Strip Extensiometry for Comparison of the Mechanical Response of Bovine, Rabbit, and Human Corneas

Specimens of bovine, rabbit, and human corneas were systematically tested in uniaxial tension to experimentally determine their effective nonlinear stress-strain relations, and hysteresis. Cyclic tensile tests were performed over the physiologic load range of the cornea, up to a maximum of 10 percent strain beyond slack strain. Dimensional changes to corneal test specimens, due to varying laboratory environmental conditions, were also assessed. The measured stress-strain data was found to closely fit exponential power function relations typical of collagenous tissues when appropriate account was taken of specimen slack strain. These constitutive relations are very similar for rabbit, human and bovine corneas; there was no significant difference between the species after preconditioning by one cycle. The uniaxial stress strain curves for all species behave similarly in that their tangent moduli increase at high loads and decrease at low loads as a function of cycling. In the bovine and rabbit data, there is a general trend towards more elastic behavior from the first to second cycles, but there is little variation in these parameters from the second to third cycles. In comparison, the human data demonstrates relatively little change between cycles. Increases in width of corneal test specimens, up to a maximum of 2 percent were found to occur under 95 percent relative humidity test conditions over 10 minutes elapsed time test periods, while specimens which were exposed to normal laboratory conditions (45 percent RH) were found to shrink in width up to a maximum of 9.5 percent over the same elapsed time period. The thickness of the test specimens were observed to decrease by 3 percent in 95 percent relative humidity and by 12 percent in 45 percent relative humidity over the same elapsed time period.

Introduction

The cornea has been defined by Kuwabara [21] as "a clear window comprising the most anterior portion of the eye." Anatomically, the cornea is comprised of five distinct layers that include the epithelium, Bowman's membrane, the stroma, Descemet's membrane, and the endothelium. Figure 1 depicts the relative position and thickness of each of these layers of the cornea. The shape and refractive capability of the cornea is important for establishing the proper focal distance of light rays entering the eye, in tandem with the lens to the retina. Mechanical properties may play a significant role in establishing corneal topography and its effect on focal distance to the retina, particularly following surgical modification.

The objective of the present study was to develop a careful protocol and systematic measurement procedure enabling us to directly quantify the effective nonlinear stress-strain characteristics of cornea using the strip extensiometry method. Reliance on previously published data was avoided, particularly in light of the variability of the measurement techniques employed by, and data acquired from, these earlier studies. Animals corneas were compared for use as an approximate model for human corneas in mechanical property characterization. This is particularly important in light of the need to acquire statistically significant material property data, which is extremely difficult to obtain from the normal human population.

The viewpoint adopted herein is that the cornea behaves as a continuum [22], whereby the relationships between gross phenomenon can be explained with appropriate regard to the structure of the material on a smaller scale. We believe that "strips" of the cornea can adequately represent the "relative" (species-to-species following a uniaxial tensile test protocol), "effective" (averaged over the thickness) local behavior of the tissue. While we have elected to use excised "strips" of corneal tissue for the measurement of Young's modulus, we believe that the membrane inflation technique offers promise for reliable determination of the more "intrinsic" (independent of sample geometry [22]) properties of the cornea, on a layer by layer basis.

Contributed by the Bioengineering Division for publication in the Journal of Biomechanical Engineering. Manuscript received by the Bioengineering Division December 19, 1988; revised manuscript received September 16, 1991.
By "effective" Young's modulus \( [1, 3] \) we mean the instantaneous stress as calculated from the original unstrained cross sectional area of the specimen, divided by the instantaneous strain (grip-to-grip) calculated as the change in specimen length over the original unstrained specimen length. A membrane finite element model incorporating variable thickness consistent with that observed in the cornea was employed for the determination of Young's modulus (see Appendix). Clearly, the multiple layer or composite structure nature of the cornea, whereby the fibrils in each layer run in alternating directions, implies that stress is supported in some complex manner that is governed by the fibril orientation angle relative to the direction of applied force application. We have not addressed issues related to the variation in corneal thickness, the possible variation in mechanical properties of individual corneal layers due to variation in fibril orientation and cross linking, nor the variation in cornea properties along the corneoscleral border (inhomogeneity). However, we are directly comparing corneas of different species to one another, and these effects are present in all corneas, although to different and as yet undetermined amounts.

It should be noted that a number of critically important but subtle complexities exist which can seriously affect the testing protocol for soft biological tissues. These include 1) removal of the specimen from its natural environment, 2) attachment of a specimen to grips, 3) specification of initial test conditions (zero stress, zero strain), and 4) the ability to adequately measure specimen dimension, among others. As a result, extreme care must be exercised if one is to obtain accurate tensile stress-strain data and in order to avoid the occurrence of significant measurement errors that may occur due to discrepancies in assumed 1) initial condition states [14, 32] and 2) boundary conditions [14, 27] that the tissue is "believed" to comply with during testing. Several investigators have advocated the use of intact corneas [14, 18, 19] for material property characterization; they believe that the removal of strips of corneal tissue effectively destroys the support from fibers not oriented in the direction of the strip, and creates boundary conditions atypical of the tissue in vivo, thereby invalidating the measured material property values.

