Surgical Intervention and Accommodative Responses, I: Centripetal Ciliary Body, Capsule, and Lens Movements in Rhesus Monkeys of Various Ages

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Purpose. To determine how surgically altering the normal relationship between the lens and the ciliary body in rhesus monkeys affects centripetal ciliary body and lens movement.

Methods. In 18 rhesus monkey eyes (aged 6–27 years), accommodation was induced before and after surgery by electrical stimulation of the Edinger-Westphal nucleus. Accommodative amplitude was measured by coincidence refractometry. Goniovideography was performed before and after intra- and extracapsular lens extraction (ICLE, ECLE) and anterior regional zonulolysis (ARZ). Centripetal lens/capsule movements, centripetal ciliary process (CP) movements, and circumlental space were measured by computerized image analysis of the goniovideography images.

Results. Centripetal accommodative CP and capsule movement increased in velocity and amplitude after, compared with before, ECLE regardless of age ($n = 5$). The presence of the lens substance retarded capsule movement by ~21% in the young eyes and by ~62% in the older eyes. Post-ICLE compared with pre-ICLE centripetal accommodative CP movement was dampened in all eyes in which the anterior vitreous was disrupted ($n = 7$), but not in eyes in which the anterior vitreous was left intact ($n = 2$). After anterior regional zonulolysis ($n = 4$), lens position shifted toward the lysed quadrant during accommodation.

Conclusions. The presence of the lens substance, capsule zonular attachments, and Wieger's ligament may play a role in centripetal CP movement. The capsule is still capable of centripetal movement in the older eye (although at a reduced capacity) and may have the ability to produce ~6 D of accommodation in the presence of a normal, young crystalline lens or a similar surrogate. (*Invest Ophthalmol Vis Sci.* 2008;49:5484–5494) DOI:10.1167/iovs.08-1916

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Presbyopia (the loss of accommodation with age) has been attributed to increased hardness of the lens,[1–7] lens growth,[6,8–14] and loss of elasticity of the ciliary muscle's posterior attachments.[15,16] No individual over the age of 45 years appears exempt, making presbyopia the most common ocular affliction in the world. Although certainly not a blinding condition and correctable by various optical means, presbyopia's cost in devices and lost productivity is substantial.

Since the accommodative apparatus of the rhesus monkey is similar to that of the human,[17–19] and both species develop presbyopia on a time scale similar to lifespan[19–21] experimental studies elucidating the role of the various structures for development of presbyopia can be performed in the rhesus. In addition, this study for the first time characterizes the function of the eye's various accommodative components after lens extraction procedures, which are undertaken in millions of human patients every year (either unilaterally, bilaterally at separate time points, or in some cases bilaterally during the same surgery day). The information generated from these studies is critical to the successful development of an accommodating intraocular lens (IOL).

We determined how surgically altering the normal relationship between the lens and the ciliary body in live rhesus monkeys 6 to 27 years of age affects the normal centripetal movements of the ciliary body and lens/capsule.

Materials and Methods

Details of all equipment and animal handling procedures for anesthesia, iridectomy, electrode implantation, central stimulation, measurement of accommodation, image calibration, goniovideography, velocity,[22] and measurements have been described previously.[20,23–26] All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were in accordance with institutionally approved animal protocols.

Monkeys

Twenty-five rhesus monkeys (*Macaca mulatta*) of either sex, aged 6 to 27 years and weighing 5.0 to 13.4 kg, were studied. The monkeys all had normal phakic eyes with no signs of ocular disease (other than age-related lenticular opacification), as assessed by slit lamp examination. Animals were evaluated for corneal clarity and anterior chamber and anterior vitreous inflammatory reaction before and after surgery.

Eight of the monkeys, aged 17 to 26 years and weighing between 7.2 and 9.5 kg, were designated solely for measurements used in the calculation of the normal velocity of the ciliary process (CP) and lens centripetal movements during accommodation and disaccommodation at supramaximum central stimulation.

The remaining 17 monkeys underwent surgical procedures. Baseline measurements of the normal accommodative response in the eyes of each animal were taken before the surgical procedures were performed. Three lens surgery procedures (described later) were used in the study: intracapsular lens extraction (ICLE), extracapsular lens extraction (ECLE), and anterior regional zonulolysis (ARZ).

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Table 1. Division of Study Animal Eyes According to Age and Surgical Procedure

	ICLE	ECLE	ARZ
Young eyes (n = 9; 6–13 y)	5*	3	1
Older eyes (n = 9; 17–27 y)	4†	2	3
Total (n = 18)	9	5	4

ARZ, anterior regional zonulolysis.

* This group was further divided according to whether Wieger's ligament was intact (n = 2) or disturbed (n = 3).

† ICLE eyes in which Wieger's ligament was disrupted.

From the 17 monkeys designated for surgery, 18 eyes underwent successful surgical procedures. These 18 eyes were divided into two groups, according to age, to allow comparisons of young (6–13 years) versus older (17–27 years) eyes (Table 1). The two age groups were then further subdivided into three groups, each comprising both young and older monkeys, according to which surgical procedure they underwent (Table 1). No surgical group had two eyes from the same monkey.

Ten monkeys (five young; five older) each provided one eye for surgical intervention (ICLE, n = 4; ECLE, n = 2; ARZ, n = 4). The opposite eye was iridectomized but was otherwise surgically untouched and served as a contralateral control eye for morphologic examination.

One additional young monkey also provided one eye for the ICLE procedure (ICLE, n = 1), with its opposite eye iridectomized but otherwise surgically untouched; however, this monkey was not euthanatized and was retained for another study. Therefore, this eye did not undergo morphologic examination. Another older monkey contributed one eye to the ICLE group and, in a subsequent surgery, contributed the opposite eye to the ECLE group. Surgery in this monkey's second eye was allowed by the veterinary staff of our institution and the Institutional Animal Care and Use Committee, since the surgery was performed at a separate time point from the first eye, and cognitive behavior was observed by laboratory personnel and veterinary staff, to ensure that the animal was functioning normally before and after surgery. If signs of visual or other distress had been observed, the animal would have been euthanatized. However, no overt signs of distress were noted in any animal. Both of these eyes underwent morphologic examination.

In each of the five remaining monkeys (three young; two older), surgical procedures were performed initially in one eye (ICLE, n = 3; ECLE, n = 2). However, the postsurgical clinical examination of these five eyes uncovered surgical or technical complications that likely would have affected the accommodative apparatus in ways not intended by the surgery protocols, which were designed to disrupt specific parts of the accommodative apparatus. These complications were ciliary body degeneration (n = 2, ICLE), severing of the posterior zonular attachments (n = 1, ICLE), and perforation of the posterior capsule, capsular fibrosis, and lens cell regrowth with pronounced presence of pearls and Soemmering's ring (n = 2, ECLE). Therefore, the decision was made, before postsurgical imaging, not to include postsurgical imaging data for these five eyes in the study. Subsequently, in each of these five monkeys, the contralateral eye also underwent surgery (ICLE, n = 3; ECLE, n = 2). These eyes were free of postsurgical complications, based on clinical examination, and the postsurgical imaging of these eyes was completed according to protocol. Again, cognitive behavior was observed for overt signs of distress after surgery in the second eye. However, the animals' function in their cage environment appeared normal. For the older animals, the loss in accommodative ability (through either ECLE or ICLE) was not really a change since most, if not all, of their ability to accommodate had already been lost. For the younger animals, this meant an adjustment to the presbyopic condition at an earlier age. These eyes (young and older), being aphakic after surgery, lost distance acuity as well. However, because the monkeys were housed in a room within the animal care facility, their visual space and need for far distance vision was limited accordingly, and thus any aftereffects on the monkeys of this surgically induced loss of distance vision were reduced. The second eye for surgery did not appear to adversely affect the monkey's ability to function normally within the caged environment, as determined by daily observation by both laboratory and veterinary staff. The strategy of using the second eye in these monkeys, under the careful constraints indicated, avoided major intracranial surgery in additional monkeys. All the eyes included in the study underwent morphologic examination.

