

# Age-Related Loss of Ciliary Muscle Mobility in the Rhesus Monkey

## Role of the Choroid

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• **Ciliary muscle topography was studied in rhesus monkey eyes (aged 6 to 29 years) bisected meridionally through cornea and optic nerve head. Half of each eye was incubated in atropine sulfate, the other in pilocarpine hydrochloride, and both were then processed for histologic study. Several ciliary muscle sections from the original cut margin and the middle of the half eyes were traced and compared quantitatively. In sections from the middle, where the attachments of the muscle were presumably intact, the pilocarpine effect on ciliary muscle topography was lost with age. In sections near the cut margin, where some of the posterior attachments were disrupted and the choroid had detached from the sclera, the pilocarpine effect persisted with age. These findings suggest that loss of ciliary muscle movement with age is caused by decreased compliance of its posterior attachment.**

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With increasing age, the ciliary muscle in the intact rhesus monkey (*Macaca mulatta*) eye largely ceases to move antero-inwardly in response to cholinomimetic drugs<sup>1,2</sup> or central electrical stimulation,<sup>2</sup> paralleling a similar decline in accommodative responses to these stimuli.<sup>2,3</sup> The minor age-related structural alterations in the muscle cells and nerve fibers and the small increase in connective tissue between the muscle fiber bundles seen by light and electron microscopy seem insufficient to explain the almost complete immobility of the muscle.<sup>4</sup> The parasympathetic ciliary

neuromuscular mechanisms seem normal since the overall number and binding affinity of muscarinic receptors and the activity levels of choline acetyltransferase and acetylcholinesterase in the ciliary muscle do not change with age.<sup>5</sup> External restriction of the muscle by the enlarging lens does not occur, because a space between the lens equator and the ciliary processes is always present, even in the oldest animals.<sup>2</sup>

Elasticity of the ciliary muscle's posterior attachment allows forward-inward movement of the pharmacologically or neuronally stimulated muscle, producing accommodation. The posterior elastic tendons of the rhesus monkey ciliary muscle exhibit marked age-related structural changes and in old monkeys are surrounded by dense layers of collagen fibrils.<sup>6</sup> An age-related increase in inelastic fibrillar material could cause decreased compliance of the posterior insertion of the ciliary muscle, thereby restricting muscle movement and preventing accommodation. We have tested this hypothesis by observing histologically the effects of varying sectioning, drug treatment, and tissue fixation strategies on ciliary muscle configuration in young and old rhesus monkeys.

### MATERIALS AND METHODS

#### Animals and Eyes

Eleven rhesus monkeys (*Macaca mulatta*; three male, eight female), aged 6 to 29 years, were studied. The young and middle-aged animals were killed in conjunction with various nonocular experimental protocols, while the elderly animals were debilitated and killed to spare them suffering. All eyes were enucleated in vivo under surgical depth anesthesia achieved by intramuscular ketamine as hydrochloride, 15 mg/kg, followed by intravenous pentobarbital sodium, 25 mg/kg. Immediately after enucleation, the animals were killed by exsanguination or pentobarbital overdose. This study conforms to the Association for Research in Vision and

Ophthalmology Resolution on the Use of Animals in Research,<sup>7</sup> and all National Institutes of Health (Bethesda, Md) and University of Wisconsin (Madison) guidelines regarding animal experimentation.

**Group 1.**—Three monkeys, aged 6, 12, and 20 years, received one drop (40  $\mu$ L) of commercial ophthalmic 10% pilocarpine hydrochloride topically in the right eye and (25  $\mu$ L) 1% atropine sulfate in the left eye 30 and 15 minutes before enucleation. In each case, the pupils responded appropriately. Immediately after enucleation, each eye was bisected equatorially, the zonule was cut with scissors (Vannas) over 270° of the circumference, and the anterior segment was quadrisectioned meridionally, leaving the lens attached to the temporal quadrant. The right eye quadrants were then immersed in Ito's fixative<sup>8</sup> containing 10-mmol/L pilocarpine hydrochloride, while the left eye quadrants were placed in fixative containing 10-mmol/L atropine sulfate.

**Group 2.**—Four monkeys, aged 8, 10, 10, and 27 years, received no topical drugs in vivo. Following enucleation, each eye was bisected meridionally, with the cut passing through the center of the optic nerve head, and the lens was removed. The two halves of each eye were immersed in 0.9% saline containing either 1-mmol/L pilocarpine hydrochloride or 1-mmol/L atropine sulfate, respectively, for 5 minutes, and then immersed in Ito's fixative containing that same drug concentration.