A number of studies have been undertaken in an attempt to characterize the nonlinear (tangent) modulus and creep response of the cornea and sclera. Most notable among these studies is the work by Sato [31], Greene [14], Nash [27], and Woo [36, 37]. In an earlier ARVO abstract [15], we directly compared our data against that produced by Woo [36] and Greene [14]. Based on this comparison, we believe that precisely measured low load stress-strain data (stress levels below 30 \( \text{N/cm}^2 \)) is lacking in the literature. In vivo conditions are simulated in humans with an intraocular pressure of 14 to 20 mm Hg (Woo [36]) which can increase ten fold by rubbing the eye or squinting (Ku and Green [20]). The load range over which our low load material property data was measured was expressly chosen to correlate with these physiological intraocular pressure levels which correspond to stress levels up to 18 \( \text{N/cm}^2 \) if we approximate the cornea as a spherical shell with thickness \( = 0.6 \text{ mm} \) and radius \( = 0.8 \text{ cm} \).

Within the last 5 to 7 years little progress has been made in the area of biomechanics of the cornea, particularly with regard to careful characterization of its material properties and structural behavior. Recently, Ju and Maurice [19] employed an ingenious membrane inflation technique to determine relations characterizing intraocular pressure versus strain for both intact and isolated layers of the stroma and Descemet's membrane. Conclusions drawn by these authors indicating that the stroma is much less extensible than Descemet's membrane in both species is open to question however, since the important parameter of specimen thickness required for the calculation of stress was not taken into account.

Bowman's membrane is typically an acellular structure that is part of the stroma. If its modulus is greater than a typical layer of the stroma, it could create a variation in the modulus and strength of the different species' corneas. The absence of Bowman's membrane in the bovine and rabbit model must be considered in detail, and was not evaluated in this study. Further insight into this issue could be gained by other strip extensometry studies. The thickness of Bowman's membrane, 12 or 30 \( \mu \text{m} \), depending on the source [19, 21] comprises 2 to 6 percent of the corneal thickness. Because of this, Bowman's membrane would probably not raise or lower the effective modulus of the cornea drastically even if its modulus was somewhat higher than that of the stroma.

Since the endothelium and the epithelium are not load bearing layers of the cornea, and since Descemet's membrane is believed to have a lower modulus than a typical layer of stromal lamellae [14, 34] this leaves the stroma as the primary load bearing layer. With regard to the stroma's ability to bear tensile loads, McPhee [25] concludes that the intraocular tension in rabbit corneas is probably distributed across all corneal stromal lamellae as opposed to being borne primarily by the anterior or posterior layers, implying that the tensile load acting on each layer through the thickness of the cornea is nearly the same, and hence the stress is likewise very nearly the same. Furthermore, since the cornea does not support shear forces [23], and since it exhibits relatively little if any "bending rigidity" (see Appendix), the deformation experienced by each differential layer due to an increase in intraocular pressure must be the same for adjacent neighboring layers. Although in uniaxial testing, the strain will tend to decrease slightly from the inner to the outer layers due to the slight increase in corneal radius, it is reasonable to assume that the actual strain undergone by each layer will be approximately equal. This idea is in agreement with classical membrane theory [34], in which the state of stress through a thin membrane (i.e., thickness \( \ll \text{radius} \)) is very nearly uniform.

Given that the strain and stress through the corneal thickness remain relatively uniform, one would conclude that the material properties are probably very similar across all stromal layers. Knowledge of variation in lamellae size and levels of interweaving have been observed as a function of stromal depth [23], and yet McPhee's study implies that stress is borne equally by all levels of the stroma. Variation in microstructures as determined through histological analysis should correspond to the variation in mechanical properties, but this has yet to be demonstrated. Variation in corneal tissue on a layer by layer basis was not explored in this study, but should be an important consideration for future work.

On a more theoretical note, Fung [11] has advocated the use
of exponential power functions to analytically characterize the relationship between stress and strain in soft biological tissues [10, 11]. Fung emphasized the importance of a unified mathematical theory, which can account for: 1) static (very slow) elasticity, 2) dynamic elasticity (finite strain rate), 3) stress relaxation under fixed strain, 4) creep deformation under fixed stress, 5) strain cycle hysteresis, and 6) cycle stress fatigue, in developing experimentally verifiable models for such tissue.

