy and associates have measured tissue displacement by introducing a platinum wire along the sclera and across the optic nerve and subsequently monitoring its deformation using X-ray radiography. This method is ingenious but may suffer from artifacts in that the platinum wire may actually provide reinforcement to the tissue and limit its displacement. Moreover, the method requires many steps (radiography, film placement, displacement measurements on negatives), which makes it cumbersome to perform and susceptible to errors. For these reasons,
and to obtain a method capable of yielding independent results, we developed a new procedure. Perpendicularly placed steel probes were implanted at the center of the nervehead and in the adjacent sclera and then visually monitored to assess their displacement as a function of pressure. Specifically, a sharpened steel probe, 75 μm in diameter and 700 μm in length, was affixed to a steel rod 400 μm in diameter. The glue used to connect the rod and the probe was shaped into a flange, which served as a stop when the probe was introduced into the tissue. This probe was used for the center of the optic nervehead. A stronger probe than this was needed to penetrate the nasal sclera, so one was fashioned with a 200 μm diameter. Both locations were identical to those used for the laser Doppler velocimetry measurement. As shown in Figure 1, the probes were implanted vertically into the tissue, one at the center of the disc and the other ½ to 1 disc diameter from the rim. The probes were held vertically by two openings in a lucite plate placed on the globe holder. The globe holder was attached to the table of a microscope (Olympus BH-2; Tokyo, Japan). The top of the probe was imaged with X40 magnification, and a sharp detail was chosen as a target on which to focus. The pressure was varied by changing the height of the saline reservoir and monitoring the transducer. Each time the pressure was changed, the probe was brought into focus by adjusting the table level, and a reading was taken from the micrometric dial. The readings were repeated three times. The microscope was defocused between each reading and the dial was masked from the operator. At each pressure the procedure was performed for the two probes and the relative displacement of the optic nervehead was obtained by subtracting the means of the results of the two probes. The pressure was changed from the baseline level of —10 mm Hg to —30, —50, and -70 mm Hg and then back again. This cycle was repeated three times.

To determine if the method caused damage to the globe, the reversibility was evaluated by comparing the micrometer readings each time the baseline pressure was reached. The reproducibility was evaluated by calculating the standard deviation of the readings at the same pressure.

**Histologic preparation:** The half-globe was kept in the holder in a configuration similar to that during the measurement, and saline was replaced with Trump’s fixative. Some fixative also was maintained inside the globe. Two or more hours later, the globe was removed and refrigerated in Trump’s solution for at least 12 hr before processing.

After dehydration, the tissue was embedded in a paraffin block in a well-defined orientation, using a specially designed procedure. A cubical mold was constructed with a microtome holder on one side. Liquid paraffin was poured in the mold, and the tissue was placed in it, with its rim flush with the bottom and its nasal side facing away from the microtome holder. The preparation was allowed to cool and harden. The mold was then removed, leaving the tissue embedded in a cube attached to the microtome holder. Serial sections, 5 μm thick, were made. Every other section was stained with hematoxylin and eosin.

**Results.** Noninvasive measurement of elastic compliance by laser Doppler velocimetry: The measurement was performed on 13 eyes from 12 different donors, 25 to 76 years of age (mean, 56 years). Nine were men and three were women. The measurements were made between 10 to 400 hr after death (mean, 105 hr). The reproducibility of the optic nervehead displacement was ±4, ±6, and ±5 μm for changes in IOP of 30, 45, and 65 mm Hg, respectively. This corresponded to respective coefficients of variation of 17%, 21%, and 15%.

The results of the optic nervehead displacement as a function of IOP are summarized in Figure 2. The bars represent the standard error, or the error in the estimate of the mean.

The reversibility was ±0.8 μm, namely, the last measurement of optic nervehead displacement differed from the first one by this value. This amounted to 2% of the displacement at the highest pressure increment.

A correlation with age was tested at the three pressures and was found to be nil (r < 0.07).
Invasive measurement of elastic compliance by monitoring implanted probes and its comparison with the noninvasive measurement: Measurements were performed on five eyes that were used for laser Doppler velocimetry. All five came from different donors, 36 to 76 years old (mean, 60 years), of whom three were men and two were women. The measurements were made between 6 and 120 hr after death (mean, 47 hours).

The reproducibility of the optic nervehead displacement was ±3.5, ±4.0, and ±5.6 μm for IOP pressures of 30, 50, and 70 mm Hg, respectively. These values corresponded to coefficients of variation of 16%, 12%, and 13%. The reversibility was ±1.1 μm.

