Age-Related Loss of Morphologic Responses to Pilocarpine in Rhesus Monkey Ciliary Muscle

Elke Lütjen-Drecoll, MD; Ernst Tamm, MD; Paul L. Kaufman, MD

• Ciliary muscle topography and connective tissue distribution were studied by light microscopy in atropinized, pilocarpinized, or untreated eyes from rhesus monkeys of various ages. With age, the connective tissue ground plate between ciliary muscle and ciliary processes thickens, while there is very little increase in connective tissue within the ciliary muscle. With age, the atropinized muscle becomes shorter and smaller in area while it remains unchanged in width and position. In pilocarpinized eyes, the ciliary muscle is shorter, narrower, smaller in longitudinal and total area (ie, more circular and compact), and positioned more anteriorly than in contralateral atropinized eyes. These contractile responses to pilocarpine diminish with age at a rate similar to that for accommodative decline. According to these topographic findings, physicians seeking the pathophysiologic characteristics of presbyopia, which occurs in humans and rhesus monkeys on a comparable relative time scale, should redirect their attention toward the ciliary muscle.
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Presbyopia, the age-related loss of accommodative amplitude, is a seemingly universal and invariant human affliction the cause and pathophysiologic characteristics of which are unknown.[1-3] Earlier studies that

suggested that lenticular factors rather than decreased ciliary muscle contractility were responsible for presbyopia employed indirect methodologies,[4-9] while more direct experimental approaches were severely hindered by the lack of an animal model.[1-3,10]

The accommodative apparatus of the rhesus monkey (*Macaca mulatta*) appears to be similar to that of the

See also p 1526.

human, and the accommodative amplitude in rhesus eyes declines with age on a comparable relative time course.[1,10-12] This species is now under multidisciplinary scrutiny as a model system for human accommodation and presbyopia. It therefore seemed important to study the age-comparative morphologic and topographic characteristics of the ciliary muscle and their possible functional correlates in rhesus monkeys over their entire life span.

We have previously shown that during pilocarpine-induced accommodation, the young monkey ciliary muscle changes its shape and position. The muscle shortens and moves anterointernally, and in sagittal sections, the area of the longitudinal portion decreases, while the area of the circular portion increases, giving the muscle a sharp and prominent inner edge.[13] We report the following: (1) the shape and position of the ciliary muscle, the sagittal area of different regions, and the content and distribution of connective tissue in rhesus monkey eyes of various ages; (2) the morphologic and topographic responses of the rhesus ciliary muscle to pilocarpine hydrochloride and atro-

pine sulfate at various ages; and (3) the effect of different tissue-processing strategies on the information obtained.

MATERIALS AND METHODS
Animals and Eyes

Twelve rhesus monkeys received, in vivo, one drop of commercial ophthalmic 10% pilocarpine hydrochloride topically in one eye and 1% atropine sulfate in the other eye, 45 minutes and 30 minutes, respectively, before fixation began. In each case, the pupils responded appropriately.

Five of these monkeys then underwent perfusion from the left ventricle with heparinized physiologic saline followed by fixative. The eyelids and orbital bones were cut and rongeured away, and the globes were removed with minimal external pressure. In three of these animals (aged 8, 10, and 26 years), after enucleation, full-thickness 3 × 3-mm windows were cut in the posterior sclera adjacent to the optic nerve and in the central cornea using a razor-blade knife and Vannas scissors; the globes were then placed whole in fixative until final processing in West Germany two weeks later. In the other two animals that underwent perfusion (aged 15 and 34 years), the eyes were removed immediately and then bisected equatorially, quadrisected anteriorly, and fixed by immersion.

Seven monkeys did not undergo perfusion; rather, their eyes were fixed only by immersion immediately after enucleation. The eyes of four of these animals that did not undergo perfusion (aged 4, 8, 23, and 26 years) were enucleated in vivo, scleral and corneal windows were cut, and the eyes were placed whole in fixative for 15 minutes. The eyes were then dissected as described above, and the pieces were returned to the fixative. The eyes of the remaining three animals (7, 12, and 20 years) were enucleated, dissected at once, and fixed by immersion.
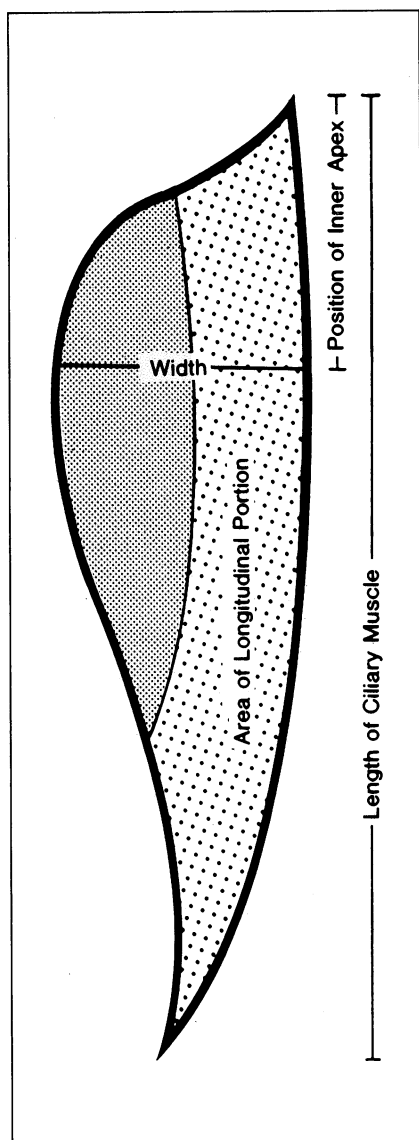
Another group of three young animals

Fig 1.—Schema for topographic analysis of ciliary muscle. By digitization of ciliary muscle drawings, measurements were made of ciliary muscle length (anteroposterior distance along outer longitudinal edge from posterior tip to anterior insertion at scleral spur), width (perpendicular distance between inner apex and outer longitudinal edge), position (anteroposterior distance from inner apex to scleral spur, perpendicular to width), and area of entire muscle and longitudinal component.

