Accommodative Ciliary Body and Lens Function in Rhesus Monkeys, I: Normal Lens, Zonule and Ciliary Process Configuration in the Iridectomized Eye

Mary Ann Croft,1 Adrian Glasser,2 Gregg Heatley,1 Jared McDonald,1 Timothy Ebbert,1 David B. Dahi,3 Nivedita V. Nadkarni,3 and Paul L. Kaufman1

PURPOSE. The underlying causes of presbyopia, and the functional relationship between the ciliary muscle and lens during aging are unclear. In the current study, these relationships were studied in rhesus monkeys, whose accommodative apparatus and age-related loss of accommodation are similar to those in humans.

METHODS. Centripetal ciliary body and lens equator movements were measured during accommodation in 28 eyes of 21 rhesus monkeys (ages, 5.7–26 years) by goniovideography. Ultrastructural microscopy was performed in 21 eyes of 17 monkeys. Narrowing of the angle between the anterior aspect of the ciliary body and the inner aspect of the cornea was used as a surrogate indicator of forward ciliary body movement during accommodation.

RESULTS. Average centripetal ciliary body movement in older eyes (age ≥17 years, n = 16) was ~20% (0.09 mm) less than in young eyes (age, 6–10 years, n = 6), but not enough to explain the 60% (0.21 mm) loss in centripetal lens movement nor the 76% (10.2 D) loss in accommodative amplitude. Average forward ciliary body movement was 67% (49°) less in older (n = 11) versus young (n = 6) eyes. Maximum accommodative amplitude correlated significantly with the amplitude of centripetal lens movement (0.02 ± 0.003 mm/D; n = 28; P < 0.001) and with forward ciliary body movement (3.34 ± 0.54 deg/D; n = 21; P = 0.01).

CONCLUSIONS. Decreased lens movement with age could be in part secondary to extralenticular age-related changes, such as loss of ciliary body forward movement. Ciliary body centripetal movement may not be the limiting component in accommodation in the older eye. (Invest Ophthalmol Vis Sci. 2006;47: 1076–1086) DOI:10.1167/iovs.04-1523

Fundamental elements of the accommodation mechanism (focusing from far to near objects) have been debated, notably in regard to the change in lens equatorial diameter. The classic Helmholtz theory of accommodation postulates that the ciliary muscle moves forward and inward, releasing tension on the zonula, allowing the lens equator to move away from the sclera.1 The lens equatorial diameter decreases and the lens anterior–posterior (A-P) thickness increases.1

The functional relationships between the ciliary muscle, zonular fibers, and lens and their changes with age are unclear, impeding elucidation of the pathophysiology of presbyopia (the loss of accommodative ability with age). Rhesus monkeys have an accommodative apparatus virtually identical with the human2–10 and exhibit an age-related decline in pharmacologically and centrally stimulated accommodation that, adjusted for lifespan, is comparable to the loss of visual stimulus-driven accommodation in the human.5–7,9,10 The rhesus monkey is therefore an excellent model for accommodation and presbyopia.

In living rhesus monkeys, after surgical iridectomy, the tips of the ciliary processes (CPs), the zonular fibers, and the lens equator can be observed, and accommodative movements can be measured by goniovideographic imaging upon onset of electrical stimulation to the Edinger-Westphal (E-W) nucleus.5–9 The tips of the CPs and the lens equator move away from the sclera.5,9 Both lens equator and CP movement are highly linearly related to refractive change.9 These techniques also permit studying age-related changes in the accommodative structures. Herein, we describe age-related changes in the functional interaction between the ciliary muscle and lens and the direction and extent to which these structures move during accommodation.

MATERIALS AND METHODS

Animals

Twenty-eight eyes of 22 rhesus monkeys (Macaca mulatta) of both sexes weighing 5.7 to 14.8 kg, with normal eyes (assessed biomicroscopically) were studied. Their age range at entry was 5.7 to 24 years, with experiments conducted between 2 weeks and 5 years thereafter. One eye of a 6-year-old cynomolgus monkey (Macaca fascicularis) weighing 4.5 kg was also included. The ages given are those at which the particular experiment was performed and thus range from 5.7 to 26 years. Longitudinal comparisons were not performed. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgical and Experimental Preparations