The results of the optic nervehead displacement obtained by this method are presented in Figure 3 with the results of laser Doppler velocimetry performed on these same five eyes. The bars represent standard errors of the mean. The curve has been extended to zero change in pressure (baseline IOP, −10 mm Hg), since measurements were made at this point. The laser Doppler data can only be extrapolated below 30 mm Hg, because no data were obtained below this value. The two methods yielded results that were similar to within 5% at low pressure increments (30 mm Hg). At 65 mm Hg, the laser Doppler velocimetry yielded values 20% lower than those of the invasive measurements at 60 mm Hg. A pairwise t-test showed, however, that the difference, even at that pressure, was not statistically significant ($P > 0.26$).

Assessment of possible artifacts: There was no significant correlation between the displacement measurements obtained at all pressure ranges and the time interval after death ($r < 0.3$).

To assess the potential damage by the probe, we examined by light microscopy several sections obtained from the center portion of the optic nervehead. The prelaminar tissue, the lamina cribrosa, and the retrolaminar tissue were evaluated for disruptions caused by the probe. In some eyes the structure of the prelaminar nerve tissue was disturbed. We could not determine if this had been caused by the dissection or by the probe. However, none of the eyes showed any damage at or posterior to the level of the lamina cribrosa.

Discussion. The main purpose of this study was to compare, in the same eye, measurements of optic nervehead mechanical compliance obtained by two methods, one invasive and one noninvasive. We first evaluated the methods themselves. The reproducibility of the optic nervehead displacement relative to the sclera was satisfactory for both: between 3.5 and 6.0 μm or 12% to 21% of the displacement. It was encouraging that the reproducibility of the noninvasive method was similar to that of the invasive one.

Therefore, we could be confident that the two methods we used were adequate. The tests performed to evaluate gross damage caused by the procedures showed no effect on compliance: the elastic range was not exceeded, as demonstrated by the reversibility of the two methods. In addition, the histologic examination disclosed no gross damage in the lamellar sheets in the eyes that were implanted with steel needles. Finally, the lack of correlation between the results and the elapsed time between death and the measurements demonstrated that time was not a significant factor. The protocol for handling the tissue thus seemed appropriate for reliable measurements of elastic compliance.

A comparison of the two methods showed that they yielded similar results for IOP increments up to 60 mm Hg, which is very encouraging for future use of the noninvasive method: It indicated that the use of backscattered light and a dynamic pressure measurement do not prevent the calculation of bulk motion of the optic nervehead. This ability to determine bulk motion was probably due to the fact that red light sufficiently penetrates the optic nervehead tissue. The ability to obtain, by dynamic pressure increments, results similar to those obtained by static increments indicated that the tissues are capable of deforming in 100 to 200 msec. The situation in vivo differed somewhat, in that the pressure increment is caused indirectly by deforming the eye with a suction contact lens. This aspect should be studied separately. Another difference in vivo was the presence of moving blood. It was reasonable to expect that the dynamic
and static processes should yield similar results in areas supplied only by capillaries. However, it would be less clear if the choroid behaved similarly under both modes of pressure increments, since the thickness of the choroid may decrease due to expulsion of blood under a sustained elevated IOP, which may not occur in the presence of a fast increment.

A second intent of our study was to begin an evaluation of normal values of optic nervehead displacement. Our results showed that a large portion (73%) of the displacement at 60 mm Hg was already attained at 20 mm Hg above the resting baseline pressure. It was interesting that, as shown in Figure 2, our values are in excellent agreement with those reported by Levy and coworkers (ref. 12, Table 2, row 4) with a different method.12

In conclusion, this study indicates that by using red light with laser Doppler velocimetry one can measure noninvasively bulk motions of the optic nervehead and obtain results similar to those recorded with invasive techniques. In addition, this line of research has allowed the acquisition of normal optic nervehead displacement measurements following an elevation of IOP, information that will be useful in the study of glaucomatous eyes.

Key words: optic nervehead, laser Doppler velocimetry, displacement, glaucoma

Acknowledgments. Yuichiro Ogura, MD, performed the histologic examination, Lisa Molnar processed the tissues for histology, Marlene Heneghan provided secretarial assistance, Marek T. Mori assisted in the preparation and execution of the experiments, Maxine Gere provided editorial services, Linda Warren executed the artwork, and Norm Jednock and the photography department provided photographic services.

From the Applied Physics Laboratory, Department of Ophthalmology, Lions of Illinois Eye Research Institute, University of Illinois College of Medicine, Chicago, Illinois. Supported in part by research grant EY-03841 and Ophthalmic Research Center Core Grant EY-1792 from the National Eye Institute, Bethesda, Maryland. Submitted for publication: December 10, 1986. Reprint requests: Ran C. Zeimer, PhD, Applied Physics Laboratory, Department of Ophthalmology, University of Illinois College of Medicine, 1905 West Taylor Street, Chicago, IL 60612.

References