(all aged 3 years) received no topical drugs in vivo, but after enucleation and dissection, quadrants of each eye were placed in fixative containing 123 mmol/L of pilocarpine hydrochloride, 43 mmol/L of atropine sulfate, or no drug to compare drug actions within the same eye.

The third group, consisting of 29 animals ranging in age from 137-day fetuses and a 3-week-old neonate to 34 years, was not exposed to pilocarpine or atropine either in vivo or in the fixative. In these animals the eyes were enucleated after the animals were killed, and they were then dissected and fixed by immersion.

Tissue Processing and Evaluation

The fixative for all monkeys was Ito's solution.[14] The eyes were further dissected, embedded in epoxy resin, and processed for light-microscopic examination using Richardson's and periodic acid–Schiff stains.[15] In the three rhesus monkeys that underwent initial perfusion and then immersion fixation of the whole bulb, two crescent strips running from the anterior to posterior pole that were 5 mm wide at the equator were cut from opposite sides of the globe with a razor-blade knife and scissors; this assured that the ciliary muscle remained anchored anteriorly and (via Bruch's membrane) posteriorly. These sectors were then embedded in paraffin, after which 6-μm sections were cut and stained with hematoxylin-eosin. The rest of the globe was dissected and processed as usual.

In the 15 pairs of rhesus eyes exposed to pilocarpine or atropine, drawings outlining the perimeter and longitudinal component of the ciliary muscle were made from eight epoxy resin-embedded sections per eye (two sections per quadrant); in the nine pairs that were fixed whole, drawings were also made from two immediately adjacent paraffin-embedded sections using a drawing microscope (Wild, Heerbrugg, Switzerland). These drawings permitted measurement by digitization (MOP/17 MO1 system, Contron, Munich) of the length, width, position, and area of ciliary muscle. In Fig 1 the measurements are schematized and defined. In all sections of all 41 animals, the amount and distribution of connective tissue within the ciliary muscle was subjectively evaluated (Fig 2).

The drawings were done by E.L.-D., and the measurements were done by E.T.; neither knew the age, drug treatment status, or fixation protocol of the material they were evaluating.

Accommodation and Anesthesia

In a few animals, the maximum accommodative responses to topical pilocarpine or carbachol had been determined previously. In each case, the accommodative amplitude was consistent with the animal's age based on the known age-accommodation relationship for a large, representative sample of this rhesus colony.[10]

Anesthesia for in vivo enucleation and systemic perfusion was 15 mg/kg of ketamine hydrochloride administered intramuscularly followed by 25 mg/kg of pentobarbital sodium administered intravenously. Anesthetized animals were killed by exsanguination or a pentobarbital overdose.

This study conforms to the guidelines of the Association for Research in Vision and Ophthalmology's Resolution on the Use of Animals in Research.[16]

RESULTS
Ciliary Muscle Topography

In sagittal sections from adult rhesus eyes dissected before fixation, there were no apparent systematic differences in muscle length, width, position, overall area, or area of specific regions related to age or to treatment with pilocarpine or atropine, interindividual variations notwithstanding. In the nine animals in which the globes were fixed intact, there were systematic age- and drug-related differences. However, the measurements obtained from the epoxy resin-embedded and adjacent paraffin-embedded sections in the *individual* eyes that were fixed intact were identical, and the sections were virtually superimposable. This eye-specific comparability existed in both the pilocarpine- and atropine-treated eyes. We conclude that dissecting the eyes before fixation altered muscle shape and position, obscuring or obliterating drug and age effects, whereas dissection and fixation in epoxy resin of the globes that were initially fixed intact did not. Therefore, histometric analysis is presented in Table 1 only for the latter material.

The general shape of the ciliary muscle and the size of its subdivisions were essentially the same in all nine atropinized eyes (Figs 2 through 4). There was no circular portion and only a small reticular zone. The outer longitudinal region predominated, and the individual muscle fibers in its anterior part appeared narrow, with optically empty spaces visible between the muscle bundles. The angle formed by the borders of the muscle at its insertion to the scleral spur was acute. The reticular fibers formed an obtusely angled inner apex located approximately 0.5 to 0.9 mm behind the scleral spur, where the muscle attained its maximum width of approximately 0.6 to 0.8 mm.