In summary, 26 eyes from 25 monkeys were used in the study: eight eyes were used solely to obtain measurements for calculating normal CP velocity and lens centripetal movements; 18 eyes from the remaining 17 monkeys underwent successful surgical procedures: ICLE (nine monkey eyes; five young, four older), ECLE (five monkey eyes; three young, two older), and ARZ (four monkey eyes; one young, three older).

Surgical Procedures

Intracapsular Lens Extraction. ICLE, entailing removal of the entire lens and capsule, was performed by the standard clinical technique in nine monkey eyes. After a fornix-based conjunctival flap and a 150° corneoscleral limbal groove were formed, the anterior chamber was entered, the incision was extended within the groove for the full 150°, the zonule was lysed with α-chymotrypsin (83 U/mL; Sigma-Aldrich, St. Louis, MO) injected into the anterior chamber, and the lens and capsule were removed intact with a cryoprobe. In seven (three young, four older) of the monkey eyes undergoing ICLE, the α-chymotrypsin was allowed to remain in the anterior chamber for 1 to 2 minutes before rinsing and removal of the lens. Mechanical anterior vitrectomy (Ocutome) and toileting of the wound were performed as needed, and the wound was closed with interrupted sutures of 9-0 or 10-0 nylon. In the remaining two monkey eyes (both young) in this group, the α-chymotrypsin was allowed to remain in the eye for <30 seconds before fluid rinsing and removal of the lens with a cryoprobe. Wieger's ligament remained intact in these two young eyes post-ICLE, and mechanical vitrectomy was not required.

Extracapsular Lens Extraction. ECLE, entailing removal of the lens substance from within the capsule, leaving an empty capsule bag still attached to the zonula, was performed in five monkey eyes (three young; two older). The standard clinical ECLE technique was used, involving a fornix-based conjunctival flap; intracameral instillation of viscoelastic; large anterior capsulotomy (~4 mm); irrigation/aspiration ± phacoemulsification with phacoemulsification unit (series 20000 Legacy model STTL; Alcon, Ltd., Fort Worth, TX); removal of viscoelastic; and wound closure.

Regional Zonulolysis of the Anterior Zonule. In four monkey eyes (one young, three older), 2 μL of 40 U/mL of α–chymotrypsin were dissolved in heavy sucrose medium (10% sucrose solution) and injected into the anterior chamber near the anterior zonular fibers, to regionally dissolve 1 to 2 clock hours of the anterior zonule[27] ("anterior zonular fibers" in this article refers to the fibers that course to the anterior, posterior, and equatorial lens surfaces). The head of the monkey was oriented so that the heavy solution fell with gravity to the lens/anterior–zonule/muscle interface (see Movie S1, http://www.iovs.org/cgi/content/full/49/12/5484/DC1) in either the nasal (three eyes) or temporal (one eye) quadrant. The end of the needle was visualized so that when the injection was made, the solution was seen falling onto the anterior zonular fibers.

Accommodation, Stimulation, and Response Measurements

Refractometry. A Hartinger coincidence refractometer (Jena, Germany) was used to measure resting refractive error and accommo-

Table 2. CP and Lens Velocity in Normal and Postsurgical Eyes

A. Normal Eyes

	Young (n = 8 eyes; 15 quadrants)	Older (n = 15 eyes; 30 quadrants)
Accommodation		
CP	0.77 ± 0.054	0.67 ± 0.072
Lens	0.68 ± 0.047	0.34 ± 0.032*
Disaccommodation		
CP	−0.98 ± 0.15	−1.28 ± 0.18
Lens	−0.73 ± 0.11	−0.46 ± 0.05†

B. Surgically Treated Eyes

	Young (n = 3 eyes; 6 quadrants)		Older (n = 4 eyes; 7 quadrants)	
	Baseline	**Post Surgery**	**Baseline**	**Post Surgery**
ICLE + WD				
Accommodation				
CP	0.69 ± 0.082	0.41 ± 0.087*	0.32 ± 0.049	0.22 ± 0.045
Disaccommodation				
CP	−1.16 ± 0.297	−0.87 ± 0.190	−0.67 ± 0.163	−0.34 ± 0.075‡

	Young (n = 2 eyes; 4 quadrants)	
	Baseline	**Post Surgery**
ICLE + WI		
Accommodation		
CP	0.70 ± 0.120	0.72 ± 0.060
Disaccommodation		
CP	−1.11 ± 0.201	−1.47 ± 0.284

	Young (n = 3 eyes; 5 quadrants)		Older (n = 2 eyes; 4 quadrants)	
	Baseline	**Post Surgery**	**Baseline**	**Post Surgery**
ECLE				
Accommodation				
CP	0.75 ± 0.180	0.94 ± 0.238†	0.54 ± 0.119	0.68 ± 0.196
Lens/Capsule	0.69 ± 0.137	0.87 ± 0.172§	0.24 ± 0.008	0.50 ± 0.044‖
Disaccommodation				
CP	−0.97 ± 0.153	−0.85 ± 0.046	−0.68 ± 0.377	−0.98 ± 0.173
Lens/Capsule	−0.72 ± 0.093	−0.75 ± 0.039	−0.26 ± 0.071	−0.70 ± 0.035

Data represent the mean ± SEM of gonioscopically measured CP and lens velocity (mm/s)[22] in normal and postsurgical eyes during supramaximum stimulation. Comparisons between young versus older eyes (A) or between pre- and post-surgical measurements (B) by the two-tailed paired *t*-test: *P = 0.034; †P = 0.082; ‡$P < 0.054$; §P = 0.052; ‖P = 0.088. The SEMs are pooled values from two quadrants for each eye (nasal and temporal) with two exceptions: one older ICLE + WD eye provided data from one quadrant and one young ECLE eye provided data from one quadrant.

dation in response to electrical stimulation of the Edinger-Westphal (E-W) nucleus. Accommodation was stimulated centrally via the implanted electrode. Supramaximum stimulus settings were chosen (as defined later) that induced maximum accommodative responses (i.e., maximum centripetal CP and lens/capsule movement, maximum forward ciliary body movement) and maximum accommodation, allowing comparisons to be made between pre- and postsurgical accommodative responses. The forward ciliary body movements are beyond the scope of this article and are reported in the companion article in this issue.[28]

Maximum accommodative amplitude was induced, measured, and tabulated for each monkey eye during four to five separate experimental sessions before surgery.

Maximum stimulus is defined as the level of E-W stimulus current necessary to induce maximum accommodative change, measured refractometrically. *Supramaximum stimulus* is a level of E-W stimulus current ~25% (or ~0.10–0.20 mA) above the maximum stimulus that ensures maximum centripetal CP and lens movement. *Circumlental space* is the average distance from the tips of four to five CPs to the equatorial edge of the lens or capsule, as measured in the goniovideography images[23] in both the nasal and temporal quadrants (two separate locations 180° from each other).

Comparisons of circumlental space were made at rest, at maximum stimulation, and at supramaximum stimulation, before and after ECLE. All other pre- and postsurgical comparisons were made at rest versus supramaximum stimulation.