Total iridectomy was performed11 in both eyes, and a bipolar stimulating electrode was implanted into the E-W nucleus of each monkey.12 The accuracy of electrode placement during surgery was ensured by obtaining the maximum accommodative response.13 After surgery, the monkeys behaved normally with no evidence of neurologic deficit or phtotic discomfort. Anesthesia was induced before all surgical and
experimental procedures: (1) total iridectomy, slit lamp examination, routine photography, and refractions were performed in animals under intramuscular (IM) ketamine 10 mg/kg with or without IM diazepam 1 mg/kg, supplemented by ketamine 5 mg/kg every 20 to 30 minutes as needed; (2) midbrain electrode implantation was performed under IM ketamine 10 mg/kg + inhalant isoflurane 1% to 2%; and (3) central electrical stimulation and video recording of accommodative apparatus were performed under IM ketamine 10 mg/kg + IM or intravenous (IV) pentobarbital sodium (35 mg/kg IM, supplemented by 10 mg/kg IM per hour beginning at 2 to 3 hours, as needed; 10 to 15 mg/kg IV, supplemented by 10 mg/kg IV per hour beginning at 0.5 to 1.0 hours). A Hartinger coincidence refractometer (aus Jena, Jena, Germany) was used to measure resting refractive error and accommodation in response to stimulation of the E-W nucleus. Goniovideographic imaging and ultrasound biomicroscopy of the ciliary body, CPs, and lens equator were recorded during accommodation between 3 weeks and 5 years after electrode implantation. In 20 eyes of 16 monkeys, these experimental sessions were undertaken between 3 weeks and 10 months after electrode implantation. Eight eyes of five other monkeys were imaged at 1 (n = 2), 2 (n = 4), 4 (n = 1), and 6 (n = 1) years after electrode implantation. One monkey did not undergo goniovideographic imaging because the stimulating electrode stopped working.

Goniovideographic Imaging

For the experimental imaging sessions, the anesthetized monkey was held in a head holder with the head upright and facing forward. The body was placed prone on heating pads to maintain temperature at 36 °C to 38°C. When necessary, the medial and either the superior or inferior rectus muscles were paralyzed with an injection of botulinum A toxin at least 2 days before the recording session, to minimize eye movements; the effect typically lasted 3 to 6 months. Any residual eye movements not eliminated by the toxin injections were dampened by applying tension to a 5-0 Dacron suture passed beneath the lateral rectus muscle. Suture tension sufficient to minimize eye movements, but without dampening the accommodative response, was applied.

Accommodation was stimulated centrally via the implanted electrode. Goniovideography images (using a Swan-Jacob gonioscopy lens) were obtained with an infrared-sensitive, high-resolution CCD video camera (model 4915; Advanced Video Technology, COHU, Inc., San Diego, CA). The camera was mounted on a modified stereo photomicroscope (Carl Zeiss Meditec, Inc., Dublin, CA), and recordings were made with an SVHS videocassette recorder (SVO-9500MD; Sony Medical Systems, Montvale, NJ). A time-date generator (aus Jena, Jena, Germany) was used to record the E-W stimulus onset and termination and to place a time-date stamp on the videotape. Care was taken to ensure that the observation tube of the slit lamp was always aligned with the AP axis of the eye, allowing the circumpapillary space to be visualized through the Swan-Jacob gonioscopy lens. This standardization allowed detection of CP movement centripetally and comparisons between experimental sessions on the same or different days. Details of all equipment and procedures for iridectomy, electrode implantation, and central stimulation have been described.7,11-15

To account for movement of the eye (if any), a 9-0 nylon suture placed in the cornea at the nasal or temporal limbus served as a reference point (Fig. 1) from which to measure centripetal movement of the lens equator and the CPs during accommodation. The amount of residual eye movement induced for each quadrant was quantified as the absolute amount the suture moved in the image from the baseline unaccommodated state to the maximally accommodated state.

Further, to determine whether residual eye movement affected the amplitude of the measured CP and lens centripetal accommodative movement, artificial eye movements were induced without accommodation by pulling on the extraocular muscle sutures in two rhesus eyes. Artificial movement was induced several times for each quadrant, and the position of the CPs and lens equator relative to the corneal suture position was measured.

Recording of the motion of the CPs was limited by the presence of prismatic distortion, but this did not affect overall results or conclusions, because the CPs and lens were always oriented in the middle of the field of view through the gonioscopy lens. For calibration of the extent of CP and lens movements, a 30-gauge needle was inserted into the anterior chamber just slightly anterior to the level of the zonular fibers in one eye, and the image was recorded goniovideographically. The gonioscopy image of the known-diameter 50-gauge needle in the anterior chamber was then measured to provide a calibration in pixels per millimeter. This calibration technique used the true in vivo situation (i.e., true anterior chamber depth and distance to CP/lens equator interface) and was also validated by ultrasound biomicroscopy (50 mHz).14