The shape and size of subdivision of ciliary muscle differed markedly among the nine pilocarpinized eyes. In the four youngest animals, a well-developed circular portion formed an acutely angled inner apex, which was located nearly as far forward as (and in some sections anterior to) the scleral spur. Thus, the muscle generally resembled a right triangle, with the right angle at its anterior insertion into the spur (Figs 2 and 3). Compared with the contralateral atropine-treated eyes, the muscle tended to be narrower at the inner apex, smaller in overall area, and shortened (Table 2). The longitudinal portion, which in the atropinized eyes constituted approximately three fourths of the overall muscle area, was smaller and constituted a smaller fraction of the total muscle area in the pilocarpinized eyes (Table 2). The individual muscle-fiber
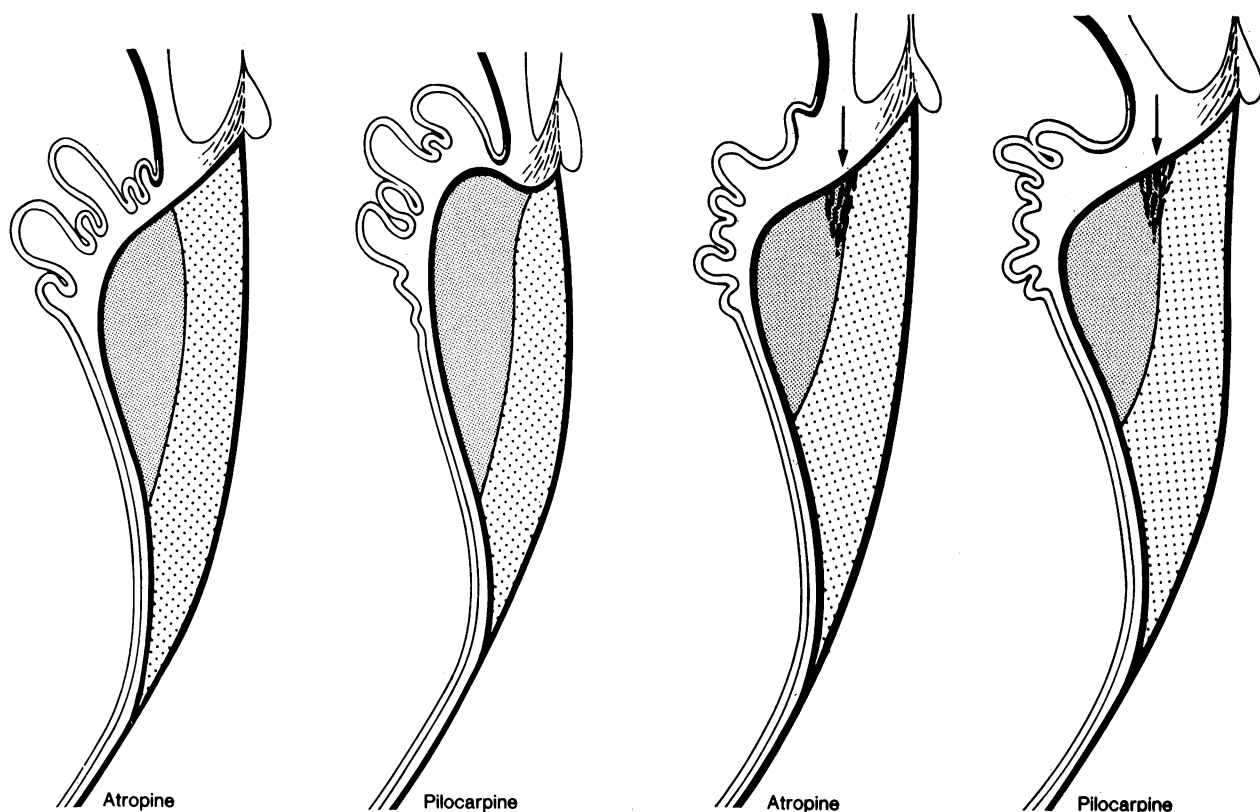
Fig 2.—Ciliary muscle topography and connective tissue distribution in rhesus monkeys. Representative sections are depicted schematically. Left, 8-year-old rhesus monkey exhibits essentially no intramuscular connective tissue. Right, 34-year-old rhesus monkey exhibits connective tissue (arrows) only anteriorly between longitudinal and reticular zones. Age- and drug-related differences in muscle topography are described in "Results" section.

Table 1.—Ciliary Muscle Topography in Pilocarpine- or Atropine-Treated Rhesus Eyes Fixed Intact at Various Ages[*]

Animal No./ Age, y	Eye	Drug	Length, mm	Width, mm	Inner Apical Position, mm	Area of Longitudinal Portion, mm²	Total Area, mm²	Longitudinal Area/ Total Area Ratio
127/4	L	Atropine	3.33 ± 0.08	0.57 ± 0.02	0.49 ± 1.00	0.85 ± 0.03	1.18 ± 0.03	0.72 ± 0.02
	R	Pilocarpine	3.00 ± 0.06	0.56 ± 0.01	0.09 ± 0.02	0.76 ± 0.03	1.10 ± 0.03	0.69 ± 0.02
61/8	L	Atropine	3.25 ± 0.07	0.75 ± 0.04	0.74 ± 0.14	0.99 ± 0.15	1.48 ± 0.04	0.67 ± 0.04
	R	Pilocarpine	2.74 ± 0.06	0.64 ± 0.08	0.15 ± 0.11	0.66 ± 0.04	1.20 ± 0.03	0.55 ± 0.03
89/8	L	Atropine	3.12 ± 0.07	0.79 ± 0.02	0.66 ± 0.07	1.11 ± 0.03	1.53 ± 0.02	0.73 ± 0.03
	R	Pilocarpine	2.68 ± 0.17	0.75 ± 0.05	0.11 ± 0.10	0.83 ± 0.06	1.53 ± 0.09	0.54 ± 0.03
60/10	L	Atropine	3.36 ± 0.06	0.80 ± 0.01	1.52 ± 0.06	1.25 ± 0.04	1.52 ± 0.04	0.82 ± 0.01
	R	Pilocarpine	2.91 ± 0.10	0.60 ± 0.02	0.09 ± 0.03	0.78 ± 0.04	1.30 ± 0.05	0.60 ± 0.02
56/15	L	Atropine	3.31 ± 0.05	0.70 ± 0.02	0.65 ± 0.03	0.96 ± 0.04	1.33 ± 0.05	0.72 ± 0.01
	R	Pilocarpine	3.20 ± 0.04	0.70 ± 0.02	0.53 ± 0.04	0.89 ± 0.03	1.29 ± 0.04	0.69 ± 0.02
129/23	L	Atropine	2.90 ± 0.18	0.61 ± 0.04	0.68 ± 0.06	0.76 ± 0.09	1.00 ± 0.11	0.76 ± 0.02
	R	Pilocarpine	2.76 ± 0.20	0.62 ± 0.04	0.35 ± 0.04	0.60 ± 0.04	0.85 ± 0.06	0.71 ± 0.03
221/26	L	Atropine	2.80 ± 0.08	0.62 ± 0.04	0.87 ± 0.07	0.79 ± 0.04	0.99 ± 0.05	0.80 ± 0.02
	R	Pilocarpine	2.60 ± 0.04	0.56 ± 0.02	0.63 ± 0.04	0.61 ± 0.02	0.84 ± 0.03	0.73 ± 0.05
59/26	L	Atropine	2.91 ± 0.07	0.68 ± 0.04	0.67 ± 0.04	0.94 ± 0.03	1.15 ± 0.03	0.82 ± 0.02
	R	Pilocarpine	3.10 ± 0.07	0.64 ± 0.03	0.66 ± 0.04	0.95 ± 0.06	1.22 ± 0.05	0.78 ± 0.02
43/34	L	Atropine	2.63 ± 0.11	0.74 ± 0.01	0.77 ± 0.05	0.72 ± 0.04	0.97 ± 0.02	0.74 ± 0.04
	R	Pilocarpine	2.54 ± 0.08	0.74 ± 0.02	0.69 ± 0.04	0.59 ± 0.04	0.90 ± 0.03	0.66 ± 0.03