Materials and Methods

Fresh samples of bovine eyes were obtained in refrigerated storage containers from Max-Insel Cohen, Inc. [24], and prepared for testing between 36 and 48 hours postmortem. Fresh samples of rabbit eyes were obtained from the Department of Internal Medicine, College of Physicians and Surgeons of Columbia University and were prepared for testing within several hours after sacrifice. The moist chamber method used in this study is valid in the range of 36 to 63 hours postmortem [33]. If the epithelium or the endothelium was slightly damaged, the specimens would still be valid from a mechanical viewpoint due to their small contribution to corneal strength. In this study, the importance of these layers is to protect the stromal lamellae just prior to the final preparation of the strip specimen.

There was a great deal of difficulty in obtaining fresh donor corneas that had not been removed from the globe, and that had not been stored in a preservation medium, in such a short period of time. It was desired to observe the effects of human donors within 48 hours of death, and this was done for four (4) eyes. These eyes were acquired from two donors of ages 59 and 51 years. The eyes were obtained within 36 hours of death and immediately tested. Other eyes tested, were not tested within the 48 hours of death as was required in the protocol. There was definite edema in all tissues due to the moist storage method; this is known because specimen thicknesses measured are greater than those that appear in the literature. The average central thickness of the eyes tested are:

Human: 0.82 mm 4 eyes
Bovine: 1.53 mm 3 eyes
Rabbit: 0.50 mm 3 eyes

All test samples within a given group (bovine or rabbit) were prepared simultaneously and were subsequently stored in a refrigerated bath at 4° Celsius, in small 20 ml air tight vials containing only the natural body fluid from the sample. Equilibrium of this fluid with the air in the vial typically occurred within 15 minutes following the time the samples were placed in the vials as determined by humidity and temperature probes. As a result, these vials effectively served as miniature, equilibrated, humidity chambers. All tensile testing for a given group of samples commenced within one half hour of corneal test sample preparation and storage.

The preparation of the corneal specimen to be used for uniaxial testing was the same for the bovine, rabbit, and human eyes and typically required 5 to 10 minutes. The desired specimen was a parallel strip, typically 2 mm in width that ranged in length from 10 mm to 20 mm. The first step in the dissection procedure involved removal of a corneal "disk" containing a small outer "ring" of scleral tissue. While holding the intact eye between thumb and forefinger on a smooth flat surface, a scalpel was used to gently create an incision in the sclera, approximately one half centimeter below the corneal-scleral junction. While the scalpel blade was used to create an initial incision in this region, a pair of curved blade scissors was subsequently used to completely separate the corneal scleral disk containing the iris, ciliary body, choroid and lens from the remainder of the eye. The vitreous fluid typically clings to this portion of the eye, but can easily be pinched off, separated and removed. To remove the remainder of the corneal disk and the scleral rim, the flat side of the scalpel blade was gently inserted between the choroid and the sclera. The choroid was then separated from the cornea. This effectively removed the iris, ciliary body, choroid and lens from the corneal-scleral sample.

Following this dissection, final trimming of the sclera, to a thin 1 or 2 mm ring around the cornea, was accomplished using the scissors. The next step in the dissection process involved the removal of a strip of cornea with small scleral ends attached. The corneal-scleral discs were subsequently placed in temperature controlled, humidity-equilibrated vials and stored for subsequent testing. This tended to prevent swelling and degradation of the samples.

The corneal-scleral disk is gently flattened on a wax platform. This deformable platforms permits the blades of the specimen cutter to penetrate completely through the sample thereby providing a nicely trimmed specimen of uniform width. The cutting tool consists of an aluminum block which holds two parallel cutting blades. This tool is placed lightly on the corneal scleral disk through its center. Angular orientation of the corneal-scleral disk relative to the cutting tool was ignored under the assumption that the value of Young's modulus of the sample demonstrates symmetry about an axis passing through the center of the optical zone (i.e., axisymmetric) in a macroscopic sense in accordance with anatomical studies describing the ultra structure of the cornea [21]. Following placement of the cutting tool on the specimen, it is struck firmly with a hammer so that the blades penetrate the tissue and embed in the wax base. The wax is subsequently removed. The final specimen is shown in Fig. 2.

Cyanacrylate cement [26] was used to attach the tensile specimens to the grips at both ends. The technique prevented undue squeezing and subsequent damage to the delicate tissues and collagen fibrils of the cornea while ensuring nearly uniform attachment of each corneal layer to the grips. It is important to note that the samples were cemented along both their upper and lower surfaces (epithelium and endothelium) at either end over a distance of approximately 4 mm, as well as through their thickness (Bowman's membrane, stroma and Descemet's membrane) at the ends, thereby ensuring attachment of each corneal layer to the grips by way of the cement. A SEM analysis of the attachment points of the specimens to the grips was conducted following completion of tensile testing, verifying that the corneal layers remained bonded to the grips through the cyanoacrylate cement which was absorbed into the corneal tissue 75 percent of the total thickness (Figs. 3, 4, 5). This method of specimen attachment also reduced the possibility of specimen slippage at the grip, an important consideration, since grip to grip distance was used to determine specimen strain.