The movement values presented are the millimeters of change from baseline. The lens and CP movements were measured from 3 to 4 seconds of recorded videocassette with a frame-by-frame analysis spanning the ~2.2-second stimulus period. An image-management program (Optimas; Media Cybernetics Inc., Silver Spring, MD) was used to save images from tape to hard drive and to analyze movements. Time to the nearest one thirtieth of a second and onset and termination of electrical stimulation were electronically encoded on the videotape. The measurements, together with the corresponding video frame numbers and the times of onset and termination of the stimulus, were recorded in a data file. CP movements were measured from the corneal marking suture to the tips of three to five well-defined CPs (Fig. 1). Lens movement was measured from the corneal marking suture to the vertical position corresponding to the lens equator (Fig. 1). Lens equator and CP movements were measured on the same images. Data were collected only from image sequences where the measurement reference points could be viewed throughout the entire accommodation/disaccommodation sequence. Video sequences of three responses were analyzed for each stimulus amplitude and were averaged. The
maximum amplitude of the centripetal CP and lens movement was calculated by averaging the measurements from 20 consecutive frames beginning 25 frames before termination of the stimulus (i.e., when the eye was in the stable accommodated state) and subtracting the measurements of the eye in the unaccommodated state (taken from a single image, 11 frames before the stimulus onset).

E-W Stimulation

The stimulus current amplitude (at constant frequency of 100 Hz, with a 500-μs pulse duration) versus accommodative response relationship was established for each monkey. Goniovideography recordings of the CPs, zonule, and lens equator movements were then made during stimulation at several current amplitudes. Beginning at threshold (the level needed to induce a minimum level of accommodation), the stimulus was increased by consistent increments available on the stimulus isolation unit until maximum accommodation was reached (maximal stimulus level). The stimulus level was then increased again by the same increment above the maximal stimulus (supramaximal stimulus level). This method was used, not only to ensure that maximum CP and lens movement had been achieved. Because of technical limitations, the amount of current increase above the maximal stimulus was not immediately known during each experiment but was retrospectively calculated. The supramaximal stimulus was calculated to be a current 0.1 to 0.2 mA or 26.2% ± 3.9% above that necessary to induce maximum accommodation.

Ultrasound Biomicroscopy

The anesthetized monkeys were placed supine with the head stabilized facing upward in a head holder and a saline fluid-well was placed around the eye.15,16 The eye was rotated using a suture passed beneath the lateral rectus muscle.16 A 50-MHz ultrasound biomicroscopy (UBM) instrument (model 840; Carl Zeiss Meditec, Inc., Dublin, CA) was used to image the lens, zonule, and ciliary body configuration at rest and during accommodation16 while recording to SVHS tape. Measurements taken from the tape are accurate to within a few pixels (i.e., 4 pixels ≈ 0.046 mm), as a result of the ultrasound’s passing through tissues of different densities. However, the error should not influence the results appreciably, since the anterior–posterior ends of the ciliary body were oriented in a horizontal direction within all images. Comparisons were made between the images in the unaccommodated and accommodated states. The angle between the anterior aspect of the ciliary body (CB) and the inner aspect of the cornea (CB–cornea angle) was measured in the unaccommodated (resting) eye and during supramaximal stimulation. CB–cornea angle change was defined as the CB-cornea angle in the unaccommodated (resting) state minus the CB-cornea angle in the supramaximally stimulated state. Narrowing of the CB-cornea angle in the accommodated versus the unaccommodated state was used as a surrogate indicator of forward CB movement and will be referred to as such hereafter.

All analyses, images and data refer to the rhesus monkey eyes except where the single cynomolgus eye is specifically indicated (UBM images only).

Statistical Analysis

Simple linear regression (i.e., CP movement versus age, lens movement versus age, CP movement versus accommodation, and lens movement versus accommodation) and multiple regression analysis (i.e., accommodation versus age and CP movement; accommodation versus age and lens movement) were undertaken in all monkeys. The multiple regression analysis adjusted for the relatedness between two observations (i.e., instances where there were two eyes from the same monkey). Regression analysis that adjusts the relatedness between two observations has no associated correlation coefficient. The model due diligence diagnostics were performed, and, based on the residual plots, the proposed models are not unduly affected by variance fluctuations. There was no specific pattern in the residual plots and therefore no concern as to the validity of the model.

The expected lifespan of the rhesus monkey in captivity is ~35 years. In a separate analysis, monkeys up to 10 years of age (at ±25%–30% of their expected lifespan) were grouped into a ‘young’ age category, and monkeys 17 to 26 years of age were grouped in an ‘older’ age category (at ±45% of their expected lifespan). The mean ± SEM CP and lens movement were calculated for each group. The intermediate age group (11–16 years) was not included in this analysis.