[*]Data are mean ± SEM values from eight sections. Each section contributed one value for each measurement.
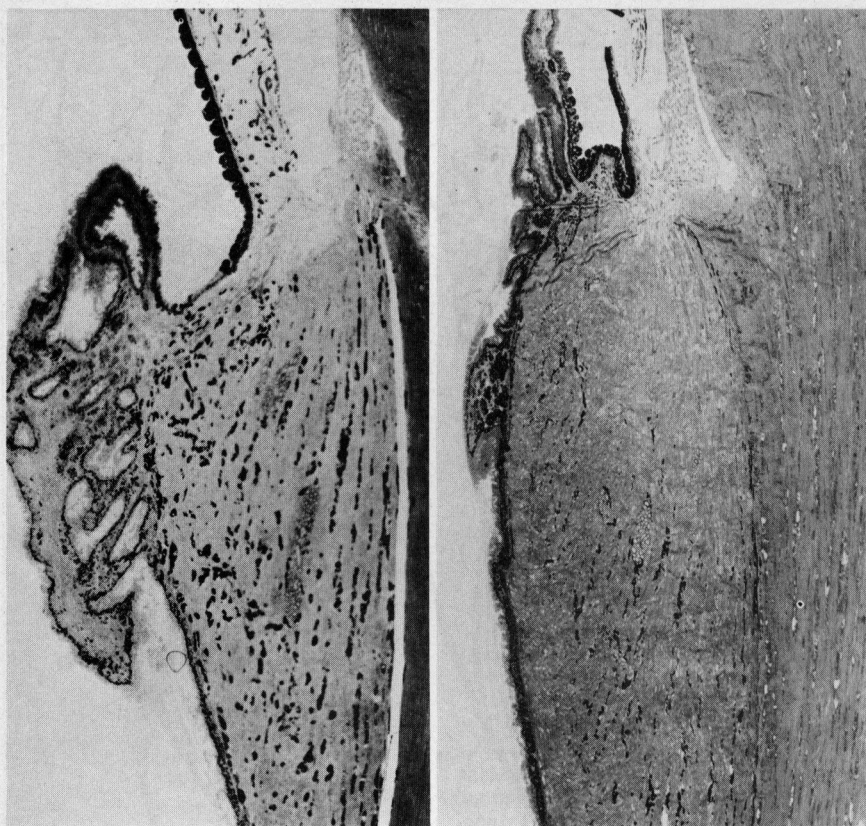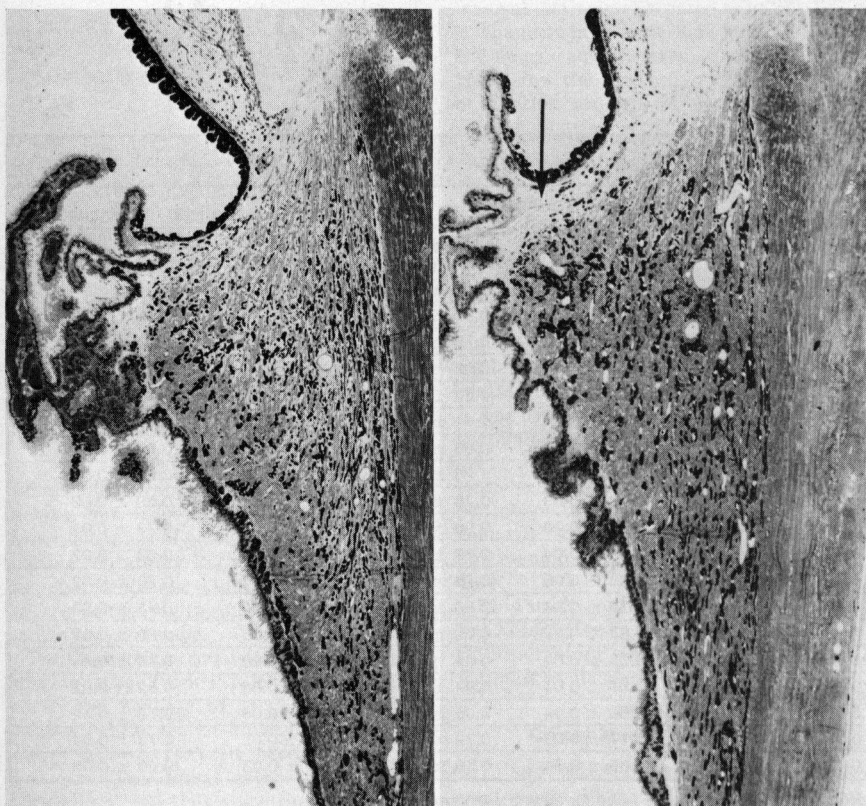
Fig 3.—Ciliary muscle of 8-year-old rhesus monkey was treated topically with atropine (left, left eye) or pilocarpine (right, right eye) before enucleation. Note extremely anterior position of inner apex of ciliary muscle in pilocarpine-treated eye and absence of intramuscular connective tissue (Richardson's stain, original magnification ×120).