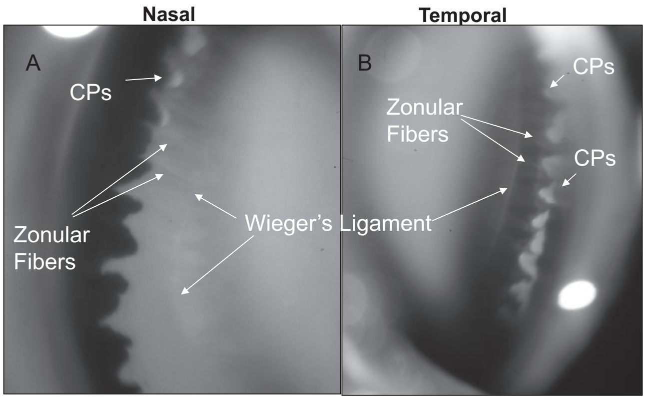

FIGURE 1. Photograph slitlamp images obtained through a gonioscopy lens, taken after (ICLE, showing intact Wieger's ligament and zonular attachments between Wieger's ligament and the CP in both the nasal (**A**) and temporal (**B**) quadrants of a 13-year-old rhesus monkey.

Goniovideography Imaging. Dynamic goniovideography images (using a Swan-Jacobs gonioscopy lens) were obtained during stimulation of accommodation and then recorded to videotape (30 frames per second).[20] Goniovideography[20] was performed before and after each surgical procedure. The postsurgical imaging sessions were performed 2.5 weeks to 3 months after surgery. Measurements were taken at the beginning of the study in all 25 monkeys, using goniovideography, to enable calculation of the normal velocity[22] of the CP and lens centripetal movements at supramaximum stimulation. In the 18 monkey eyes that underwent surgical procedures, goniovideography allowed measurement of the centripetal lens/capsule and CP movement (i.e., velocity[22] and amplitude[20]) and measurement of the circumlental space width[23] before and after surgical intervention.

Computerized analysis of the goniovideography images was used to measure the extent and dynamics of the centripetal lens and CP movements during accommodation and disaccommodation in both the nasal and temporal quadrants (180° from each other) and an average value calculated for each eye.[20] The mean ± SEM dynamic centripetal movements of the capsule edge or lens equator (if present) and four to five adjacent CPs during 2.2-second stimuli were plotted.

Histology

The animals were perfusion fixed through the heart with 4% paraformaldehyde, after perfusion with 1 L of 0.1 M PBS (phosphate-buffered saline). After enucleation, slits were cut in the posterior sclera, and a window was cut in the anterior cornea, to enhance fixative penetration and preserve the architecture of the ciliary muscle and its posterior attachment to Bruch's membrane. The eyes were then immersed in Ito's fixative[29] until they were sent to Erlangen, Germany, for morphologic investigation. Small sectors of the anterior globe, including the entire ciliary body and adjacent cornea and sclera, were embedded in Epon, and 1-μm semithin sections were cut and stained with Richardson's stain.[30]

Statistical Analysis

Average CP and lens movement amplitudes were calculated for two quadrants (nasal and temporal) of each eye at each time point (one thirtieth of a second) during stimulation. The mean ± SEM centripetal CP and lens movement amplitudes were calculated by using the movement amplitude from 20 consecutive frames, beginning 25 frames before termination of the stimulus (i.e., when the eye was in the stable accommodated state). The initial velocity of CP and lens or capsule movements was determined by the movement amplitude change from 5 to 15 frames after the beginning of the stimulus. Disaccommodative velocity was measured between 5 and 13 frames after the stimulus was discontinued. The area under the entire stimulus response curve of CP and lens or capsule movement was also calculated. The response curve included the 2.2-second stimulus duration plus the time during which the CPs or lens/capsule returned to baseline position. Comparisons were made between pre- and postsurgery velocity, amplitude, and area under the curve for both lens and CP movement.

A two-tailed paired *t*-test was used to detect significant differences. $P \leq 0.05$ was considered significant; $0.05 \leq P \leq 0.10$ was considered to indicate a trend, given the small number of monkeys.

RESULTS

To give an indication of the range in accommodative amplitudes and variability within each eye for each group, the mean ± SEM accommodative amplitude was calculated for all presurgical experimental sessions within each monkey eye. The resulting mean values for each of the young monkey eyes ranged from 16.4 ± 1.5 to 18.2 ± 0.2 D, whereas the resulting mean values for each of the older monkey eyes ranged from 0.9 ± 0.1 to 6.9 ± 0.5 D.

Centripetal CP Movement as Measured by Goniovideography

During accommodation and disaccommodation, the velocity of the older lens was less than in the young eyes (accommodation, $P = 0.034$; disaccommodation, $P = 0.082$; Table 2). In contrast, the velocity of the CPs during the same period was similar in the young and older eyes.

Intracapsular Lens Extraction. Post-ICLE, CP movement depended on whether the anterior vitreous (i.e., Wieger's ligament) was intact. In the eyes in which α-chymotrypsin stayed for <30 seconds and no vitrectomy was performed, Wieger's ligament was intact. In these eyes, we also observed zonular attachments between Wieger's ligament and the CPs (Fig. 1, Movie S2, http://www.iovs.org/cgi/content/full/49/12/5484/DC1). Eyes were grouped according to whether Wieger's ligament was disrupted (ICLE+WD) or intact (ICLE+WI), for the sake of comparison.

ICLE+WI (Intracapsular Lens Extraction + Intact Wieger's Ligament and Zonular Attachments between the Ciliary Processes and Wieger's Ligament). The entire lens and capsule were removed from one eye of each of two young monkeys in the ICLE group, leaving Wieger's ligament intact (Fig. 1). (Croft MA, et al. *IOVS* 2004;45:ARVO E-Abstract 2187) The presence of Wieger's ligament and the zonular arrangement (shown in

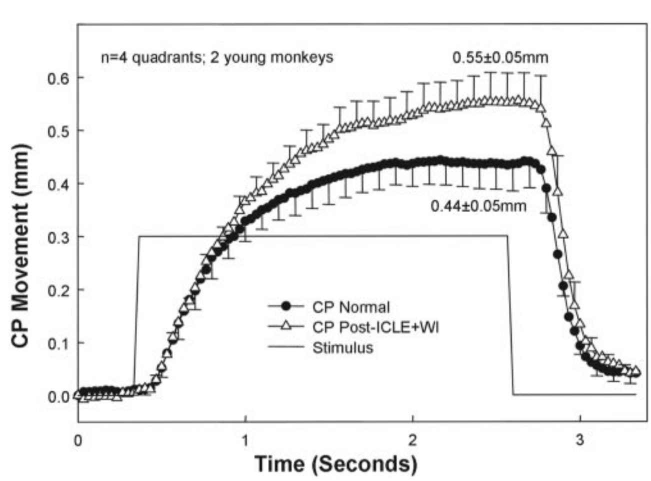

FIGURE 2. ICLE+WI. Mean ± SEM of gonioscopically measured centripetal CP movement in response to a 2.2-second electrical stimulus of the E-W nucleus before and after ICLE+WI. The data are the average of four quadrants of two young monkeys (ages, 6 and 11 years).