**Group 3.**—Four monkeys, aged 22, 23, 28, and 29 years, also not treated topically in vivo, were enucleated and each eye was bisected sagittally as above, but the lens was left attached to one half. In three of these animals (aged 22, 28, and 29 years), the phakic half of one eye and the aphakic half of the contralateral eye were initially immersed in 0.9% saline containing 1-mmol/L pilocarpine hydrochloride, while the other two halves were immersed in saline containing 1-mmol/L atropine sulfate for 5 minutes before being immersed in Ito's fixative containing 1 mmol/L of the same drug. The remaining animal (aged 23 years) was processed similarly except that the half eyes were initially immersed in Ito's fixative containing 10-mmol/L pilocarpine hydrochloride or atropine sulfate. All tissue remained in

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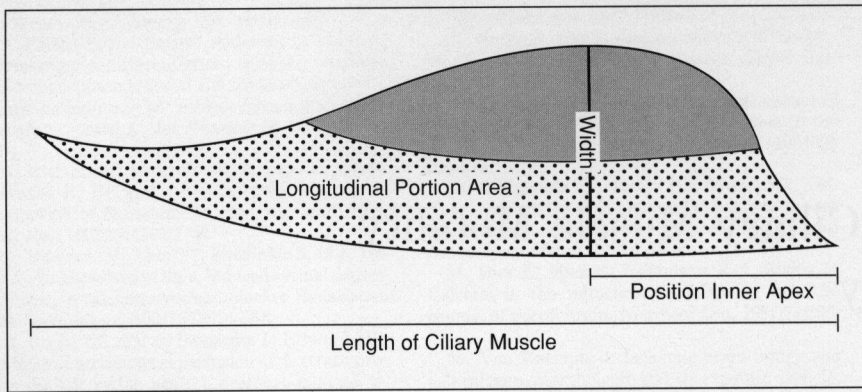


Fig 1.—Schema for topographic analysis of ciliary muscle. By digitization of ciliary muscle drawings, measurements were made of ciliary muscle length (anterior/posterior distance along outer longitudinal edge from posterior tip to anterior insertion at scleral spur), width (perpendicular distance between inner apex and outer longitudinal edge), and position (anterior/posterior distance from inner apex to scleral spur, perpendicular to width) (from Lütjen-Drecoll et al<sup>1</sup>).