bundles in the anterior longitudinal region appeared thicker than in the atropinized eyes, and no empty spaces were visible between the fiber bundles (Fig 3).

In the older animals, the differences between eyes treated with pilocarpine and atropine were much less pronounced. In the 15- and 23-year-old animals and in one 26-year-old animal, only a small circular portion was formed in the ciliary muscle of the pilocarpinized eyes (Figs 2 and 4). In these three eyes, the inner apex of the muscle was located farther anteriorly than in the contralateral atropinized eyes, but in contrast to the younger animals, the inner apex of the muscle remained relatively far posterior to the scleral spur (Table 1). In the other 26-year-old animal and in the 34-year-old animal, the muscle in the pilocarpinized eyes exhibited no circular portion (Figs 2 and 4), and there was essentially no difference in muscle position between the eyes treated with pilocarpine and atropine (Table 1).

Some other age-related trends were most apparent in the eyes treated with atropine but were somewhat obscured in the eyes treated with pilocarpine by the more pronounced muscle contraction in the younger eyes (Fig 5). With age, the ciliary muscle became shorter (Fig 5, part 1) and smaller in area (Fig 5, part 13). The longitudinal component also decreased in area (Fig 5, part 10), so that it occupied approximately the same proportion of total muscle area throughout the animal's life (Fig 5, part 16). The width of the muscle apex (Fig 5, part 4) and its position relative to the scleral spur (Fig 5, part 7) were not related to age in the atropinized eyes.

Ciliary Muscle Connective Tissue

Intramuscular Connective Tissue.—In youth (ages 1 to 4 years) and early and middle adulthood (ages 6 to 20 years) the muscle bundles were packed closely together; connective tissue was only seen in small amounts surrounding nerve fibers and blood vessels, and the

Fig 4.—Ciliary muscle of 34-year-old rhesus monkey was treated topically with atropine (left, left eye) or pilocarpine (right, right eye) before enucleation. Note similar position of inner apex of ciliary muscle, well posterior to scleral spur, in both eyes. In right eye, arrow indicates increased amount of connective tissue between ciliary muscle and iris root (ground plate) compared with younger animals (Fig 3); no increase in connective tissue is seen within muscle (Richardson's stain, original magnification ×120).

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Measurement	Drug	Age, y	
		≤10 (n = 4)	≥15 (n = 5)
Length, mm	Pilocarpine	2.832 ± 0.074	2.840 ± 0.132
	Atropine	3.265 ± 0.054	2.910 ± 0.112
	Pilocarpine/atropine ratio	0.867 ± 0.012†‡	0.976 ± 0.023‡
	Difference between pilocarpine and atropine in fellow eyes	−0.433 ± 0.038§‖	−0.070 ± 0.068‖
Width, mm	Pilocarpine	0.637 ± 0.041	0.652 ± 0.031
	Atropine	0.727 ± 0.054	0.670 ± 0.025
	Pilocarpine/atropine ratio	0.884 ± 0.052	0.972 ± 0.021
	Difference between pilocarpine and atropine in fellow eyes	−0.090 ± 0.042¶	−0.018 ± 0.013
Inner apical position, mm	Pilocarpine	0.110 ± 0.014	0.572 ± 0.062
	Atropine	0.853 ± 0.229	0.728 ± 0.041
	Pilocarpine/atropine ratio	0.153 ± 0.033§#	0.787 ± 0.080**#
	Difference between pilocarpine and atropine in fellow eyes	−0.742 ± 0.233††‡‡	−0.156 ± 0.057§§‡‡
Area of longitudinal portion, mm²	Pilocarpine	0.757 ± 0.036	0.728 ± 0.079
	Atropine	1.050 ± 0.085	0.834 ± 0.049
	Pilocarpine/atropine ratio	0.548 ± 0.091‖‖¶¶	0.864 ± 0.046††¶¶
	Difference between pilocarpine and atropine in fellow eyes	−0.292 ± 0.079††‡‡	−0.106 ± 0.034††‡‡
Total area, mm²	Pilocarpine	1.283 ± 0.092	1.020 ± 0.097
	Atropine	1.428 ± 0.083	1.088 ± 0.068
	Pilocarpine/atropine ratio	0.900 ± 0.042**	0.931 ± 0.040
	Difference between pilocarpine and atropine in fellow eyes	−0.145 ± 0.064¶	−0.068 ± 0.041
Longitudinal area/total area ratio	Pilocarpine	0.595 ± 0.035	0.714 ± 0.020
	Atropine	0.735 ± 0.031	0.768 ± 0.018
	Pilocarpine/atropine ratio	0.813 ± 0.053††‡‡	0.929 ± 0.012††‡‡
	Difference between pilocarpine and atropine in fellow eyes	−0.140 ± 0.042††##	−0.054 ± 0.009†##