Table 3. CP and Lens/Capsule Movement Data in Young and Older Eyes Combined before and after Surgery

	Baseline	Post ICLE + WD	Difference	P
ICLE + WD (n = 7 [young eyes: n = 3; ages 6, 8, 9 y; older eyes: n = 4; ages 18, 19, 19, 27 y])				
CP movement				
Amplitude (mm)	0.35 ± 0.056	0.26 ± 0.053	−0.09 ± 0.042	0.072
Area under curve (mm-sec)	0.72 ± 0.118	0.52 ± 0.107	−0.19 ± 0.084	0.060
Accommodation velocity (mm/sec)	0.48 ± 0.086	0.31 ± 0.056	−0.17 ± 0.062	0.03
Disaccommodation velocity (mm/sec)	−0.88 ± 0.173	−0.57 ± 0.135	0.31 ± 0.109	0.03
	Baseline	**Post ICLE + WI**	**Difference**	***P***
ICLE + WI (n = 2; ages 8, 11.5 y)				
CP movement				
Amplitude (mm)	0.44 ± 0.05	0.55 ± 0.05	0.11 ± 0.038	0.22
Area under curve (mm-sec)	0.91 ± 0.097	1.09 ± 0.155	0.18 ± 0.058	0.20
Accommodation velocity (mm/sec)	0.70 ± 0.120	0.72 ± 0.060	0.02 ± 0.05	NS
Disaccommodation velocity (mm/sec)	−1.11 ± 0.201	−1.47 ± 0.284	−0.37 ± 0.25	NS
	Baseline	**Post ECLE**	**Difference**	***P***
ECLE (n = 5 [young eyes: n = 3; ages 6, 7, 9 y; older eyes: n = 2; ages 22, 23 y])				
CP movement				
Amplitude (mm)	0.39 ± 0.054	0.47 ± 0.075	0.08 ± 0.036	0.094
Area under curve (mm-sec)	0.81 ± 0.124	0.98 ± 0.178	0.17 ± 0.079	0.092
Accommodation velocity (mm/sec)	0.67 ± 0.118	0.84 ± 0.158	0.17 ± 0.043	0.016
Disaccommodation velocity (mm/sec)	−0.85 ± 0.146	−0.90 ± 0.069	−0.05 ± 0.131	NS
Lens/capsule movement				
Amplitude (mm)	0.26 ± 0.071	0.38 ± 0.072	0.12 ± 0.027	0.012
Area under curve (mm-sec)	0.56 ± 0.153	0.80 ± 0.163	0.24 ± 0.067	0.022
Accommodation velocity (mm/sec)	0.51 ± 0.135	0.72 ± 0.134	0.21 ± 0.032	0.002
Disaccommodation velocity (mm/sec)	−0.54 ± 0.127	−0.73 ± 0.028	−0.20 ± 0.111	0.152

Data represent mean ± SEM of gonioscopically measured ciliary process (CP) and lens/capsule movement velocity, amplitude, and area under the movement curve (averaged from both nasal and temporal quadrants) in normal and postsurgical eyes during supramaximum stimulation, grouped regardless of age. *P* was obtained from a two-tailed paired *t*-test. The paired observation was between the presurgical response (baseline) versus postsurgical response within each eye. The SEM reported in this table are pooled values from two quadrants for each eye (nasal and temporal) with two exceptions: one older ICLE + WD eye provided data from one quadrant and one young ECLE eye provided data from one quadrant.

Movie S2, Fig. 1) was confirmed by a vitreoretinal specialist (Michael Nork, MD, University of Wisconsin-Madison) and a cataract surgeon (author GH) by slit lamp examination. Further, the Wieger's ligament structure seen in Figure 1 and Movie S2 has a smooth edge and cannot be the edge of a torn capsule remnant, as that would appear ragged rather than smooth. Wieger's ligament appeared to move centripetally in accordance with the CP accommodative response, and the zonular fibers appeared to relax (see Movie S2). After ICLE+WI (Fig. 2), the initial CP velocity was similar to that at baseline (Table 2, Fig. 2), but the area under the CP movement curve and the amplitude of CP movement that were achieved near the end of the stimulus train were greater after ICLE+WI (Table 3, Fig. 2) than at baseline before lens removal, but the difference was not statistically significant, given the small sample size.

ICLE+WD (Intracapsular Lens Extraction + Disruption of Wieger's Ligament). In the three other young monkeys, and the four older monkeys that underwent ICLE, the entire lens and capsule were removed from 1 eye and Wieger's ligament was disrupted (Fig. 3). After ICLE+WD, CP velocity declined significantly, whereas CP movement amplitude, and the area under the CP movement curve tended to decline (Table 3) compared with the same eyes presurgically, regardless of age (Fig. 3, Table 2).

Extracapsular Lens Extraction. In all five monkey eyes (three young, two older) that underwent ECLE, the lens substance was removed from the capsule, leaving an empty capsule bag (CPs, zonules, and posterior capsule left intact; Fig. 4).

At the time of postsurgical imaging, the eyes were examined for pearls, adhesions, and any age-related differences. In the young eyes, the empty capsular bags did not have folds and appeared smooth and semitransparent, with no evidence of pearls or of anterior–posterior capsule adhesions (Fig. 4A compared to 4B; Movie S3, http://www.iovs.org/cgi/content/full/49/12/5484/DC1). In the older eyes, the empty capsule bags had several folds, but also appeared semitransparent and without evidence of pearls or of anterior–posterior capsule adhesions (Fig. 4B). There was no evidence of fibrosis in or around the capsulorrhexis or inside the capsular bag in either age group at the time of postsurgical imaging. Six months after ECLE the capsular bags (young and older) began to exhibit the presence of pearls, fibrosis around the capsulorrhexis, and adhesions between the anterior and posterior capsular bag surfaces.

Lens Capsule/CP Movement

After ECLE, CP accommodative velocity increased significantly ($P = 0.02$), while movement amplitude, and the area under the

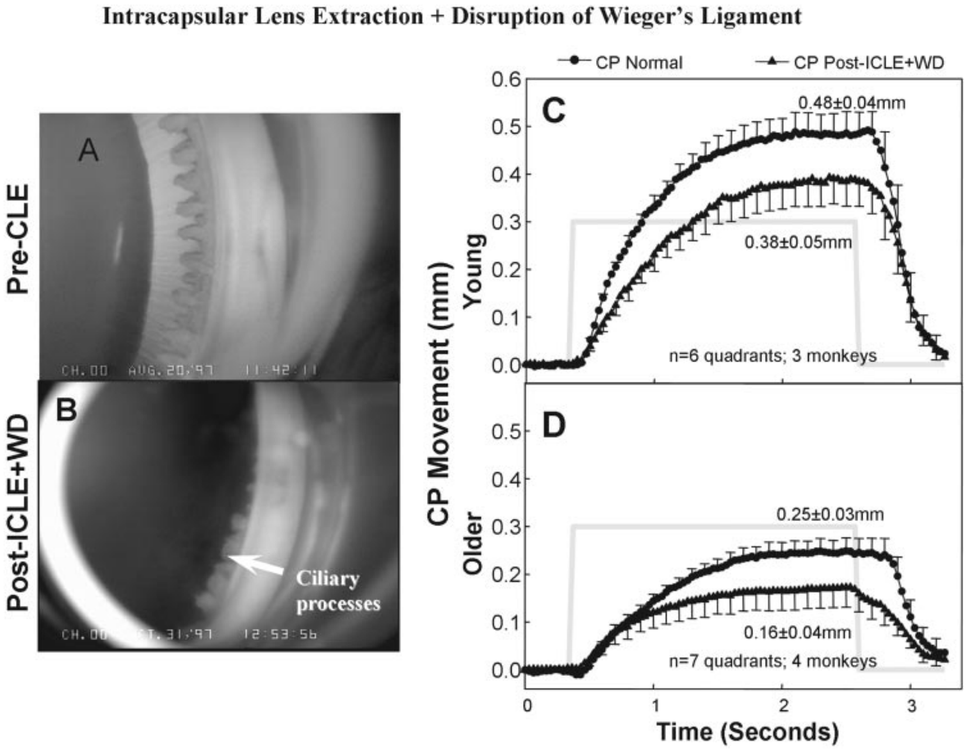

FIGURE 3. (**A**, **B**) Goniovideography images before (phakic state) and after ICLE in a 6-year-old monkey's eye. (**C**, **D**) Mean ± SEM of gonioscopically measured centripetal CP movement (in millimeters) in response to a 2.2-second electrical stimulus of the E-W nucleus before and after-ICLE in which Wieger's ligament was disrupted (ICLE+WD). A core vitrectomy was performed, which aspirated or disrupted ~75% of the anterior vitreous. (**C**) Average of six quadrants of three young monkeys (ages, 6, 8, and 9 years). (**D**) Average of seven quadrants of four older monkeys (ages, 18, 19, 19, and 27 years).