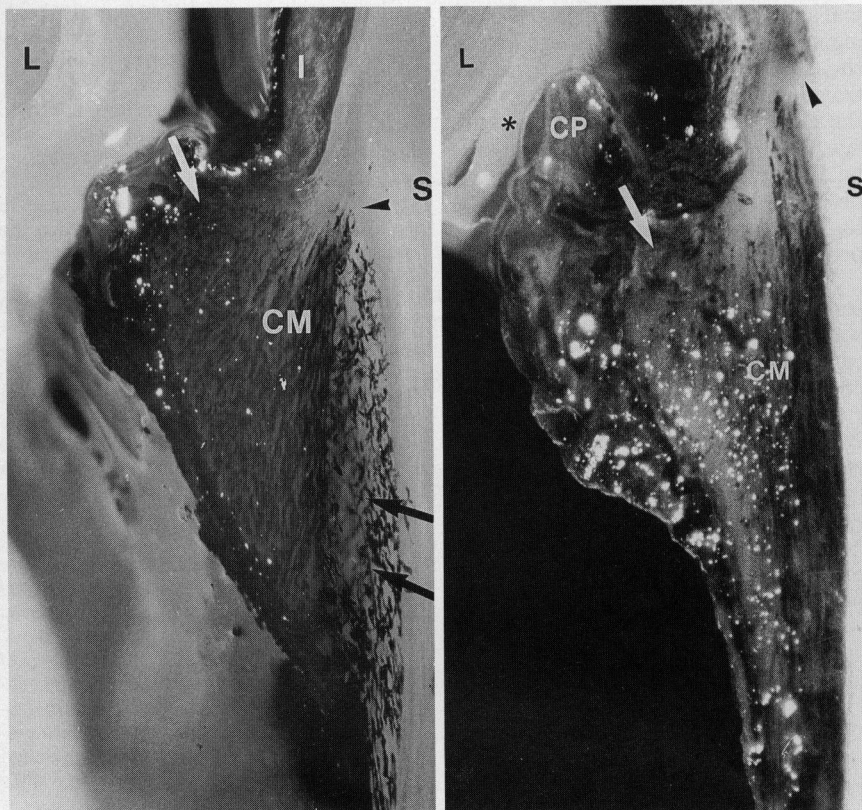


Fig 2.—Meridionally bisected rhesus monkey half eyes were immersed for 5 minutes in saline containing 1-mmol/L pilocarpine hydrochloride and then fixed and examined under the dissecting microscope before embedding and staining. Left, Original cut margin, 22-year-old eye. Ciliary muscle (CM) and choroid have detached from the sclera (S); black arrows indicate the supraciliary/suprachoroidal cleft. The muscle's inner apex (white arrow) is situated as far forward as the scleral spur (arrowhead). I indicates iris; L, lens. Right, Cut edge from a "middle" tissue strip, 29-year-old eye. Ciliary muscle and choroid remain attached to the sclera (S), the muscle's inner apex (white arrow) is 0.28 mm behind the spur (arrowhead), and a space (asterisk) is present between the ciliary processes (CP) and the lens (L) (original magnification  $\times 100$ ).

fixative until final processing in the Federal Republic of Germany 2 weeks later.

#### Tissue Processing and Evaluation

**Group 1.**—Two sectors were cut from each quadrant and embedded in epoxy resin (Epon), from which 1- $\mu$ m sections were cut and stained with Richardson's.<sup>9</sup> The rest of

the globe was dissected and processed as usual for transmission electron microscopy.<sup>6</sup>

**Groups 2 and 3.**—In seven of the eight animals, four marquise-shaped strips running from the anterior to the posterior pole and 5 mm wide at the equator were cut from opposite sides of each half eye with a razor blade knife; in the eighth animal (29 years old), eight

2.5-mm-wide strips were cut. Care was taken that each cut passed through the center of the optic nerve head to assure that the ciliary muscle remained anchored posteriorly via its elastic tendons to Bruch's membrane,<sup>1</sup> as well as anteriorly to the scleral spur, trabecular meshwork, and cornea.<sup>10</sup> Each strip was labeled as to its clock-hour location and its proximity to the original cut edge of the half eye. Complete meridional portions of each strip were embedded in paraffin, after which 5- $\mu$ m sections were cut and stained with Crossmon's.<sup>11</sup> The remaining portions were embedded in epoxy resin and 1- $\mu$ m sections were cut and stained with Richardson's.

Tracings outlining the perimeter and the longitudinal portion of the ciliary muscle were made from one section taken from each strip of each eye, using a drawing microscope (Wild, Heerburg, Switzerland). These tracings permitted measurement by digitization (MOP/17 M01 system, Contron, Munich, Federal Republic of Germany) of the length, width, and position of the ciliary muscle.<sup>1</sup> The tracings were done by one of us (E.T.) and the measurements were done by one of us (W.J.); neither of us knew the age, drug treatment status, or fixation protocol of the material.

To evaluate the roles of age, drug (pilocarpine or atropine), and proximity of section to the cut edge (margin of strip or middle of strip) in determining ciliary muscle configuration as seen in meridional histologic section, three-way analysis of variance (split plot design) was performed using the general linear model procedure for unbalanced data, with a statistical program (SAS Institute Inc, Cary, NC) on a mainframe computer (VAX, Digital Equipment Corp, Maynard, Mass).<sup>12</sup> The monkeys were classified as young (8, 10 years), medium-aged (22, 23 years), or old (27 to 29 years). Two histologic sections from each half eye were evaluated, one at the margin and one at the middle, closest and farthest respectively from the original sagittal cut. The muscle configuration factors evaluated were length, width at the apex, and position of the apex relative to the scleral spur, as defined in Fig 1.<sup>1</sup> The resulting F statistics were tested for significance, and where overall significance was found for the interaction terms (ie, age by drug by location), the Tukey test was used to determine which means differed significantly.