*Data are mean ± SEM values for one pilocarpine-treated eye and one atropine-treated eye from each animal.
†Difference between pilocarpine- and atropine-treated eyes was significant (P < .005 by two-tailed, paired Student t test).
‡Difference in response to pilocarpine between younger and older animals was significant (P < .01 by two-tailed, two-sample Student t test).
§Difference between pilocarpine- and atropine-treated eyes was significant (P < .001 by two-tailed, paired Student t test).
‖Difference in response to pilocarpine between younger and older animals was significant (P < .005 by two-tailed, two-sample Student t test).
¶Difference between pilocarpine- and atropine-treated eyes was significant (P < .12 by two-tailed, paired Student t test).
#Difference in response to pilocarpine between younger and older animals was significant (P < .001 by two-tailed, two-sample Student t test).
**Difference between pilocarpine- and atropine-treated eyes was significant (P < .10 by two-tailed, paired Student t test).
††Difference between pilocarpine- and atropine-treated eyes was significant (P < .05 by two-tailed, paired Student t test).
‡‡Difference in response to pilocarpine between younger and older animals was significant (P < .05 by two-tailed, two-sample Student t test).
§§Difference between pilocarpine- and atropine-treated eyes was significant (P < .06 by two-tailed, paired Student t test).
‖‖Difference between pilocarpine- and atropine-treated eyes was significant (P < .01 by two-tailed, paired Student t test).
¶¶Difference in response to pilocarpine between younger and older animals was significant (P < .02 by two-tailed, two-sample Student t test).
##Difference in response to pilocarpine between younger and older animals was significant (P < .06 by two-tailed, two-sample Student t test).

amount of connective tissue did not increase through age 20 years (Figs 2 and 3). Beyond age 20 years (late adulthood), connective tissue was present between the muscle bundles to a slightly greater extent, mainly anteriorly between the longitudinal and reticular portions (Figs 2 and 6). Some quantitative interindividual differences occurred in these older animals. In two cases the individual muscle fibers were smaller and there was a greater increase in connective tissue, but in most animals the increase in connective tissue was slight (Figs 4 and 6). Hyalinization of the connective tissue bundles was found only in one 24-year-old animal.

Connective Tissue Ground Plate.—The portion of the connective tissue ground plate between the ciliary muscle and the pars plana ciliary epithelium remained unchanged throughout life. However, beyond age 15 years, the connective tissue in the transition area between the iris root, ciliary muscle, and ciliary processes was thickened. Between 15 an 20 years of age, the thickening was only slight and was accompanied by hyalinization limited to the anterior ciliary process stroma. Beyond age 20 years, the thickening was substantial, and the entire ciliary process stroma was hyalinized (Fig 4).

Regional Variation

Within individual animals of all ages there were no obvious regional differences in the amount of connec-

tive tissue between the ciliary muscle bundles or in the overall shape of the ciliary muscle.

Fetal and Neonatal Eyes

In the 137-day-old fetuses and the 3-week-old neonate, the ciliary muscle appeared immature, with the muscle fiber bundles separated by loosely arranged connective tissue.