CP movement curve tended to increase ($P = 0.09$), compared with the same eyes before surgery (Fig. 4; Table 3), regardless of age. Although the sample size is relatively small, an increased velocity after versus before ECLE with a $P = 0.02$ seems meaningful. It is possible that the movement amplitude and area under the movement curve, with $P = 0.09$, may also become significant with larger samples sizes (Table 3).

The pattern of increased movement occurred in both the young and older eyes (Fig. 4; Table 2), but there were age-related differences.

Young Eyes. In the young eyes during accommodation after ECLE, the lens capsule and CPs tended to move faster ($P = 0.082$ and 0.052, respectively; Fig. 4D; Table 2) and almost in a 1:1 relationship (slope of the regression line between the capsule versus CP movement data; Fig. 5B), compared to presurgical baseline measurements (Figs. 4C, 5A). During disaccommodation, the lens capsule and CPs did not move in a 1:1 relationship in either the normal or surgically altered eyes (Figs. 5A, 5B), but the capsule versus CP movement slope in the surgically altered eyes was significantly higher than in the normal eyes ($P < 0.002$; Figs. 5A, 5B).

Older Eyes. In the two older rhesus eyes, the capsule velocity tended to increase after ECLE ($P = 0.088$, Table 2) but the CP velocity increase was not significant ($P = 0.32$). During accommodation after ECLE, the CPs and capsule moved faster (Table 2, Fig. 4F) compared with presurgical baseline measurements (Figs. 4E, 5C), but not in a direct 1:1 relationship (Fig. 5D). After ECLE, the area under both the CP and capsule movement curves tended to be greater ($P = 0.09$, $P = 0.096$ respectively), and the amplitude of capsule movement tended to be greater (by 0.16 ± 0.021 mm; $P = 0.086$) than baseline before ECLE. After ECLE, the amplitude of CP movement achieved near the end of the stimulus train was greater than baseline before ECLE (by 0.09 ± 0.019 mm; $P = 0.13$), but the difference was not significant.

The increase in CP and capsule movement amplitude after ECLE was more pronounced in the older eyes (Figs. 4E, 4F) versus the young eyes (Figs. 4C, 4D). Further, in the older eyes during accommodation, the slope of the capsule versus CP movement data steepened (Figs. 5C, 5D) after ECLE, compared to the presurgical baseline measurements and did so more dramatically than that in the young eyes.

Lens Capsule/CP Movement Analysis: Young Versus Older

The average movement of the lens/capsule equator in the young eyes was 0.34 mm before ECLE and 0.43 mm after ECLE (Figs. 4C, 4D) and in the older eyes was 0.10 mm before ECLE and 0.26 mm after ECLE (Figs. 4E, 4F). Thus, the presence of the lens substance retarded capsule movement by ~21% in the young eyes and by ~62% in the older eyes. After ECLE, the ratio of capsule to CP movement was 0.89 in the young eyes and 0.65 in the older eyes. Taking the ratio of these ratios and calculating the following formula: $[(0.65/0.89) - 1] \cdot 100 = 27\%$ gives the percentage of decline in capsular movement in the older eyes compared with the young eyes.

Circumlental Space Versus Age

In both the young and older eyes, the circumlental space (CPs to capsule edge) at rest (unaccommodated) diminished after ECLE in both the nasal and temporal quadrants (Table 4). However, during accommodation at supramaximum versus maximum settings, differences in the circumlental space were observed between the young and older eyes. In the young eyes, the circumlental space at the supramaximum stimulus setting did not diminish any further from the decrease observed at the maximum stimulus setting, before or after ECLE. In the older eyes, however, the circumlental space at the supramaximum stimulus setting did diminish further compared with that at the maximum stimulus setting, both before and after ECLE. At the supramaximum stimulus setting after ECLE in the two older eyes, the calculated average circumlental space value was actually negative because the tips of the CPs overlapped the capsule edge in one monkey eye and in the eye of

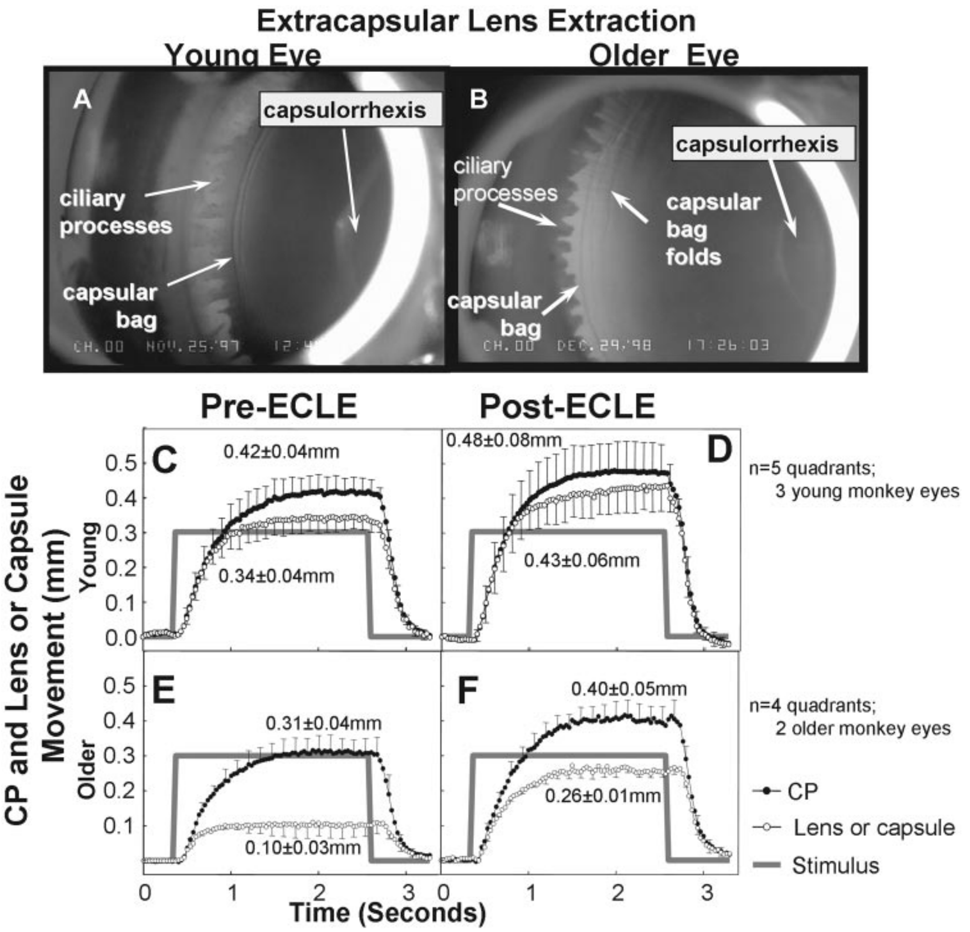

FIGURE 4. (A) Goniovideography image after ECLE in a young (age, 6 years) monkey's eye, 11 weeks after surgery. (B) Goniovideography image after ECLE in an older (age, 23 years) monkey's eye, 5 weeks after surgery. Both the young and older capsules appeared semitransparent, with no evidence of pearls or anterior–posterior capsule adhesions or fibrosis and no obvious scarring. (C–F) Data are the mean ± SEM of gonioscopically measured centripetal CP and lens or capsule movement before and after ECLE in five quadrants of three young (ages, 6, 7, and 9 years) and four quadrants of two older (ages, 21 and 23 years) monkey eyes.

the other monkey the circumlental space was barely positive, because the CPs were so close to the capsule.