## RESULTS

### Meridional Dissection

When the margin of the meridionally cut half eyes was examined under the dissecting microscope following tissue fixation, a separation between ciliary muscle/choroid and sclera was observed in all specimens regardless of age or drug treatment (Fig 2, left). A similar separation between choroid and sclera was not observed in tissue strips obtained from the middle portion of the half eyes after further meridional dissection (Fig 2, right). In all specimens where the zonular insertion to the lens was left intact, a space between lens equator and ciliary processes was ob-

Analysis of Variance\*

	N	Means			Tukey Test Results											
		Api Pos	Width	Length	Summary of the Test Results Using Underscores‡											
<b>Main Effects</b>																
Age																
Young	24	0.37	0.73	2.62												
Medium	16	0.62	0.72	2.60												
Old	24	0.54	0.70	2.63	Api Pos											
					0.62	0.54	0.37									
Mean, square error		0.0392	0.1065	0.25856												
Significance		0.025†	NS	NS												
Drug																
Atropine sulfate	32	0.68	0.70	2.78												
Pilocarpine hydrochloride	32	0.32	0.74	2.46												
Mean square error		0.0158	0.0025	0.00485												
Significance		.005	.025	.005												
Location of section																
Margin	32	0.42	0.76	2.54												
Middle	32	0.58	0.67	2.70												
Mean, square error		0.0065	0.0009	0.03516												
Significance		.005	.005	.005												
<b>Interactions</b>																
Section location																
Margin																
Young, atropine	6	0.51	0.80	2.75	Api Pos											
Young, pilocarpine	6	0.12	0.80	2.34	0.86	0.74	0.72	0.70	0.63	0.59	0.55	0.51	0.36	0.23	0.14	0.12
Medium, atropine	4	0.74	0.66	2.72												
Medium, pilocarpine	4	0.36	0.84	2.32												
Old, atropine	6	0.63	0.69	2.67												
Old, pilocarpine	6	0.23	0.77	2.41	Length											
Middle																
Young, atropine	6	0.72	0.74	2.96	2.96	2.85	2.75	2.74	2.72	2.71	2.67	2.50	2.43	2.41	2.34	2.32
Young, pilocarpine	6	0.14	0.60	2.43												
Medium, atropine	4	0.86	0.64	2.85												
Medium, pilocarpine	4	0.55	0.74	2.50												
Old, atropine	6	0.70	0.63	2.74	Width											
Old, pilocarpine	6	0.59	0.70	2.71	0.84	0.80	0.80	0.77	0.74	0.74	0.70	0.69	0.66	0.64	0.63	0.60
Mean, square error		0.0065	0.0009	0.00468												
Significance		.005	.05	.025												

\*NS indicates means are not significantly different. Data are mean apical position (Api Pos), length, and width (millimeter) of the ciliary muscle following meridional bisection. Where overall significant difference among the means are found, Tukey tests were conducted to determine which means were significantly different from each other. Means without a common underscore are significantly different at the .05 level. Data are graphically represented in Fig 3.

†Probability of having a larger F.

‡Means with a common underscore are not significantly different.

served regardless of age or drug treatment (Fig 2, right).

The quantitative findings were similar in phakic and aphakic half eyes for corresponding drug, age, and section location, so their data were averaged for final analysis and presentation. In the Table, analysis of variance results are shown in the left-hand side, with Tukey test results underscored in the right-hand side. Figure 3 schematizes all the meridional bisection data.

In the eyes bisected meridionally before fixation, ciliary muscle topography varied with drug treatment and age. Additionally, ciliary muscle topography showed marked differences in the individual half eyes that had been treated with pilocarpine or atropine, which depended on the proximity of the section to the original meridional cut.

**Atropine**

**Inner Apical Position.**—The ciliary muscle of the half eyes treated with atropine was obtusely angled at its inner apex in all age groups (Fig 4). In sections from the middle (farthest from the original cut), the apex of the muscle formed no circular portion and was located approximately 0.7 to 0.9 mm behind the scleral spur (Fig 4, right). In sections from the margin (closest to the original cut), the apex of the muscle tended to be farther forward than at the middle and formed a small circular portion (Fig 4, left), but the differences between margin and middle sections decreased with increasing age (Table 1, Fig 3, top part).

**Length and Width.**—Within each age group, the muscle was shorter at

the margin than in the middle, with the differences decreasing with increasing age (Table 1, Fig 3, center part). The muscle of all age groups that had been treated with atropine was slightly wider at the margin than at the middle (eg, Fig 4, left vs Fig 4, right), but the differences were small and none was statistically significant (Table 1, Fig 3, bottom part).