The effective cross sectional area (original unstrained area) of each sample was gently measured using an area micrometer,
designed so that the opening of the width of the sample groove was slightly larger than the spacing between the blades of the specimen cutting tool in accordance with the recommendations provided by Ellis [8]. Extreme care was exercised when making this measurement to ensure that the delicate corneal tissue was not in any way damaged.

Each cornea test sample was retained in a humidity chamber fed by a mild ultrasonically induced vapor mist that was held at approximately 95 percent RH and room temperature throughout the course of the tensile testing procedure. This helped to reduce any dimensional and mechanical property changes to the specimen due to fluid evaporation from the sample (see discussion of dimensional changes with environmental conditions below).

Multiple cycle (three cycles per specimen) tensile tests were carefully performed on 3 specimens of bovine and rabbit corneas and 4 specimens of human corneas. We found that three test cycles were sufficient to precondition each specimen and

---

**Fig. 3** SEM of glue penetration into the left side of corneal specimen in cross section. Darker region is area penetrated.

**Fig. 4** Enlarged SEM of specimen in Fig. 3 depicting region where glue has not penetrated.

**Fig. 5** Enlarged SEM of specimen in Fig. 3 depicting region where glue has penetrated. (Note how layers are bonded together in comparison with Fig. 4.)

**Fig. 6** Uniaxial stress-strain relations for bovine cornea.

Eyes tested within 36 hours postmortem.
to achieve consistent results. The strain values corresponding to each applied stress value have been determined, along with the standard deviation of the strain. We report values for the maximum standard deviation in strain as a percentage of the average strain for each species. This provides a succinct description of the uncertainty in our experimental strain results. A constant deformation rate of 0.05 cm/minute was applied to the specimens through the use of a model TM (mechanical drive) Instron Material Testing Machine.

The effect of changes in environmental conditions on dimensional changes of corneal specimens were measured using a Bausch and Lomb StereoZoom-4 stereoscopic microscope (28X-120X magnification) fitted with an eye piece mounted reticle scale and an X-Y translation stage to which a Starrett dial indicator (.001 in. resolution) was attached. The reticle scale was moved across the specimen, and the dial indicator measured the displacement of the stage required to traverse the dimension. Other specimens (bovine, rabbit and human)
were placed in 1) the humidity chamber in which the stress strain tensile testing was performed and subjected to 95 percent RH conditions by an ultrasonically induced vaporizer, 2) a sealed chamber containing a tray of distilled water (93 percent RH) or 3) exposed to normal laboratory conditions (15.0°C, 45 percent RH, 760 mm-Hg). Dimensional changes experienced by the specimen, over time, were subsequently measured for each of these methods of storage.

Results
Plots depicting the average stress-strain relations measured for bovine, rabbit, and human specimens cycled three times and including the slack strain are presented in Figs. 6, 7, and 8, respectively. The same curves are plotted with the slack strain removed in Figs. 9, 10, and 11 for visual comparison of the different representative species arranged by cycle. Each of these curves allows for visual presentation of the qualitative
Table 1  Maximum percent standard deviation in strain* for each stress-strain curve presented (Figs. 9, 10, and 11)

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Bovine</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>27%</td>
<td>23%</td>
<td>21%</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>20%</td>
<td>30%</td>
<td>25%</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>25%</td>
<td>27%</td>
<td>26%</td>
</tr>
</tbody>
</table>

*Note: Definition of Maximum Percent Std. Dev. The standard deviation in the strain for each data point on each stress-strain plot has been calculated. This was done for each species. The maximum standard deviation in the strain was then calculated for each species, and expressed above as a percentage of the average strain (averaged over the number of samples for each species).

relationship between stress and strain. Note that the 2nd cycle data is very similar to the 3rd cycle data of the same species (Figs. 6, 7, and 8). There is no significant difference between the 2nd and 3rd cycle data of different species in these tests based on standard deviation values resulting from specimen population variations. The maximum percent standard deviation due to population variations has been calculated for each of the nine stress-strain curves, and is presented in Table 1.

Calculations of the percent hysteresis were undertaken based on experimental measurement of the ratio of the area under the stress-strain curve during load application to that during load removal and is indicative of the nonconservative energy loss, within the specimen, due to its viscoelastic behavior. Figure 12 depicts the percent hysteresis averaged over three cycles for each of the specimens. In general, as the number of loading cycles increase, the percent hysteresis tends to decrease for all sample types.

Figures 13, 14, and 15 respectively show representative plots of the percent change in the thickness of 24 human, 15 bovine, and 15 rabbit corneal specimens in different humidity environments (standard room conditions, humidity chamber, and sealed chamber containing a tray of distilled water). The abbreviated terms “room,” “humidity” and “tray,” are used in Figs. 13–16. Shrinkage is quite dramatic when the specimen is permitted to reside in a normal laboratory environment with a relative humidity of 45 percent. In contrast, a relative humidity of 95 percent has a significantly less dramatic effect on dimensional changes to the sample. Over the duration of the testing period (less than 10 minutes for all three loading cycles), dimensional changes are of minimal concern provided proper environmental control precautions are observed. Measurements of the changes in width of the specimen revealed that the width increases in the humidity chamber, and decreases in the normal laboratory (room) environment (Fig. 16).