Definitions

Maximal stimulus: the level of E-W stimulus current necessary to induce maximum refractometrically measured accommodative change. Supramaximal stimulus: a level of E-W stimulus current 3.9% above that necessary to induce maximum accommodation. 1.0 to 0.2 mA or 26.2% ± 3.9% above that necessary to induce maximum accommodation.

Results

Accommodation versus Age

Maximum accommodative amplitude ranged from 20.1 D (age, 6 years) to 3.4 D (age, 24 years). Centrally stimulated maximum accommodative amplitude declined with age by 0.76 ± 0.07 D/yr (P = 0.001; n = 22; r = 0.93; Fig. 2). Previous findings in rhesus monkeys (Fig. 2; Neider et al.7) and humans (Fig. 2; Duane19) were compared with the current findings (see the
they came in contact with the lens (Fig. 4H), causing the lens to shift nasalward (Fig. 4G), perhaps because the CPs were not in contact with the lens nasally.

**Average Centripetal CP and Lens Movement**

The Young Eye. In the young eyes (age range, 5.8–9.5 years; average, 7.2 ± 0.7 [mean ± SEM]), the lens equator moved in close association with CP movement (Fig. 3). The average maximum accommodation was 13.50 ± 0.06 D. At the maximal stimulus (Figs. 3A, 3B), the amplitude of the centripetal CP and lens movement was 0.41 ± 0.03 and 0.35 ± 0.02 mm, respectively, in the nasal quadrant and 0.39 ± 0.01 and 0.31 ± 0.02 mm, respectively, in the temporal quadrant. At the supramaximal stimulus, the older eye CP movement was 41% (0.17 mm) and 31% (0.12 mm) less in the nasal and temporal quadrants, respectively. During maximal stimulation, the older eye CP movement was 3.3 ± 0.7 D, nearly identical with group A. However, the amplitude of CP movement was less in both quadrants (nasal, P = 0.062; temporal, P = 0.01) in this group (Figs. 4K, 4L versus Figs. 4G, 4H), and the temporal, but not nasal, lens equator movement was also significantly less (nasal, P = 0.51; temporal, P = 0.006; Figs. 4K, 4L versus 4G, 4H).

In one other monkey eye (age, 22 years), the temporal CPs were in contact with the lens at rest, and the nasal CPs came in contact with the lens 0.75 second after the onset of stimulation. The lens did not shift nasalward (data not shown). The eye accommodated 4 D.

Compared with the younger eyes, during maximal stimulation the older eye CP movement was 41% (0.17 mm) and 31% (0.12 mm) less in the nasal and temporal quadrants, respectively. Lens movement was 66% (0.23 mm) and 65% (0.20 mm) less in the nasal and temporal quadrants, respectively. During supramaximal stimulation, the older eye CP movement was only 27% (0.12 mm) and 12% (0.05 mm) less in the nasal and temporal quadrants, respectively. Lens movement was 65% (0.24 mm) and 57% (0.18 mm) less in the nasal and temporal quadrants, respectively. The older eye accommodated 3.3 ± 0.4 D (76%, 10.2 D) less than the young eye (Figs. 3, 4).

**Residual Eye Movements and Variance**

The mean ± SEM amount of eye movement that occurred during supramaximal stimulation was 0.17 ± 0.03 mm in 27 monkey eyes. A small amount of variability was introduced by convergence eye movement, but the values were close to or less than the SD of the experimental measurements (±0.05 mm) and cannot account for the accommodative movements observed. The error bars (SEM) were small compared with the amplitude of movement (Figs. 3, 4). If there were an effect from eye movement, one would expect consistent, significant differences in CP and lens equator movement between nasal and temporal quadrants, irrespective of age.

Mean ± SEM centripetal lens and CP movements calculated over three successive responses at maximal and supramaximal stimulation showed a residual mean of 0.062; temporal, 0.01) in this group (Figs. 4K, 4L versus Figs. 4G, 4H), and the temporal, but not nasal, lens equator movement was also significantly less (nasal, P = 0.51; temporal, P = 0.006; Figs. 4K, 4L versus 4G, 4H).

The eye accommodated 4 D.