**Pilocarpine**

**Inner Apical Position.**—The ciliary muscle of the young age group half eyes that had been treated with pilocarpine exhibited a well-developed circular portion and an acutely angled inner apex that was located nearly as forward as the scleral spur. In these young eyes, the inner apical position was essentially identical at the margin and in the mid-

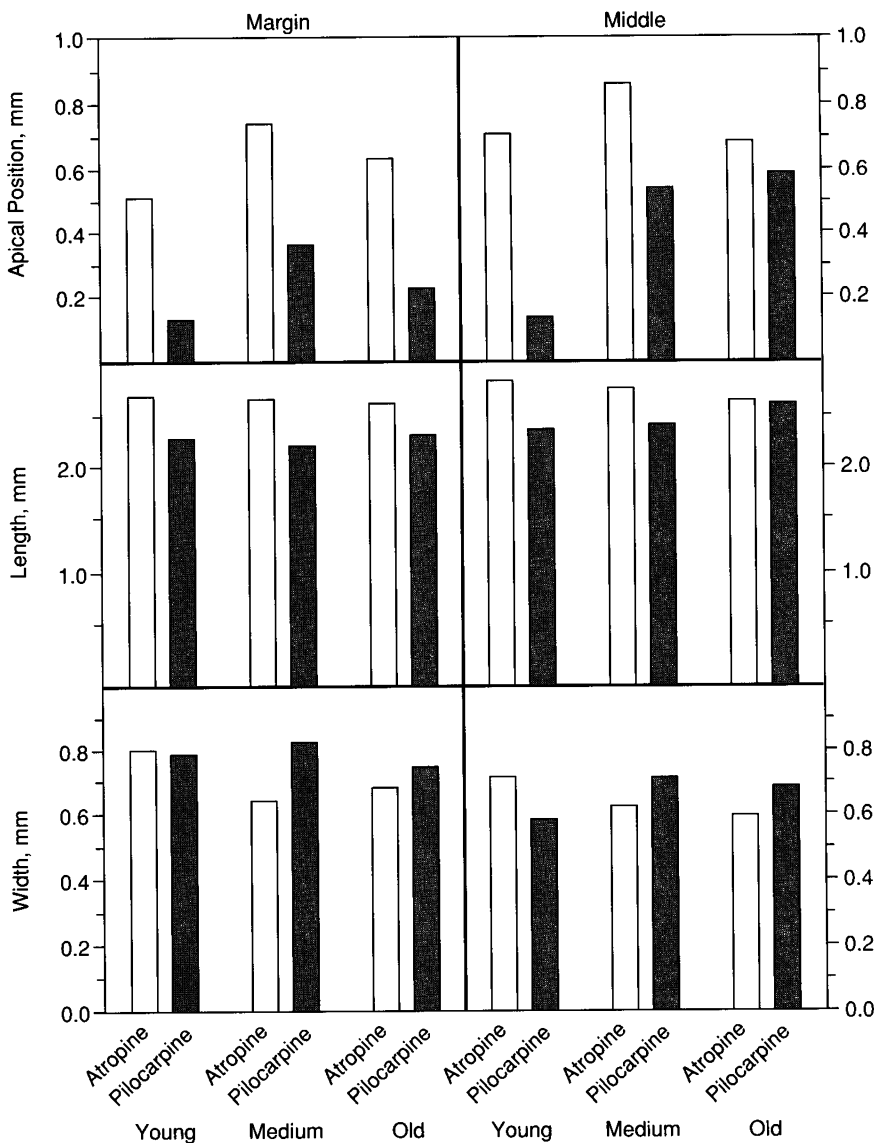


Fig 3.—Data plotted are mean apical position (top panel), length (middle panel), and width (lower panel) of the ciliary muscle grouped according to age, drug, and section location. This figure graphically represents the data presented in the second part of the Table. Shaded bars represent sections obtained from the middle (farthest from the original meridional cut), where the ciliary muscle is intact. Open bars represent sections taken from the margin (closest to the original meridional cut), where some of the posterior and outer attachments of the ciliary muscle have been severed.

dle. In the older eyes, the differences between half eyes treated with pilocarpine and atropine were much less pronounced in sections obtained from the middle (Fig 5, right vs Fig 4, right; Fig 5, left vs Fig 4, left; Table 1, Fig 3, top part). Herein, the muscle was still located somewhat more anteriorly in the half treated with pilocarpine than in the half treated with atropine, but in contrast to the younger animals, only a small circular portion was formed (Fig 5, right) and the inner apex of the muscle still remained relatively far posterior to the scleral spur (average, 0.59 mm in the oldest eyes). In sections from the margin, the muscle in the

medium-age and old-age animals was farther forward than in sections from the middle and exhibited a well-developed circular portion (Fig 5, right vs Fig 5, left Table 1, Fig 3, top part). Thus, in contrast to the sections from the middle, at the margin there was no consistent difference in the inner apical position of the ciliary muscle in half eyes treated with pilocarpine from the three age groups (Table 1, Fig 3, top part). In addition, at the margin the difference in inner apical position between half eyes treated with pilocarpine and atropine was large and virtually identical at all ages (Fig 5, left vs Fig 4, left; Table 1, Fig 3, top part).