AZR Zonulolysis

Administration of α-chymotrypsin in heavy sucrose medium achieved a localized complete lysis of the anterior zonule (lysis of all zonular fibers in 2 to 3 clock hours between the CPs and the lens edge; Fig. 6C) in one quadrant (either nasal or temporal) of all four rhesus monkey eyes (ages, 12, 23, 22, and 25 years) undergoing this procedure. The results were similar in all four monkeys, and so the data were grouped, and average lens and CP accommodative movements calculated (Figs. 6E, 6F). After anterior regional zonulolysis in the unaccommodated eyes, the lens equator became flattened in the lysed region (Fig. 6C), and the lens was pulled toward the opposite nonlysed region, where there was greater zonular tension (Fig. 6D). During accommodation after anterior regional zonulolysis, the lens equator moved toward the sclera in the region in which zonular fibers were lysed (Fig. 6E), consequent to accommodative zonular relaxation in the opposite (nonlysed) quadrant (see Movie S4, http://www.iovs.org/cgi/content/full/49/12/5484/DC1). The lens accommodative movement in the nonlysed quadrant exceeded the preanterior regional zonulolysis lens edge movement in the same quadrant (Fig 6F), probably due to the shift in resting lens position toward the nonlysed quadrant. During accommodation, the lens edge convexity returned in the lysed quadrant, due to the overall release in zonular tension and decrease in lens diameter (see Movie S4).

Histology

Histologic examination showed that the morphology of the muscle fibers and the CPs were not affected by the surgical procedures. This was true for all four quadrants of the eyes. In eyes with ICLE, the anterior zonule was present next to the pars plicata region of the CPs, and the zonule appeared clumped (Fig. 7). In eyes that received ICLE+WD, no morphologic changes were seen in the ciliary body, including the transition zone between pars plicata and pars plana adjacent to Wieger's ligament.

Discussion

In summary: (1) Centripetal accommodative CP and capsule movement increased in velocity and amplitude after ECLE in all five monkeys undergoing this procedure. (2) Centripetal accommodative CP movement was dampened in all seven monkeys undergoing ICLE when the anterior vitreous (i.e., Wieger's ligament) was disrupted. (3) Centripetal accommodative CP movement was increased post-ICLE in two young monkey eyes when Wieger's ligament remained intact. (4) ARZ consistently induced a shift in lens position toward the lysed quadrant during accommodation in all four monkey eyes. Although these accommodative postsurgical responses occurred consistently in each monkey eye, caution is warranted in generalizing the results, given the small sample sizes of some of the groups.

Wieger's ligament (a structural part of the vitreous) may play a role in the centripetal movement of the CPs and in accommodation. After ICLE, in which Wieger's ligament was disrupted in the young eyes, the reduced velocity and the reduced amplitude of the CP movement was most likely due to the loss of the zonula, of Wieger's ligament, and of the elastic capsule pulling the CPs centripetally. After ICLE in which Wieger's ligament remained intact, the increased CP move-

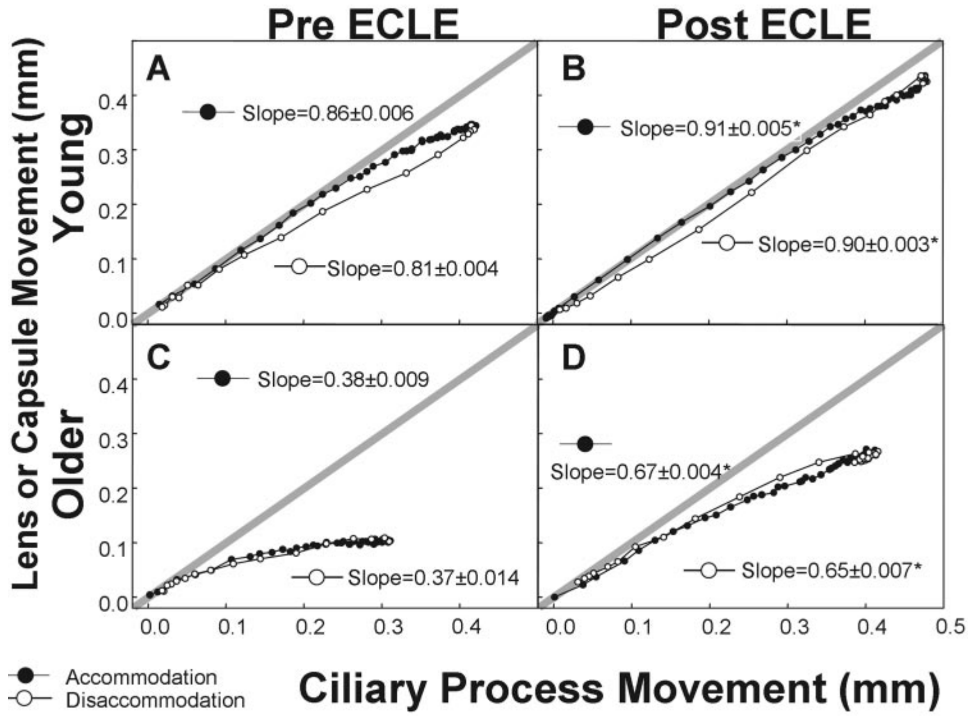

FIGURE 5. Gonioscopically measured CP movement versus lens or capsule movement, plotted using the data in Figure 4. The numbers in the panels represent the simple linear regression slope of lens or capsule versus CP movement ± SEM during accommodation and disaccommodation. All regression slopes tended to be < 1.0 ($P < 0.04$). *The slope after ECLE tended to be higher compared with baseline ($P < 0.002$).

ment was possibly due to removal of the lens substance and to the presence of intact zonular attachments between the CPs and Wieger's ligament, pulling the CPs centripetally. Movie S2 actually shows that Wieger's ligament moves centripetally during accommodation. To our knowledge, this is the first time that Wieger's ligament has been observed in vivo during the accommodative response (first shown in an ARVO 2004 presentation; Croft MA, et al. *IOVS* 2004;45:ARVO E-Abstract 2187). Bernal et al.,[31] using environmental scanning electron microscopy in later research in human eyes, showed zonular attachments to the hyaloid membrane (Wieger's ligament). The present study demonstrates and quantifies the importance of the zonular attachment to Wieger's ligament, and confirms the importance of keeping the hyaloid and anterior zonular apparatus undisturbed for the potential restoration of (some) accommodation in presbyopes (and hopefully cataract patients), a concern that Jackson Coleman alluded to in his theory.[32]

For the two groups (ECLE and ICLE with Wieger's ligament intact), the CP accommodative and disaccommodative velocities were similar at baseline before surgery. In addition, for

TABLE 4. Circumlental Space at Rest and during Stimulation before and after Extracapsular Lens Extraction