Discussion

The curves in Figs. 6 through 11 demonstrate that the relationship between stress and strain is not a linear one. Fung [10, 11] advocates that researchers should not attempt to define a single elastic modulus value for nonlinear tissues whose true modulus varies over a wide range. When a linear regression (linear least squares) curve fit was applied to the natural log of stress versus the natural log of strain minus slack strain, for each type of sample (human, bovine and rabbit), best fit straight line plots were obtained. Included are values for the coefficient of determination, denoted $R^2$, which is an indicator of the proportion of variation present in the dependent variable (strain) which has been explained by the use of the estimating Eq. (4). These values ranged from 0.095 to 0.999, and are indicative of excellent fits. A mathematical description of the relationship between stress and strain was derived through the application of this linear regression analysis applied to the experimentally measured stress-strain data and can be expressed as follows:

$$\ln \sigma = \ln \alpha + \beta \ln (\epsilon - \epsilon_s)$$

where $\sigma =$ stress, 
$\epsilon =$ strain, and 
$\epsilon_s =$ slack strain (difference between zero strain and the smallest strain to initiate load bearing in the specimen).

Taking the antilog of Eq. (1) yields the following expression for the constitutive law:

$$\sigma = \alpha(\epsilon - \epsilon_s)$$

where $\alpha$ is a scale factor, and $\beta$ represents the exponent of the nonlinear relationship between stress and strain. The closeness
of fit and the high degree of similarity demonstrated by these plots, for all samples, is indicative of the close adherence of the stress-strain relationships to an exponential functional form, typical of biological soft tissues. In short, Eq. (1) was found to approximate the experimental curves quite well. The coefficient values for each curve are listed in Table 2. Figure 17 is a typical linear regression fit of the power function to the natural log of the data values; the $R^2$ values, which are all very similar, reveal excellent correlation of the regression to the data.

The values of $\beta$ provide an indication of the rate of the increase in stress with an increase in strain, and are very close to values of 2.0 in the second and third cycles, which is the reported value for the power coefficient of collagenous tissues, such as tendon [17]. The values for $\alpha$, which are merely scaling factors, and the values for $\beta$, both increase for each cycle. In the linear regression curve fits which are defined by these parameters, the natural log of $\alpha$ is the y intercept, and $\beta$ is the slope. For larger values of $\alpha$ of strain, the effect of $\beta$ is small and conversely for smaller values of strain, the effect of $\beta$ is large. The opposite is true for $\alpha$. A low beta value dominates more at lower values of stress, than at higher values since strain

---

**Fig. 12** Percent hysteresis versus cycle (avg. of 5 runs)

**Fig. 13** Average percent change in human corneal thickness versus time

Note: Each curve represents an average of 8 tests
always takes on a value that is much less than 1. Since both α and β increase with cycle number, we found that the tangent modulus decreases for very low stress and strain values while it is increased for higher stress-strain values from the first cycle to the later cycles.

Hysteresis values are directly related to slack strain values which are the difference between zero strain and the smallest strain for load bearing in the specimen, by definition 0.0 percent for cycle 1. Slack strain signifies the permanent set in the specimen, and corresponds to deformation energy that cannot be regained. The average slack strain which developed in the bovine specimen (3 percent for cycle 2 and 3.4 percent for cycle 3) was approximately one half that found in the rabbit specimen (6 percent for cycle 2 and 7 percent for cycle 3). This is logical, as the same peak loads were applied to each specimen, although the thickness of the specimens varies. Thus, the rabbit corneal specimens underwent higher stress and resulted in higher slack strain. The slack strain induced in the human specimen was 0.9 percent for cycle 2 and 1 percent for cycle 3. This cannot be explained based upon dimensional considerations, but perhaps is due to some effect associated with the physiological differences in the cornea donors. Perhaps the greater age of the human specimens is significant in that the corneal tissue may have already undergone permanent set in vivo, which did not display in vitro.

While we recognize that the in vivo biaxial state of stress was not replicated in our uniaxial experimental protocol, our goal was to acquire basic information to assess the relative mechanical property differences among these species. Based on the work of Huang [16], Ischreyt [18], Maurice [23], and
Fig. 16 Representative plot of average percent change in corneal width versus time for bovine specimens. (Note that rabbit and human demonstrated similar results.)