Compared with the younger eyes, during maximal stimulation the older eye CP movement was 41% (0.17 mm) and 31% (0.12 mm) less in the nasal and temporal quadrants, respectively. Lens movement was 66% (0.23 mm) and 65% (0.20 mm) less in the nasal and temporal quadrants, respectively. During supramaximal stimulation, the older eye CP movement was only 27% (0.12 mm) and 12% (0.05 mm) less in the nasal and temporal quadrants, respectively. Lens movement was 65% (0.24 mm) and 57% (0.18 mm) less in the nasal and temporal quadrants, respectively. The older eye accommodated 3.3 ± 0.4 D (76%, 10.2 D) less than the young eye (Figs. 3, 4).

**Residual Eye Movements and Variance**

The mean ± SEM amount of eye movement that occurred during supramaximal stimulation was 0.17 ± 0.03 mm in 27 monkey eyes. A small amount of variability was introduced by convergence eye movement, but the values were close to or less than the SD of the experimental measurements (±0.05 mm) and cannot account for the accommodative movements observed. The error bars (SEM) were small compared with the amplitude of movement (Figs. 3, 4). If there were an effect from eye movement, one would expect consistent, significant differences in CP and lens equator movement between nasal and temporal quadrants, irrespective of age.
Stimulus levels delivered to the E-W nucleus were plotted (Supplementary Fig. S1, available online at http://www.iovs.org/cgi/content/full/47/3/1076/DC1) versus time for one young rhesus monkey eye. At each time point during the stimulation, the SEM of the three values was typically small (i.e., 0.005–0.02 mm), indicating fairly consistent CB and lens movement for a given stimulus level and monkey eye.

Stimulus Amplitudes

The older eye required lower stimulus levels to achieve maximum accommodation than did the young eye (Table 1, Fig. 5A). The average increase in stimulus current between the maximal and supramaximal stimulus settings was 0.10 \(\pm\) 0.01 mA or 26.2\% \(\pm\) 3.9\% for the young and the older monkeys combined (Table 1A). The mean difference between the supramaximal and maximal current amplitudes was 0.17 \(\pm\) 0.03 mA (21.1\% \(\pm\) 7.4\%) for the young eye and 0.08 \(\pm\) 0.01 mA (28.2\% \(\pm\) 4.7\%) for the older eye. The mean increase in absolute current amplitude (maximal versus supramaximal stimulation in mA) was higher for the young eye versus the older eye (\(P = 0.016\)) but not when expressed as a percentage of current increase (Table 1A). Although the change in stimulus amplitude from maximal to supramaximal was significantly greater in the young eye than in the older eye (when expressed in milliamperes), the magnitude of increased CP movement was significantly higher in the older eye (Figs. 3, 4, 5B; Table 1B). Further, in some older eyes only able to accommodate 2 to 4 D, the magnitude of the CP centripetal movement was in the range of that in the young monkey (Fig. 5B) during the supramaximal stimulus. There was no significant increase in lens centripetal movement between the maximal and supramaximal stimulus current in either age group (Figs. 3, 4, 5C; Table 1C). The average current necessary to induce 3.0 \(\pm\) 0.6 D of accommodation in the young eye was 0.45 \(\pm\) 0.11 mA, similar to the current (0.41 \(\pm\) 0.07 mA) necessary to induce maximum accommodation of 3.0 \(\pm\) 0.5 D in the older eye.

The amplitude of maximum accommodation correlated significantly with the magnitude of stimulus current needed to achieve it in 21 eyes of 14 monkeys ranging in age from 6 to 26 years (Fig. 5A). The maximal and the supramaximal stimulus currents were 59\% (0.60 mA) and 58\% (0.69 mA) less, respectively, in the older eye than in the younger eye.

Ciliary Body Configuration by UBM

The Young Eye.

During accommodation, the anterior aspect of the CB moved forward past the scleral spur at higher accommodative amplitudes (Fig. 6D), and formed an acute angle with the inner aspect of the cornea (CB-cornea angle) in both quadrants. We used the same supramaximal stimulus level as given during goniovideography, not only to ensure that we had reached maximum accommodative CB movement but also for comparison to the CP centripetal movement. The analysis in the temporal quadrant was used to compare forward versus centripetal (CP) CB movement, because the amount of centripetal CP movement was reduced by only 12\% (0.05 mm) in the older eye versus the young eye (Figs. 3, 4). The average temporal CB-cornea angle in the unaccommodated and supramaximally stimulated young eye was 157.5 \(\pm\) 3.8\° and 84.1 \(\pm\) 2.8\°, respectively (\(n = 6\) eyes of five monkeys; Table 2A).
In the older eye the anterior aspect of the CB did not move past the scleral spur (Fig. 6F) in either quadrant. The average temporal CB-cornea angle in the unaccommodated and supramaximally stimulated older eye was 147.5° ± 2.5° and 123.5° ± 3.1°, respectively (n = 11 eyes of eight monkeys; Table 2A).