**Length.**—The ciliary muscle in old half eyes treated with pilocarpine was significantly longer than in young eyes in sections from the middle (Table, Fig 3, center part). In sections from the margin, there was essentially no difference in muscle length in half eyes treated with pilocarpine from the three age groups. As in the half eyes treated with atropine, the muscle was shorter at the margin than in the middle. In half eyes treated with pilocarpine, however, the difference between margin and middle increased with age and was especially pronounced in the old-age group. In sections from the middle and the margin, the muscle was shorter in the half eyes treated with pilocarpine than in the half eyes treated with atropine in all age groups. However, in sections from the middle this difference decreased with age, while at the margin the difference remained substantial, significant, and constant at all ages (Table, Fig 3, center part).

**Width.**—In sections from the middle, the ciliary muscle of the half eyes treated with pilocarpine of the old- and medium-age groups was significantly wider than in young eyes. At the margin, however, there was no age-related difference in muscle width (Table, Fig 3, bottom part). The muscle was significantly wider at the margin than in the middle of the half eyes treated with pilocarpine in each age group (Fig 5, left vs Fig 5, right), but the difference between margin and middle sections decreased with increasing age (Table, Fig 3, bottom part). In margin sections, muscle width in young animals was the same in half eyes treated with pilocarpine and atropine, while in the medium-aged and elderly animals the muscle was significantly wider in half eyes treated with pilocarpine than in half eyes treated with atropine (Fig 5, left vs Fig 4, left; Table, Fig 3, bottom part). In middle sections from young animals, the muscle in the half eyes treated with pilocarpine was narrower than in the ones treated with atropine, while in medium- and old-aged animals, the opposite was true (Fig 5, right vs Fig 4, right; Table, Fig 3, bottom part). Grouping the data according to section location only, the muscle was shorter and its apex was wider and closer to the scleral spur in sections from the margin than in those from the middle (Table 1,  $P < .005$ ).

#### Equatorial Bisection

In the 6-, 12-, and 20-year-old eyes bisected equatorially before fixation, the general topography of the ciliary muscle was essentially the same regardless of age or drug treatment. The muscle was extremely short (2.4 to 2.6



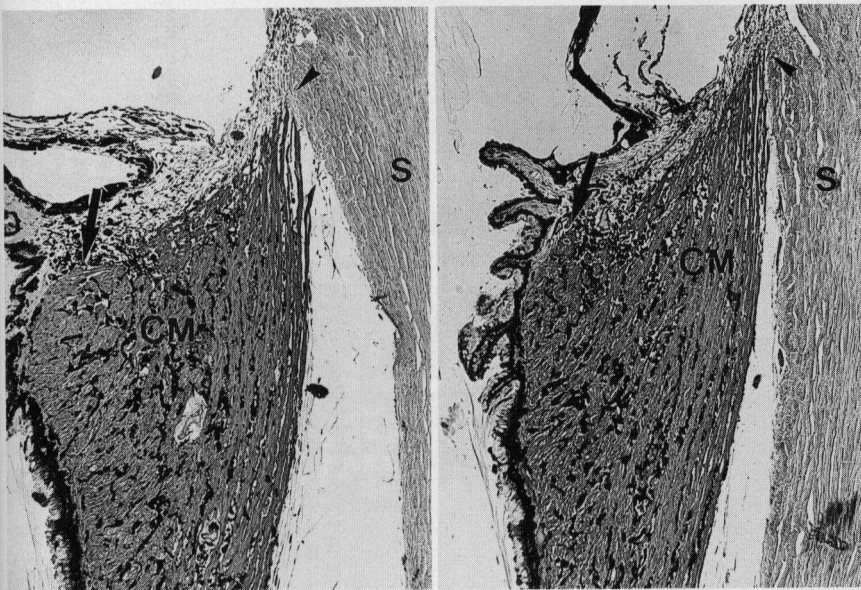


Fig 4.—Light micrographs from histologic sections of the half of the same eye treated with atropine as in Fig 2, right and Fig 5. Left, Section from the margin (nearest to the original cut). Right, Section from the middle (farthest from the original cut). In the middle section, the ciliary muscle's inner apex (arrow) is obtusely angled and does not exhibit a circular portion. In the marginal section, the inner apex is more acutely angled and forms a small circular portion. The distance between the muscle's inner apex and the scleral spur (arrowhead) is approximately the same in both of the sections. CM indicates ciliary muscle; S, sclera (Crossmon's, original magnification  $\times 150$ ).