Fig. 17 Linear regression fit: Rabbit average of cycle 1, day 1

### Table 2 Linear regression values

<table>
<thead>
<tr>
<th></th>
<th>Human Cycle 1</th>
<th>Human Cycle 2</th>
<th>Human Cycle 3</th>
<th>Human 3</th>
<th>Rabbit Cycle 1</th>
<th>Rabbit Cycle 2</th>
<th>Rabbit Cycle 3</th>
<th>Bovine Cycle 1</th>
<th>Bovine Cycle 2</th>
<th>Bovine Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>5432</td>
<td>8103</td>
<td>9897</td>
<td>1636</td>
<td>10940</td>
<td>1359</td>
<td>2441</td>
<td>9897</td>
<td>12088</td>
<td></td>
</tr>
<tr>
<td>$\beta$</td>
<td>1.84</td>
<td>1.96</td>
<td>1.98</td>
<td>1.39</td>
<td>1.89</td>
<td>1.99</td>
<td>1.99</td>
<td>1.77</td>
<td>2.01</td>
<td>2.10</td>
</tr>
<tr>
<td>R$^2$</td>
<td>.997</td>
<td>.997</td>
<td>.997</td>
<td>.999</td>
<td>.997</td>
<td>.996</td>
<td>.999</td>
<td>.995</td>
<td>.995</td>
<td>.995</td>
</tr>
</tbody>
</table>

Note: the units for $\alpha$ are N/cm² (the same as the units for $\beta$).
Table 3  Modulus values found in the literature

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Description</th>
<th>I.O.P. Equivalents</th>
<th>Modulus (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyquist [28]</td>
<td>1968</td>
<td>Pig cornea uniaxial</td>
<td>high</td>
<td>1.6 x 10⁶</td>
</tr>
<tr>
<td>Woo* [36]</td>
<td>1972</td>
<td>Human stroma bi axial shear</td>
<td>10 mm Hg</td>
<td>5.4 x 10⁶</td>
</tr>
<tr>
<td>Foster* [9]</td>
<td>1978</td>
<td>Human cornea in vivo O2 rigid</td>
<td>physiological;</td>
<td>1.0 x 10⁶</td>
</tr>
<tr>
<td>Andreasson* [12]</td>
<td>1980</td>
<td>Human cornea</td>
<td>2350 mm Hg</td>
<td>5.7 x 10⁵</td>
</tr>
<tr>
<td>Nash* [27]</td>
<td>1982</td>
<td>Human cornea uniaxial</td>
<td>30 mm Hg</td>
<td>3.9 x 10⁵</td>
</tr>
<tr>
<td>Edmund* [7]</td>
<td>1989</td>
<td>Human cornea in vivo indent</td>
<td>2350 mm Hg</td>
<td>2.1 x 10⁵</td>
</tr>
<tr>
<td>Reichel [30]</td>
<td>1989</td>
<td>Bovine cornea central-uniaxial</td>
<td>15 mm Hg</td>
<td>5.0 x 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bovine cornea biaxial-uniaxial</td>
<td>100 mm Hg</td>
<td>1.25 x 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bovine cornea central-uniaxial</td>
<td>15 mm Hg</td>
<td>1.25 x 10⁴</td>
</tr>
<tr>
<td>Our group</td>
<td>1991</td>
<td>Human cornea uniaxial</td>
<td>100 mm Hg</td>
<td>1.75 x 10⁴</td>
</tr>
<tr>
<td>Our group</td>
<td>1991</td>
<td>Human cornea biaxial</td>
<td>10 mm Hg</td>
<td>3.4 x 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>400 mm Hg</td>
<td>4.1 x 10⁴</td>
</tr>
</tbody>
</table>

* Estimated according to Laplace's equation by Edmund (1989).
* Presented by Edmund (1989) from in vivo ocular rigidity data.

Nyquist [28], we realize that while an absolute comparison may not be possible using an uniaxial test protocol, a fundamental understanding of the relative differences among the mechanical properties of the species has been obtained.

In accordance with the linear regression parameters, we found that the first cycle stress-strain data, in comparison with subsequent cycles, is the least elastic (more hysteresis) and demonstrates the lowest tangent modulus for all three specimens. As testing progressed through cycles 2 and 3, the stress-strain curves measured for the human cornea changed very little, and those of the bovine and rabbit corneas became more and more similar to the human (Figs. 9, 10, and 11). Note the remarkable similarity between cycle 3 data for each of the three species in Fig. 11. While absolute intrinsic material property values may not be obtainable from the experimental data, a correlation may exist between properties determined uniaxially and those determined from a biaxial testing protocol, depending on the extent and type of interaction between the stromal layers.