The magnitude of the forward movement in the younger eye (73.0° ± 6.4°, n = 6) was significantly greater (P < 0.001) than in the older eye (24.0° ± 3.0°, n = 11) by 67.1% (49°).

The Older Eye. In the older eye the anterior aspect of the CB did not move past the scleral spur (Fig. 6F) in either quadrant. The average temporal CB-cornea angle in the unaccommodated and supramaximally stimulated older eye was 147.5° ± 2.5° and 123.5° ± 3.1°, respectively (n = 11 eyes of eight monkeys; Table 2A).

The magnitude of the forward movement in the younger eye (73.0° ± 6.4°, n = 6) was significantly greater (P < 0.001) than in the older eye (24.0° ± 3.0°, n = 11) by 67.1% (49°).
during supramaximal stimulation. In these same eyes (stimulated at the supramaximal level) centripetal CP movement (as measured from goniovideography images) was only 22.5% (0.09 mm) less in the older eye than in the young eye (Table 2B; \( n = 5 \) young rhesus eyes, \( n = 11 \) older rhesus eyes). Centripetal lens equator movement was 49.3% (0.15 mm) less in the older eye than in the young eye. See the following section for the results of regression analysis involving CB forward movement.

### Regression Analysis

#### Centripetal Lens and CP Movement during Accommodation.

The amplitude of gonioscopically measured CP centripetal movement during maximum accommodation declined significantly with age in both the nasal (\( P = 0.05 \)) and temporal (\( P = 0.02 \)) quadrants (28 eyes, 21 monkeys; Figs. 7A, 7B). At the supramaximal stimulus level, the amplitude of the temporal CP movement did not decline significantly with age (\( P = 0.065 \)).

#### Table 2. CB-Cornea Angle and Temporal Centripetal Movement

<table>
<thead>
<tr>
<th>A. Temporal CB-Cornea Angle Measurements (degrees)</th>
<th>B. Smax Temporal Centripetal Movement (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Resting, Smax] [°]</td>
<td>[Resting-Smax, n]</td>
</tr>
<tr>
<td>Young</td>
<td>157.5, 84.1</td>
</tr>
<tr>
<td>Mean</td>
<td>5.8, 2.8</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Older</td>
<td>147.5, 123.5</td>
</tr>
<tr>
<td>Mean</td>
<td>2.5, 3.1</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Young vs. older, ( p = ) 0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Decline, Older vs. Young (%)</td>
<td>67.1</td>
</tr>
</tbody>
</table>

(A) Data are the mean ± SEM angle between the anterior aspect of the ciliary body and the inner aspect of the cornea (CB-cornea angle) in the temporal quadrant measured in degrees. Measurements were taken from UBM images in the unaccommodated (resting) eye and during supramaximal (Smax) stimulation in 5 young rhesus and 1 young cynomolgus (age range, 5.8–9.5 years), and in 11 older rhesus monkey eyes (age range, 17–26 years). The farther the ciliary body moved forward during accommodation, the more narrow the CB-cornea angle. (B) Data are the mean ± SEM centripetal CP and lens movement amplitude (mm) in the unaccommodated eye and during supramaximal stimulation as measured from goniomedical images taken in the same eyes as in (A). \( P \leq 0.05 \) denotes a significant difference between young and older monkey eye by the two-sample \( t \)-test. Percentage of decline older versus young is calculated as \([\text{older}/\text{young}] - 1\) × 100. Loss of forward ciliary body movement as represented by accommodative CB-cornea angle change was more pronounced than loss of centripetal movement in the temporal quadrant.
The amplitude of CP centripetal movement was significantly correlated with accommodation (Fig. 8A, 8B) at the maximal but not at the supramaximal stimulus level (Figs. 8C, 8D). Because CP movement and accommodation covaried with age, we modeled accommodation as a linear function of age and CP movement, by using a multiple regression analysis. The multiple regression coefficient of nasal or temporal CP centripetal movement (at either stimulus level) was not significantly different from 0.0 (Supplementary Table S1A, http://www.iovs.org/cgi/content/full/47/3/1076/DC1), indicating that age and CP movement together could not predict accommodation better than age alone.

At the maximal stimulus level, the amplitude of gonioscopically measured lens centripetal movement significantly declined with age in both nasal \((P < 0.001)\) and temporal \((P = 0.005)\) quadrants (Figs. 7A, 7B). The multiple regression coefficient of averaged (nasal and temporal) lens centripetal movement was different from 0.0 (Supplementary Table S1B, http://www.iovs.org/cgi/content/full/47/3/1076/DC1), indicating that age and average lens equator movement (nasal and temporal) could predict accommodation better than age alone. Similar results were recorded at the supramaximal stimulus level.