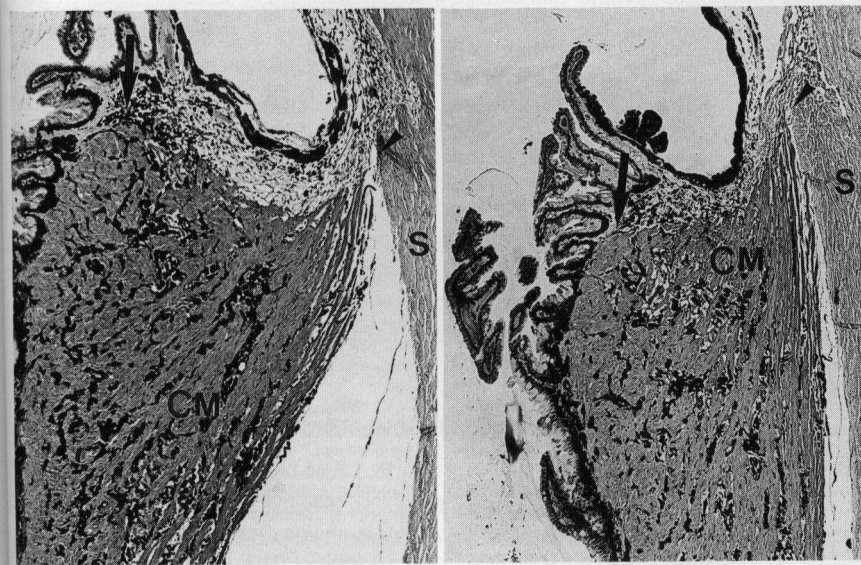


Fig 5.—Light micrographs from histologic sections of the half of the same eye treated with pilocarpine as in Fig 2, right and Fig 4. Left, Section from the margin (nearest to the original cut). Right, Section from the middle (farthest from the original cut). In the section from the middle, the distance between the ciliary muscle's inner apex (arrow) and the scleral spur (arrowhead) is only slightly shorter than in comparable sections from the half treated with atropine, but a small circular portion is formed (Fig 4, right). In sections from the margin, however, the inner apex has moved as far forward as the scleral spur and exhibits a well-developed circular portion. CM indicates ciliary muscle; S, sclera (Crossmon's, original magnification  $\times 150$ ).

mm) and thick (0.9 to 1.1 mm), and the inner apex was located nearly as far anteriorly as (only 0.1 to 0.2 mm behind) the scleral spur.

#### COMMENT

When rhesus monkey eyes are fixed intact prior to sectioning, excursions

responses of the ciliary muscle to pilocarpine may be quantitated by measuring differences in muscle configuration and position between pilocarpine- and atropine-pretreated eyes. Under such conditions, an age-related loss of excursion responses to pilocarpine is evident.<sup>1</sup> When the globes are sectioned

equatorially before fixation, no differences between pilocarpine and atropine pretreatment are seen at any age, indicating that drug-induced responses are artifactually prevented or obscured.<sup>1</sup> We have hypothesized<sup>1</sup> that the latter results from cutting Bruch's membrane, which constitutes the muscle's posterior attachment,<sup>6,13</sup> and from fixation fluid dissecting into and disrupting the connective matrix attaching the muscle and choroid to the sclera. The only fixed attachment then remaining for the muscle is at the scleral spur/trabecular meshwork/corneal periphery,<sup>10,14</sup> so the muscle is drawn forward toward that attachment by its internal elasticity and the nonspecific contraction/shrinkage produced by the fixative.