COMMENT

The size, shape and position of the primate ciliary muscle and its subdivisions change as the muscle contracts and relaxes.[13,17-19] Attempts to capture the muscle in one or another function-
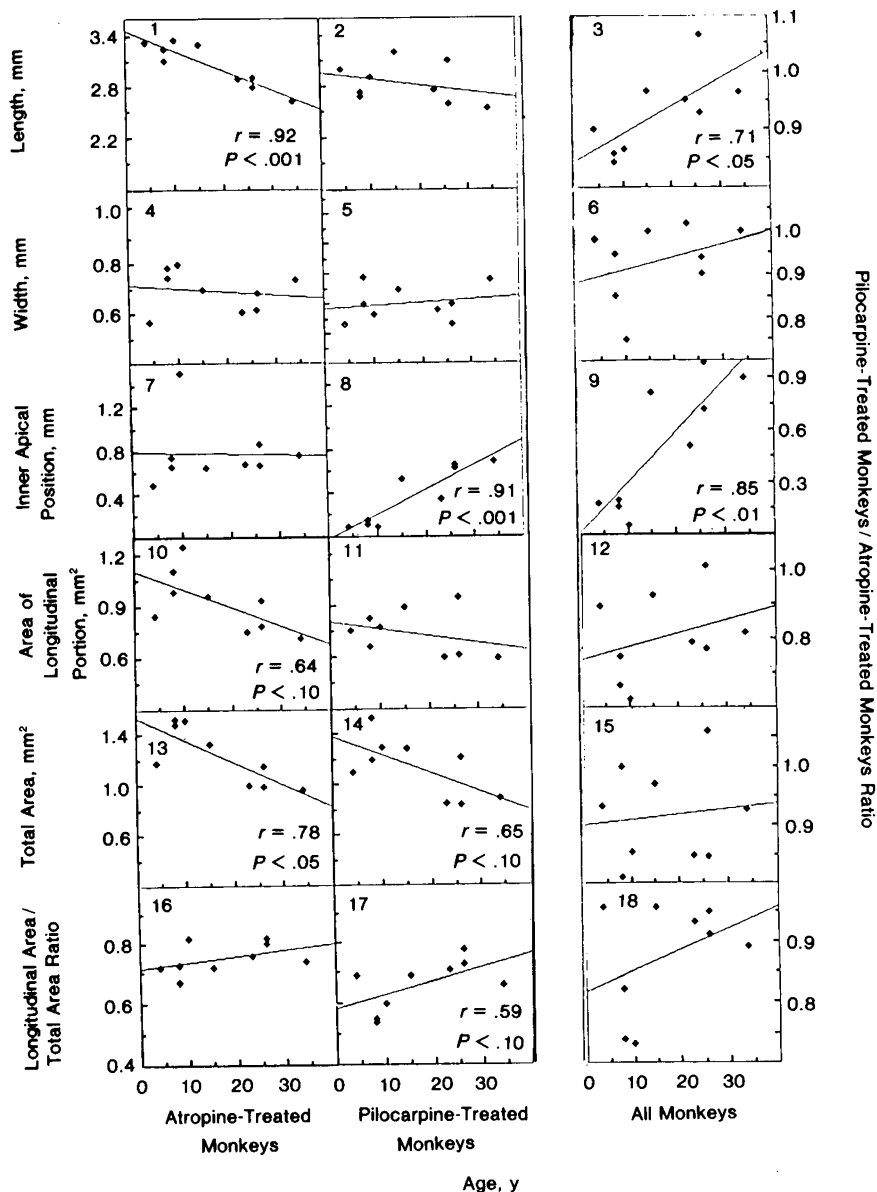
Fig 5.—Ciliary muscle topographic measurements in relation to age in rhesus eyes treated with atropine (left column) or pilocarpine (center column) that were fixed before dissection. Right column gives ratios of measurements for pilocarpine-treated animals to measurements for atropine-treated animals. Each data point is mean value for individual eye measurements given in Table 1. In some panels, line represents least-squares regression of indicated measurement in relation to age. Correlation coefficients are shown only if two-tailed statistical test gives $P < .10$.

al state are subject to artifacts induced by fixation and tissue processing. Dissecting the globe before fixation apparently alters muscle configuration and obscures or prevents putative age- and drug-related effects, perhaps by releasing the tension on Bruch's membrane, the muscle's posterior attachment.[20] Conversely, fixation of the intact globe before dissection, either by systemic perfusion or immersion, better preserves relative functional states, perhaps because the muscle remains under tension at both its anterior and posterior insertions.

The precise sequence of events that occurs during primate accommodation remains under debate.[2,3,21,22] However, there is no doubt that anterointernal movement of the zonular attachment to the valleys of the pars plicata ciliary processes is crucial in facilitating sphericization and forward translation of the crystalline lens.[22] The ciliary muscle in the pilocarpinized young rhesus monkey is strongly contracted, as evidenced by a well-developed circular portion.[13] The inner apex moved markedly forward, and the muscle tended to become nar-

rower rather than thicker. However, the ciliary ring might still have narrowed even though the muscle did not thicken, because the "anterior" movement was not linear along the anteroposterior global axis but, rather, along the spherical inner scleral surface.

The contracting young rhesus ciliary muscle also shortened and tended to decrease in overall sagittal area. Forward movement of the posterior muscle tips attached to the elastic Bruch's membrane[20] seems far more likely than backward movement of the anterior insertion at the relatively rigid scleral spur and could also account for anterior movement of the choroid and ora serrata, which putatively occurs during macaque[23] (E.L.-D., unpublished data, 1987) and human[24] accommodation. Compaction of the muscle was further evidenced by thickening of individual muscle fibers and obliteration of spaces between fiber bundles in the anterior longitudinal region, as previously described in the vervet monkey (African green, *Cercopithecus ethiops*).[13] This compaction presumably constitutes the structural basis for the pilocarpine-induced reduction in the uveoscleral drainage of aqueous humor.[25]

The most striking and unexpected finding was the loss of the ability to induce changes with pilocarpine in ciliary muscle topography with increasing age. We did not specifically study the ocular penetration of the topically applied drugs in these animals. However, the doses were large and were far above the maximal doses required to induce accommodation in other macaques,[26] and the pupils became miotic, indicating that active drug entered the anterior chamber. No drug-free eyes were fixed prior to dissection, so we could not make any observations regarding the possible age dependence of the effects of atropine on the ciliary muscle. However, such putative effects would clearly not explain the age-related loss of responsiveness to pilocarpine. Additionally, direct observation of the ciliary muscle in the living, totally iridectomized rhesus monkey indicates that muscle excursion in response to central electrical stimulation decreases with age.[11,27]

What causes the age-related loss of ciliary muscle movement in the rhesus monkey, and how does it relate to presbyopia?