In Table 3, the empirically determined tangent moduli for our human data at stress levels equivalent to intraocular pressures of 10 and 400 mm Hg are compared with values published in the literature. The values range from our low value of 3.4 x 10⁶ to the highest value of 5.7 x 10⁵ presented by Andreasson. The nearly 3 orders of magnitude of variation in the modulus values reported in the literature is due to three primary factors: 1) the nonlinearity of the stress strain relationship, 2) the quality of the tissue presented, and 3) the method of testing. Because the modulus is a function of strain, the values increase at a great deal to higher strain values. Our value at 10 mm Hg increases 6 fold at 400 mm Hg, and similarly so with other researchers. Since the measured stress value is highly dependent on the specimen dimensions at the cross section, and the specimen's ability to support strain is dependent upon the viability of the fibers, the condition of the specimen is important. Some of the researchers above froze their specimens (Nash, Andreasson), and had a great deal of edema as did our group. Lastly, as shown by Ischrey, the nature of the test can be important. A few researchers (Woo, and Jue and Maurice) have performed testing on intact tissue whereas the others (Nyquist, Foster, Andreasson, Nash, Edmund, Reichel, and our group) have performed uniaxial extensometry. It was this variation in published values seen in the literature that guided our group to repeat the uniaxial studies and look at the species together to see if our results would lie in the center of the distribution and to determine the relative moduli of different species under the same protocol.

In Fig. 16, the width of bovine corneal specimens increases in the water tray (92 percent R.H.) by 1 percent and in the humidity chamber (95 percent R.H.) by 20 percent, and the width decreases at room humidity (45 percent R.H.) by 10 percent over a testing period of 60 minutes. The increase in width in the humidity tray is coupled with a decrease in thickness, probably due to a bulging out of the tissue along the freshly cut sides. In Figs. 13, 14, and 15, it can be seen that the changes in corneal specimen thickness are all approximately the same for the different species, although changes were greatest in the rabbit specimens, and least in the bovine specimens. The thickness of the human specimens shrinks by 8 percent in the water tray, 17 percent in the humidity chamber, and 70 percent in standard room humidity conditions over a total testing period of 75 minutes. Note that the time to perform a tensile test on a sample was considerably less than this (approximately 10 minutes). We simply wanted to assess the maximum effects on the tissue due to modulus and environmental conditions. The difference between the dimensional changes found with the humidity chamber and the water tray is one of measurement technique, more so than one of humidity differential. The specimen in the humidity chamber spent 30 seconds out of every 5 minutes in normal room humidity conditions as they were being measured under the microscope, while the specimen in the water tray remained in the tray for the duration of the testing period. It can be seen that the simple action of placing the specimen in and out of room humidity causes the tissue to expand in width and shrink in height to a greater extent. The effect of the humid environment was to preserve the dimensions of the specimen during the testing procedure. Removal and reinsertion into a humid environment repeatedly has more effect on the tissue, but still a great deal less than exposing the tissue sample to low humidity laboratory (i.e., normal room) conditions.

Error in these measurements was due in part to the irregular manner in which the tissue changes shape as it loses moisture. As the cornea begins to dry under room conditions, the thickness is not uniform over the entire width of the specimen; instead, the edges begin to drop sharply almost immediately while the middle experiences some decrease in thickness. After thirty minutes, the rate of decrease at the edges slows considerably while the middle portion continues a steady decline.

Conclusion

The nonlinear stress-strain relations of bovine, rabbit, and human cornea can be described mathematically, quite succinctly and accurately, in terms of exponential power functions as determined through the use of linear regression analysis applied to the log of the measured stress and strain values. The uniaxial stress strain curves for all species behave similarly in that their tangent moduli increase at high loads and decrease at low loads as a function of cycling. There was a greater slack strain and hysteresis (which are related) in the bovine and rabbit corneas than in the human corneas. The slack strain, and hysteresis values for the rabbit cornea were twice that for the bovine cornea which makes sense based on the higher stresses undergone by the rabbit specimens. Furthermore, humidity was found to be important in preserving the integrity of the specimens through the course of the tensile tests.

Acknowledgments

This research was supported, in part, by grants from the Microsurgical Research Foundation and the Jaffin Foundation of New York. In addition, the research assistance provided by Mechanical Engineering students Kirk McIver, Steven To-
manovich and Michael Burnett, the procurement of specimens by Carol Buzard at Manhattan Eye, Ear and Throat Hospital and by Dr. Morab from Columbia University's College of Physicians & Surgeons, advice from Dr. Jan Koniarck from Columbia University's College of Physicians and Surgeons, and the SEM evaluation of corneal specimen attachment (via cyanoacrylate cement) to tensile testing grips by Dr. John L. Ricci, of the Department of Bioengineering at the Hospital for Joint Diseases Orthopedic Institute, are gratefully acknowledged.