**Forward CB Movement.** In the temporal quadrant (the only quadrant thus far studied), the amount of UBM-measured
CB forward movement (measured by accommodative CB-cornea angle change; 21 eyes, 17 monkeys) declined significantly with age (−2.71 ± 0.39 deg/y; \( P = 0.01; \; n = 21; \; \text{Fig. 9B} \)). Further, there was a significant relationship between the amplitude of UBM-measured CB forward movement and the amplitude of gonioscopically measured centripetal lens movement (0.0031 ± 0.00085 mm/deg; \( n = 19; \; P < 0.002; \; \text{Fig. 9C} \)), and between forward movement and maximum accommodative amplitude measured refractometrically (3.34 ± 0.54 deg/D; \( P = 0.01; \; n = 21; \; \text{Fig. 9A} \)). Thus, the greater the CB forward movement, the greater the lens equator movement and the higher the maximum accommodative amplitude. The multiple regression coefficient of CB forward movement was not different from 0.0 (Supplementary Table S1C, http://www iovs.org/cgi/content/full/47/3/IOVS41076/D1C), indicating that age and temporal CB forward movement could not predict accommodation better than age alone.

**DISCUSSION**

Presbyopia has been attributed to increased hardness of the lens,\(^1\)\(^{-}\)\(^2\)\(^{-}23\) lens growth,\(^22,24^{-}30\) and loss of elasticity of the ciliary muscle's posterior attachments.\(^31,32\) The ciliary muscle-body configurational change required to induce accommodation of the lens includes both inward and forward movement, allowing the lens to thicken and the lens curvatures to increase. The loss of elasticity of the ciliary muscle's posterior attachments may cause the age-related loss of the CB's ability to undergo configurational change to induce accommodation. In the enucleated old rhesus monkey eye, ciliary muscle configurational change in response to cholinergic agonist drug stimulation is lost, but is restored when the posterior attachments of the ciliary muscle are cut.\(^33\) In the present study, centrally stimulated maximum accommodative amplitude declined linearly with age by 0.76 ± 0.07 D/y (\( P = 0.001; \; r = 0.93 \)). This is similar to previous findings in rhesus monkeys (Fig. 2)\(^7\) where centrally stimulated accommodation declined by 0.57 ± 0.10 D/y (\( r = 0.85; \; n = 14 \)), and, when adjusted for lifespan, resembles the age-related decline in voluntary accommodation in humans (Fig. 2).\(^10\) Combining the previous data\(^10\) with the current data, centrally stimulated accommodation declined by 0.65 ± 0.05 D/y (\( r = 0.91; \; n = 36 \)).

The centripetal CP movement data suggest that the neurologic pathway in the older monkey functions to induce centripetal CP accommodative responses at least as well as in the young monkey. The cholinergic neuromuscular mechanisms subserving the ciliary muscle remain intact with age.\(^34\) Ciliary muscle contractile responses to pharmacological stimulation in vitro did not vary markedly with age in the coronal or longitudinal vector,\(^35\) suggesting that the age-related decrease in ciliary muscle mobility in vivo was due to extramuscular restrictive factors rather than diminished muscular contractility.

In the present study, we used a consistent viewing angle along the A-P axis of the eye, important for quantitative comparisons. Prior gonioscopy studies of accommodation in the rhesus monkey\(^7\) used a viewing angle of 35° from the A-P axis, which can result in the appearance of anomalous accommodative lens equator movements.

CP movement in older monkeys increased significantly at supramaximal versus maximal stimulation, but lens equator movement did not. Also, the amplitude of lens equator centripetal movement still declined significantly with age at both maximal and supramaximal stimulus levels. In addition, across the entire age range studied, age and lens centripetal movement together could predict accommodation better than age alone. Collectively, these data support the lens playing a role in presbyopia. However, the decreased centripetal lens movement may also be a consequence of decreased forward CB movement, given the significant correlation between them (\( P < 0.002 \)).