It is possible that the ciliary muscle cannot move for secondary mechanical reasons; ie, it becomes progressive-
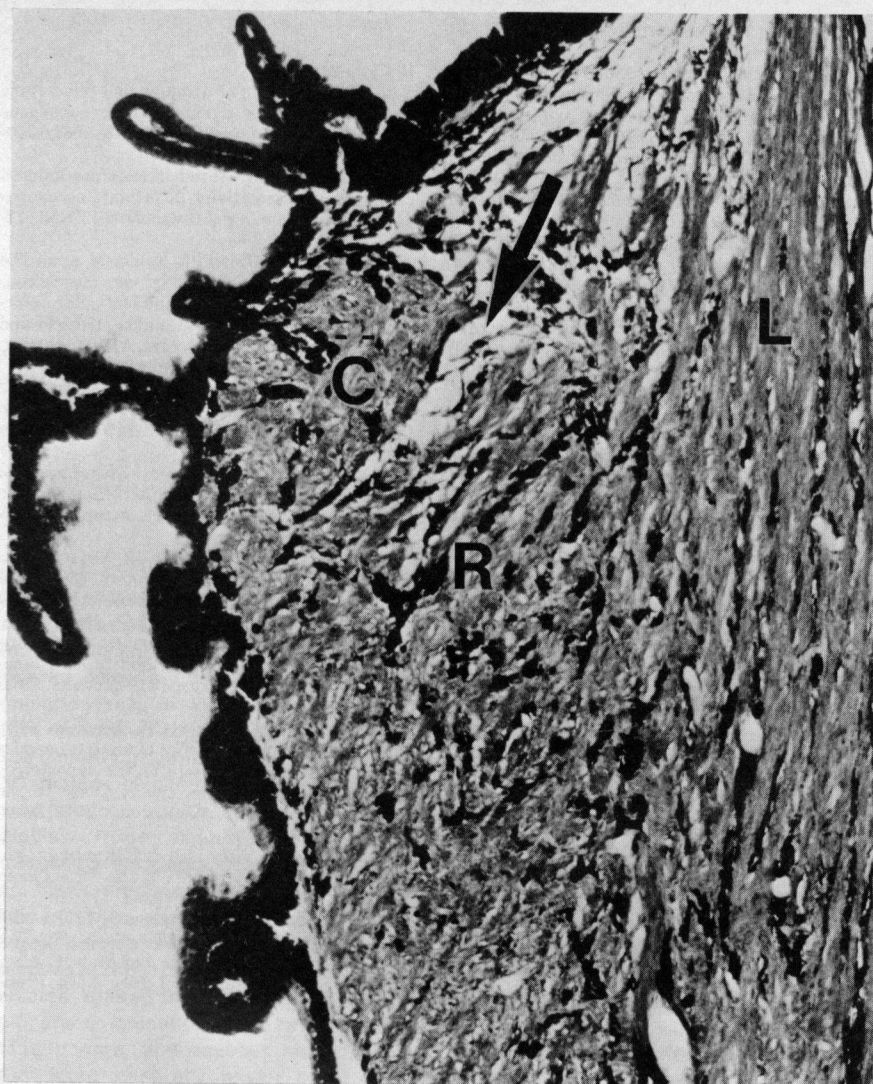
Fig 6.—Anterior part of ciliary muscle in 30-year-old rhesus monkey. There is slight increase in amount of connective tissue (arrow) between longitudinal (L), reticular (R), and circular (C) portions in 30-year-old rhesus monkeys compared with younger animals (Richardson's stain, original magnification ×160).

scopic examination showed alterations in the intramuscular nerves,[28,29] but the functional significance of these alterations is unknown. There was no striking age-related decline in overall ciliary muscle muscarinic receptor concentration or affinity as measured by specific tritiated quinuclidinyl benzilate binding,[30] but relevant subpopulations[31,32] could have been affected and escaped notice. No age-related alteration in ciliary muscle activity of choline acetyltransferase or acetylcholinesterase per milligram of protein was observed.[30] While these receptor and enzyme data suggest that the cholinergic neuromuscular mechanisms remain intact with age, other biochemical or metabolic changes within the muscle fibers could be functionally relevant but undetectable by our morphologic and biochemical methods.

Recently, reports have stated that the human ciliary muscle does not weaken or otherwise functionally decline with age[6,8,9] and that presbyopia is primarily a consequence of lenticular rather than ciliary muscle factors.[2,3,6,8,9] However, these studies utilized indirect methods, such as impedance cyclography or in vitro passive stretch and lens deformation, to make inferences about ciliary muscle function. Despite their unquestioned technical innovativeness and other merits, they do not provide direct and specific information about possible age-related changes in ciliary muscle position, configuration, and contractility.[2] In the rhesus monkey, the age-related decline in structural response parallels the loss of the functional accommodative response to pilocarpine or carbachol eye drops,[10] carbachol administered by corneal iontophoresis,[33] and central electrical stimulation.[11] While our findings do not indicate that one specific mechanism is responsible for presbyopia, they redirect attention toward pathophysiologic patterns involving the ciliary muscle.

ly restrained in the "relaxed" position, so that its contractions are, in effect, isometric. However, the age-related increase in connective tissue within and adjacent to the muscle seems far too small to immobilize it, with the muscle remaining compact and nonhyalinized. Furthermore, if the globe is sectioned equatorially prior to fixation, the muscle often takes on the topography associated with contraction, regardless of age or the presence or absence of cholinergic or anticholinergic drugs. Therefore, internal "rigidity" of the muscle cannot explain the findings. Our present observations do not exclude external restriction of muscle movement by an increasingly inelastic posterior fixation (by Bruch's membrane or choroid) or zonule or by an enlarging inelastic lens.

It is also possible that the ciliary muscle cannot move because of the loss or alteration of relevant muscle or nerve fibers. The area of the ciliary muscle decreases by perhaps one third with age. Since there is so little age-related increase in intramuscular connective tissue, the upper limit of actual muscle loss is probably around 50%, with the circular/reticular zone perhaps slightly more affected than the longitudinal zone. We do not know whether this is sufficient to affect dioptric accommodative amplitude or whether a larger selective loss of muscle fibers especially critical to accommodation occurs. Overall, the muscle appeared qualitatively normal by light-microscopic examination. There were age-related ultrastructural alterations within individual muscle fibers, and light- and electron-micro-

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