References

24. Max Insel-Cohen, Inc., Suppliers of animal organs and tissues for research, Livingston, NJ.

Appendix

The Effect of Straightening Initially Curved Corneal Specimens

The fact that an initially curved specimen of the cornea has been straightened prior to the application of a uniaxial tensile test load raises the important question as to whether any significant variation in the stress distribution through the thickness of the specimen (i.e., bending stress) is induced in the specimen when it is straightened, and whether this effect is sufficiently small to be overshadowed by the subsequent application of a uniaxial tensile load, even at the beginning stages of loading (less than 5 percent strain). In accordance with the

<table>
<thead>
<tr>
<th>Table A1</th>
<th>Determination of elastic modulus values based on a variable thickness membrane model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load step</td>
<td>Stress (N/mm²)</td>
</tr>
<tr>
<td>I</td>
<td>2.5E-2</td>
</tr>
<tr>
<td>I</td>
<td>6.5E-2</td>
</tr>
<tr>
<td>I</td>
<td>1.9E-1</td>
</tr>
<tr>
<td>I</td>
<td>2.4E-1</td>
</tr>
</tbody>
</table>

Fig. A1 Membrane models of a strip of corneal tissue loaded in tension, (A) before straightening and (B) after straightening.
laws of mechanics, the specimen of tissue assumes a localized shape that is consistent with the stresses induced in, whether due to bending or externally applied tensile loads. We have observed under microscope magnification (180x) that the tissue undergoes small scale localized buckling as a mechanism for relieving compressive stresses which it appears unable to sustain. This is indicative of the fact that the modulus in compression is lower than that in tension, and points to the existence of membrane behavior by the tissue. Greene [14] applied analytical curved beam theory to this problem and estimated that the maximum error in the stress and strain (i.e., deviation from a uniform stress state through the thickness of the specimen) could be as large as 142 percent based on the assumption that the tissue behaves similarly in tension and compression. Since we observed that the tissue behaves quite differently in compression than in tension, we undertook a numerical solution to this problem in order to gain further insight into its behavior at low stress levels, where the effect of bending could significantly alter the values we obtained for its modulus.

The finite element method was employed to ascertain the effect that a variation in stress through the thickness of the cornea could have on the uniaxial test results, and to obtain "best-fit" elastic tangent moduli values. We believe that this approach should provide a more accurate value for the modulus than that obtainable with the simple uniaxial stress strain relationship (stress = modulus \times strain), which assumes that the cross sectional area of the specimen remains constant. This was accomplished by varying the modulus of elasticity in the finite element model until the maximum strain value calculated by the model was equal to that measured experimentally. A two dimensional membrane model [12, 29] with initial curvature, and of variable thickness, equal to that in the human
Table A2: Comparison of intact cornea finite element membrane model results with experimental measurements for maximum deflection at the apex of rabbit cornea

<table>
<thead>
<tr>
<th>Intraocular Pressure (mm H₂O)</th>
<th>Elastic Modulus (N/mm²)</th>
<th>Maximum Deflection (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment</td>
<td>FDM Model</td>
</tr>
<tr>
<td>150</td>
<td>0.03-0.04</td>
<td>0.058</td>
</tr>
<tr>
<td>200</td>
<td>0.05-0.07</td>
<td>0.066</td>
</tr>
<tr>
<td>250</td>
<td>0.06-0.07</td>
<td>0.073</td>
</tr>
<tr>
<td>300</td>
<td>0.08-0.10</td>
<td>0.080</td>
</tr>
<tr>
<td>350</td>
<td>0.12-0.17</td>
<td>0.085</td>
</tr>
</tbody>
</table>

The intact cornea was developed, in accordance with our data and that presented by Woo (1972). The best-fit (tangent) modulus values are provided in Table A1 for each of four different load (stress) levels. The boundary conditions and loading distribution to which the model was subjected are shown in Fig. A1 (before and after straightening), and replicate those employed in our experimental protocol.

A second finite element model of an intact cornea was developed, to corroborate our experimental results and to justify our uniaxial experimental protocol. The model is depicted in Fig. A2. The intact cornea finite model is a nonlinear (geometrically and materially) membrane model. Results from the finite element model were compared against experimentally determined deformation data for maximum corneal deformation, that is the deformation at the apex of the cornea (i.e., at the center of the optical zone) under physiological loading conditions (15-35 mm H₂O). A membrane inflation apparatus fitted with a micrometer was employed to obtain these results (Fig. A3). A comparison of the results is given in Table A2. The difference between the finite element results and the experimental results is quite small, particularly over the 20-30 cm H₂O pressure range, and in general demonstrates good agreement.

Although the results from these finite element models do not provide direct evidence that the cornea is unable to sustain any appreciable bending stresses, they clearly point to the existence of membrane behavior. We therefore conclude that the cornea can be considered to behave very nearly as a membrane, with little ability to resist bending stresses. As expected, the membrane model displayed a relatively uniform stress variation throughout its length and thickness. In fact, the uniformity of the stress state as observed in the membrane model serves as a necessary prerequisite for validation of the uniaxial tensile test protocol for determining Young's modulus. Based on these findings, we therefore conclude that the effect of straightening initially curved corneal specimens is quite small in comparison with the other factors that can cause error in the final calculation of the modulus. These factors include initial specimen cross-sectional area and length measurements, changes in area with loading, and specimen gripping.