	Baseline (Phakic eye)						Post-ECLE					
	Unaccommodated		Max		Smax		Unaccommodated		Max		Smax	
	Nasal	Temporal	Nasal	Temporal	Nasal	Temporal	Nasal	Temporal	Nasal	Temporal	Nasal	Temporal
Circumlental Space (mm)												
Young ($n = 3$)												
Mean	0.54	0.45	0.43	0.38	0.41	0.36	0.32	0.27	0.23	0.25	0.22	0.25
SEM	0.040	0.056	0.039	0.077	0.049	0.068	0.051	0.002	0.045	0.060	0.049	0.065
Older ($n = 2$)												
Mean	0.46	0.35	0.38	0.21	0.25	0.14	0.17	0.10	0.05	0.00	−0.02	−0.06
SEM	0.08	0.05	0.02	0.10	0.04	0.07	0.02	0.11	0.06	0.19	0.11	0.21

	Unaccommodated		Max		Smax	
	Nasal	Temporal	Nasal	Temporal	Nasal	Temporal
Circumlental Space Baseline Minus Post-ECLE Differences (mm)						
Young	0.23	0.18	0.20	0.14	0.19	0.12
Older	0.29	0.25	0.33	0.21	0.27	0.20

Data are mean ± SEM of gonioscopically measured circumlental space (mm) in the resting, maximum (Max), and supramaximum (Smax) stimulated states before and after ECLE. In the two older eyes at the Smax stimulus setting post-ECLE, the calculated average circumlental space value was actually negative, because the tips of the ciliary processes overlapped the capsule edge in one monkey.

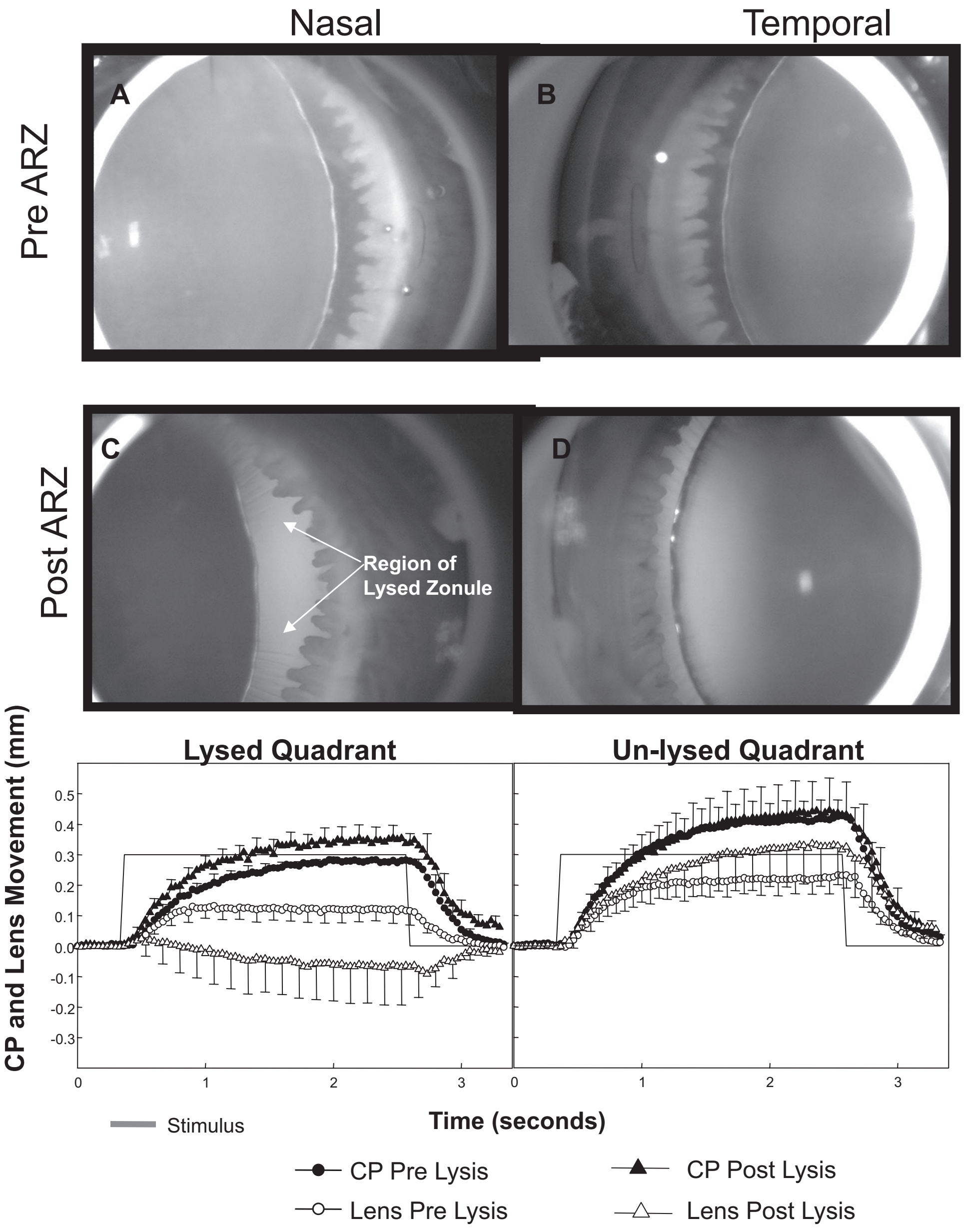

FIGURE 6. CP and lens movement before (**A**, **B**) and after (**C**, **D**) ARZ in one quadrant of one rhesus monkey's eye (age, 23 years). (**E**, **F**) Data are the mean ± SEM of gonioscopically measured CP and lens movement in both quadrants (nasal and temporal) of four rhesus monkey's eyes (ages, 13, 23, 23, and 25 years) before and after ARZ in one quadrant.

both groups there was an increased amplitude of accommodative CP movement compared to presurgical baseline (phakic eyes; Figs. 2, 4), but the velocity of disaccommodative CP movement tended to be slower (by ~40%; $P = 0.066$) in the ECLE eyes versus the ICLE eyes with Wieger's ligament intact (Table 2). Collectively, the data suggest that the capsule dampens disaccommodative velocity. It is thus plausible that the capsule aids centripetal CP accommodative velocity; indeed, the velocity of accommodative CP movement averaged 24% faster after ECLE versus post-ICLE with Wieger's ligament intact, but the difference was not statistically significant ($P = 0.458$) (Table 2).

The enhanced accommodative centripetal CP and capsule dynamics in the young ECLE eyes relative to the normal eyes were most likely due to the centripetal capsular elasticity's being unopposed by resistance from the lens substance. The edge of the empty capsular bag moved at a faster rate during accommodation than the equator of the intact crystalline lens (Tables 2, 3), suggesting that the lens substance, even in young accommodating monkeys, resisted capsular force. This observation is based on the following: (1) In the young eyes, the relationship between CP and capsule movement during accommodation and disaccommodation improved slightly after ECLE (slope in Fig. 5); and (2) removal of the lens substance from the older eyes significantly improved ($P < 0.002$), but did not fully restore, accommodative capsule movement relative to that seen in the young monkeys. This suggests that the lens was stiffer in the older monkeys and that, after the lens was removed, age-related loss of capsular elasticity, and/or stiffening of the posterior attachment of the ciliary muscle or the posterior zonule, still prevented the CPs in the older monkey eyes from moving as much as in the young monkey eyes.