The amplitude of CP centripetal movement that occurred with maximum accommodation declined significantly with age in both the nasal and temporal quadrants, corroborating previous qualitative observations.\(^7\)\(^8\) However, the maximum possible centripetal CP movement (during supramaximal stimulation) was significantly greater (0.09 mm; \( P < 0.05 \)) than that necessary to produce maximum accommodation in the older eye. The increase between supramaximal and maximal stimulus amplitudes produced a more dramatic increase in centripetal CP movement in the older eye versus the young eye (Fig. 9).
tive changes in the lens are induced by vitreous pressure,41–43 and the theory of accommodation, which suggests that accommodation involves both the centripetal and forward movement of the CB. Loss of forward CB movement in the older versus the young eye was more pronounced than loss of centripetal movement, at least in the temporal quadrant. Temporal CB forward movement (as measured by CB-cornea angle) change was 67.1% (49°) less in the older eye than in the young eye at supramaximal stimulation, perhaps sufficient to explain the 76% (10.2 D) loss in accommodative amplitude. The magnitude of the decline is similar and there is a significant correlation between the two parameters. In light of these findings, it may be that there are differences in the age effect on forward versus centripetal CB movement, perhaps consequent to stiffening of the ciliary muscle’s posterior attachments (posterior tendons and/or the elastic lamina of Bruch’s membrane) with age.52–53 Morphologic studies showed an age-related decline in forward muscle movement (76.3%; as measured by apical position), but not centripetal movement (as measured by muscle width),53 and that the ciliary muscle in the older monkey can move forward as in the young monkey when posterior attachments are cut.53

An alternative theory of accommodation postulated by Schachar et al.50 and Tscherning51 speculate that the lens equator moves toward, rather than away from, the sclera during accommodation. Schachar50 also speculates that presbyopia is due to a putative age-related increase in lens equatorial diameter. These ideas have led to surgical interventions such as anterior ciliary sclerotomy and scleral expansion.28 Recent reports, however, show that accommodation is not restored in these patients.37–40

Collectively, these data show that accommodation is related to both centripetal and forward CB movement. The fact that the age-related loss of centripetal movement is not as dramatic as the loss of forward movement suggests that the age-related loss of accommodation may be caused in part by something else, in addition to the loss of centripetal muscle movement. In relation to the Helmholtz theory,1 accommodation requires both centripetal and forward movement of the CB, and the age-related loss of accommodation may be related to the loss of forward movement of the CB. In relation to the Coleman theory of accommodation, which suggests that accommodative changes in the lens are induced by vitreous pressure,41–43 the age-related loss of centripetal CB movement may affect the vitreous support of the peripheral lens. However, normal accommodative changes occur in eye bank eyes that are devoid of vitreous forces.16,44

Researchers have reported that the forces necessary to mold the lens into a conoid shape are greater than the capsule could exert.45 Coleman and Fish43 suggest that the capsule does not have the elastic properties to round up the lens reproducibly and rapidly during accommodation but provide no evidence to support this statement. Krag et al.36,43 suggest that the viscoelastic characteristics of the lens capsule are sufficient to perform the molding of the lens during accommodation. Their findings are supported by recent studies suggesting that the accommodative changes in the lens are produced by the capsule’s molding the lens.18,22,44,48,49

The asymmetry of the lens equator movement in group A older monkeys (in which the CPs touched the lens in the temporal quadrant) resulted from (1) the loss of lens centripetal accommodative movement with age; (2) the smaller resting temporal CLS compared with the nasal quadrant14; (3) the still-substantial temporal CP centripetal accommodative movement; and (4) the nasaward shift in lens position. This asymmetry does not seem to affect accommodative amplitude, because accommodative amplitude was almost the same whether the asymmetry was present or not (group A versus group B older animals). During accommodation, before the lens movement reached a plateau or began its nasaward shift in position, there was greater lens movement per unit of CP movement in the nasal versus the temporal quadrant of the older eye. This could be due to nasal versus temporal CB configurational differences.16

Although the lens no doubt plays a major role in presbyopia, altered lens movement could be in part secondary to extraintracranial age-related changes, such as loss of CB forward movement. The CB centripetal movement may not be the limiting component in accommodation in the older eye. The results show that accommodative centripetal movements of the ciliary processes and therefore the ciliary muscle are still present in the old rhesus monkeys, as they are in the presbyopic human,50 despite the reduced accommodative amplitudes. Whether the remaining movement is sufficient to power the system and produce accommodation post-IOL implantation depends on the approach and the characteristics of the accommodating IOL material. Accommodating IOls may be more effective in restoring accommodation in the presbyopic eye if they rely on centripetal CB movement rather than forward CB movement.

Acknowledgments
The authors thank James Reed for providing technical expertise with the image-analysis systems; Kathy DePaul for computer programming of the image analysis systems; and Christopher Mullin, Jared Heine, and Christopher Swoboda for assistance with image analysis, documentation, and data management.

References