The present dissection strategy was designed to maintain the posterior and outer attachments of the ciliary muscle in some sections, while partly disturbing them in others. In "middle" meridional sections remote from the cut margins, the posterior attachment of the muscle to the elastic fiber network of Bruch's membrane and the outer attachments of ciliary muscle/choroid to the sclera should remain intact, as in whole globes fixed before dissection. In meridional sections close to the margins of the incision, some (but not all) of the posterior muscle tendons, and the elastic fiber network of Bruch's membrane along its entire meridional extension, would be cut. Additionally, the attachment of the primate uvea to the sclera is very scant in the region of the ciliary body<sup>15</sup>; entry of saline (containing pilocarpine or atropine) into the supraciliary or suprachoroidal cleft would support an artifactual detachment of ciliary muscle/choroid from the sclera near the cut margin. After detachment, the ciliary muscle/choroid would no longer follow a curved or bent course parallel to the sclera, but would be situated more centripetally. Thus, the distance between the two major fixation points of the elastic layer of Bruch's membrane (the posterior tendons of ciliary muscle and the sclera near optic canal) would shorten, allowing the ciliary muscle to overcome any age-related decrease in compliance of its posterior attachment (see below), assuming the muscle itself is still capable of contraction. Additionally, for both locations (margin and middle), sections were available with and without the lens and the zonule in place. These distinctions between margin and middle sections facilitate examination of factors that might be responsible for the age-related loss of ciliary muscle movement.

Mechanical restriction of the muscle

by the enlarging aging lens or a change in compliance or some other characteristic of the zonular fibers cannot be involved, because the findings were the same whether or not the lens and zonule were excised before fixation. The ciliary muscle retains a substantial ability to contract even in old animals, since significant pilocarpine vs atropine differences in length and apical position were maintained and essentially constant throughout the life span in marginal sections. The small remaining young vs old differences in pilocarpine-induced muscle shortening could be consequent to the minor ciliary neuromuscular structural changes previously described,<sup>4</sup> or more likely, as we will now discuss, to mechanical restriction.

In middle sections, anterior movement of the ciliary muscle in response to pilocarpine, as denoted by measurements of muscle length and apical position relative to the scleral spur, declined with age and was almost completely lost in the oldest animals. Since middle sections maintain the muscle's posterior and outer attachments, stiffening of these attachments could restrain the muscle in place, preventing its forward movement even though the muscle cells are contracting. Recent ultrastructural studies of the muscle's posterior attachments in young and old rhesus monkeys are consistent with such stiffening.<sup>6</sup>

In middle sections from young and old

rhesus eyes, the pilocarpine-stimulated muscle remains attached to the inner scleral wall, even in the absence of mechanical compression by intraocular pressure and vitreous. Apposition of these structures may, therefore, be maintained by the hyaluronate between them,<sup>16</sup> which may serve as a physiologic "glue." Elasticity of the hyaluronate or surface tension would allow anteroposterior movement of the muscle. Clinically, the ciliary muscle usually remains attached to the sclera even after near-total pars plana vitrectomy, although the intraocular pressure may contribute to muscle-sclera apposition.

In sections near the margin, where fluid can enter and dissect into the potential space between ciliary muscle/choroid and sclera, a separation of these tissues is seen. This may be analogous to the frequent development of cilio-choroidal detachment following cataract or filtration surgery in eyes following vitrectomy. In such cases, the dissecting fluid presumably originates from leaky uveal blood vessels, prostaglandins released from the inflamed tissues may mediate dissolution of the intervening hyaluronate,<sup>17</sup> and ocular hypotony prevents simple mechanical apposition.

Our observations are consistent with previously reported histologic,<sup>1</sup> ultrastructural,<sup>4,6</sup> biochemical,<sup>5</sup> and in vivo videographic<sup>2</sup> findings. Collectively, these data strongly suggest that in the rhesus monkey the posterior and per-

haps the outer attachments of the ciliary muscle stiffen with age, so that the muscle contracts isometrically. This phenomenon would be sufficient to explain presbyopia in this species,<sup>2,3</sup> without invoking any other age-related alteration in ciliary neuromuscular function or lenticular elasticity (although the latter has not yet been studied to our knowledge). Furthermore, the age-related decline in both outflow facility and the facility response to intracameral pilocarpine in the rhesus<sup>18</sup> is consistent with this hypothesis, since pilocarpine exerts its facility-increasing effect by causing the ciliary muscle to change configuration and thereby deform the trabecular meshwork.<sup>19</sup> It is unknown whether a similar phenomenon accounts for human presbyopia, but given the anatomic and physiologic similarities between the human and rhesus accommodative apparatus, it is distinctly possible.

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