The amount of centripetal accommodative capsule and CP movement after ECLE suggests that the capsule and ciliary muscle still functioned in the older eyes. Although the older capsule-to-CP-movement ratio was reduced compared with the young eyes, the older capsules still exhibited an amount of movement (0.26 mm, Fig. 4F) that would be sufficient to induce up to 10 D of accommodation if an accommodating IOL with the same properties as the young crystalline lens had been

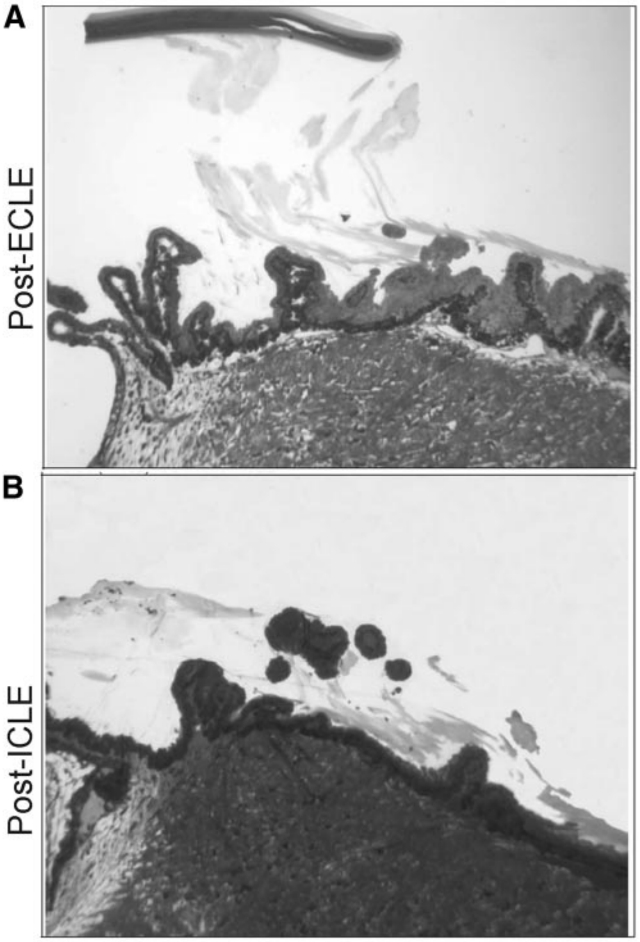

FIGURE 7. Semithin (1 μm) sagittal section through the anterior inner portion of the ciliary muscle and the adjacent CPs and zonule in a rhesus monkey's eye after (**A**) ECLE and (**B**) ICLE. Note that the muscle fibers and entire ciliary epithelium are intact. In eyes with ICLE, the zonule is still present in the attachment zones at the inner limiting membrane of the epithelium and the adjacent anterior and posterior chamber.

in place, assuming no loss in older capsular movement. Moreover, if one were to place an accommodating IOL with the same accommodative properties as the young crystalline lens within the empty capsular bag of an older eye, it is possible to predict the amount of accommodation that may be induced. Adjusting for resistance due to the presence of the lens substance, such as in the young eyes (~21%), and adjusting for loss in capsule function observed in the older eyes versus the young eyes (~27%), the predicted amount of lens/capsule movement would be 0.15 mm in the older eyes, which should yield 6 D of accommodation (Croft MA, et al. *IOVS* 2005;46: ARVO E-Abstract 713). Whether the capsule in an older eye would be able to mold an accommodating IOL depends on the characteristics of the IOL and on how much force the capsule can still exert. Of course, caution is warranted in generalizing the results, given the small sample size of some of these groups.

In the normal monkey eyes, the circumlental space diminished at the supramaximum stimulus setting in the older eyes but not in the young eyes, because of the diminished lens movement during accommodation, in line with previously published results.[23] At rest, the circumlental space tended to decrease after ECLE to a greater extent in the older eyes than in the young eyes, suggesting an enlarged lens and/or stretched, less elastic capsule in the older monkeys.

Folds in the capsular bag (Fig. 4) were always present after ECLE in the older monkeys, but never in the young monkeys (see Movie S3), suggesting that the older empty capsular bag had a larger surface area and, when emptied of the lens, was more flaccid than in young monkeys. It has been reported by others that the anterior zonulae do not change in length with age.[8] Therefore, the old-versus-young difference may be due to growth of the lens and stretching of the capsule with age, and to increased distance between zonular insertion onto the lens and the original lens equator.[8]

After ICLE with Wieger's ligament disrupted, accommodative CP velocity and amplitude were reduced, but both were increased after ECLE. This suggests that, during accommodation, the lens capsule performs a dual role of (1) reshaping the lens substance and (2) helping to pull the CPs axially and increasing the CP velocity during accommodation until zonular relaxation is achieved. The lens capsule and anterior zonule in essence form a continuous elastic sheet, acting centripetally.

Dynamic imaging of the ciliary body, lens, and zonula of the normal rhesus monkey eye with Swan-Jacob gonioscopy has provided the ability to quantitatively and qualitatively assess the normal interrelationships of the accommodative components and the normal function of the accommodative apparatus as a whole.[20,22,23,33,34] Vilupiru and Glasser[26] found that accommodative velocity was dependent on the amplitude of accommodation in living young rhesus monkey eyes; thus, the diminished lens response amplitude in the older eyes of the present study may have resulted in diminished lens velocity. In contrast, the amplitude of the CP movement during the same period was similar in the young and older eyes, and as a result, the CP velocity was not diminished in the older eyes compared with the younger eyes. Surgically altering the normal relationship between the lens and ciliary body has provided new insight into the relative roles of the lens, capsule, and ciliary body in accommodation.

Despite the complex nature of the surgical procedures and the varied amounts of time between pre- and postsurgical measurements, the results reported herein and elsewhere[28] are consistent. Comparison of these data to previous studies in the normal (iridectomized) eye[20,23] lends further credence to our findings.

Histologic examination showed that the three-dimensional structure of the ciliary muscle and the morphology of the muscle fibers were not affected by the surgical procedures. This, and the observed increases in accommodative movement after ECLE and ICLE with Wieger's ligament intact, alleviate concern that these surgical procedures caused intraocular scarring that would likely diminish accommodative movements.

After anterior regional zonulolysis, the lens position moved toward the lysed region during accommodation. This result showed that the position of the lens within the eye was dependent on zonular tension circumferentially and suggests that the apparent age-related shift in lens position toward the temporal quadrant, observed in the older resting eyes,[23] may be due to changes in the zonular tension with age.

Goniovideographically measured CP movement predominantly represents centripetal ciliary body movements, but goniovideography does not really distinguish centripetal from forward ciliary body movement. Thus, the CP movements that we report may actually be a hybrid or composite, in contrast to movements measured by UBM, which can isolate measurement of forward ciliary body movement. Nonetheless, the techniques of measuring forward ciliary body movement by UBM and of centripetal CP movement by goniovideography, clearly provide separate and distinct information about ciliary body function and its change with age.[20]

Characterization of the performance of the accommodative apparatus before and after surgical interventions may help to model the system for hypothesis testing. Information gleaned from such studies aids in understanding the accommodative mechanism itself and may also facilitate the design of accommodating IOLs. Accommodating IOLs may be more effective in restoring accommodation in the presbyopic eye if they rely on centripetal ciliary body and thereby capsular edge movement rather than forward ciliary body movement.[20] Previous studies of accommodation in the normal iridectomized rhesus monkey eye have shown a significant age-related loss of forward ciliary body movement and lens centripetal movement, but the age-related loss in CP movement was far less pronounced.[20,23] Accommodative centripetal movements of the ciliary body are also still present in the presbyopic human eye,[35] despite the reduced accommodative amplitudes. Forward muscle movement, as measured by muscle length, was still present in excised, pharmacologically stimulated postmortem human eyes.[36] Whether the remaining movement is sufficient to produce accommodation with an accommodating IOL will depend on the approach and the characteristics of the accommodating IOL.

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