DEGENERATION OF CILIARY MUSCLE AND IRIS SPHINCTER FOLLOWING RESECTION OF THE CILIARY GANGLION

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A PROMINENT DIFFERENTIATING FEATURE between skeletal and smooth muscles is their reaction to denervation. Sectioning the motor nerve to the skeletal muscle produces a series of typical trophic changes collectively referred to as atrophy and degeneration of the muscle fiber, whereas a similar autonomic denervation fails to influence the smooth muscle in this fashion or to produce any structural changes.[1] The investigation which is the subject of this report will present unequivocal evidence that resection of the ciliary ganglion produces in the monkey a characteristic degeneration of the ocular smooth muscles, especially apparent in the iris sphincter and in those fibers of the ciliary muscle that are conventionally referred to as circular and oblique fibers. In so doing, it demonstrates for the first time a truly trophic influence of the autonomic innervation on the smooth muscle and shows that such innervation is vital for its structural integrity. Chronic ciliary ganglionectomy provides a chance to investigate and elucidate the mechanism of this trophic effect for a more complete understanding of the neuromuscular interaction. In addition, it permits a more discriminating inquiry into the innervation and function of these ocular structures and into the nature and relative magnitude of the functional contributions of their different components. Ciliary ganglionectomy in the cat, however, failed to produce any such effect on the ocular smooth muscles, emphasizing the extent of species differences in this regard.

A complete review of the relevant literature will not be attempted as its volume is prohibitively large. This area has attracted the physiologist, pharmacologist, neurologist, and anatomist, as well as the ophthalmologist. I shall, therefore, confine myself to a brief summary of the more recent literature which is of immediate relevance to the correct interpretation of the results.

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The composition of the ciliary ganglion and the ciliary nerves differs markedly in the different species. In the cat, the ganglion is exclusively composed of parasympathetic neurons and has only a parasympathetic preganglionic connection.[2,3] It is not traversed by sympathetic or sensory fibers, which join its motor roots, the short ciliary nerves, somewhere between the ganglion and the eyeball. Neurons are not limited to the ganglion but are quite frequently present in its roots and branches and are called accessory ciliary ganglia.[3,4] A comparative study demonstrated that in the monkey and in man, the ganglion contains a sympathetic and a sensory root which traverse its substance and become components of the short ciliary nerves as these emerge from the ganglion.[5] Physiologic studies led to the conclusion that these components, the sensory and the sympathetic, do not synapse in the ganglion itself[6]; others, on morphologic grounds, felt that in addition to parasympathetic ganglion cells, sensory and sympathetic cells could be seen in the ciliary ganglion.[7] Recently, fluorescence staining technique demonstrated adrenergic elements in the ciliary ganglion whose significance is not yet clear.[8,9] Whether these represent truly sympathetic ganglion cells or a parasympathetic adrenergic system cannot be determined at this stage. Thus, in the cat, it may be possible to remove only the parasympathetic component by resecting the ciliary ganglion, carefully avoiding the short ciliary nerves. In the monkey, however, removal of the ciliary ganglion means in addition the loss of the sympathetic and sensory components that traverse it.

The target area of the ciliary ganglion is intraocular. Enucleation produces chromatolysis in 97 per cent of the cells in the ganglion whereas removal of the entire iris produces chromatolysis in only 3 per cent.[10] The main intraocular target seems to be the ciliary body and the ciliary muscle.

The innervation of the iris sphincter and of the ciliary muscle has been the subject of vigorous controversy, prompting renewed investigation whenever advances in methodology promised new insight into the problem. The first aspect of the controversy concerns the presence of dual autonomic motor innervation of the ciliary muscle and iris sphincter. The second pertains to the distribution of the dual innervation in the different parts of the muscle. Histologic studies in the cat, dog, monkey, and man demonstrated the impressively rich innervation of the ciliary muscle and described three types of nerve endings that were uniformly distributed over the entire ciliary muscle and were not limited to any one portion. Two of these were closely and intimately

related to the smooth muscle cell; these were the loop-like "parasympathetic" endings and the ring-like "sympathetic" endings. The third was the end-bulb "sensory" ending which did not relate to the smooth muscle cell but ended in the intervening connective tissue.[11–14] Naked axons with a series of ring-like formations were demonstrated in these studies; some of the rings appeared to be intracellular. These were presumably sympathetic axons with the rings representing the varicosities that become demonstrable by the fluorescence technique in sympathetic nerves and synapses. Again these were not limited to the longitudinal portion of the muscle but could be seen in its different parts including the circular fibers.

Fluorescence staining technique demonstrated adrenergic nerves in the ciliary muscle and iris sphincter.[8,9,15,16] The resolution of the method did not permit the identification of the innervated structure at the terminal. The majority of the nerves were around blood vessels; some, however, were definitely independent of blood vessels and could be seen in the different portions of the muscle and iris sphincter.

With electron microscopy the varicosities could be resolved into a cluster of vesicles and mitochondria.[17] This association is reminiscent of the synapses at the motor end-plate of the skeletal muscle. Two types of vesicles could be identified that were intimately related to the smooth muscle cell: the agranular solid vesicles which were 300–600 Å in diameter, and the granular vesicles that were smaller than 300 Å. Evidence points strongly to the former being the cholinergic terminal and the latter the adrenergic terminal.[18,19] The iris sphincter was found to have predominantly agranular vesicles and the dilator predominantly granular vesicles. In the ciliary muscle both types could be seen although the agranular vesicles were the predominant ones.[17,20]

In man the ciliary muscle was shown to exist not as a syncytium but as individual smooth muscle cells with individual innervation.[20] Three types of nerve terminals have been described in man. The cells exist in bundles surrounded by connective tissue elements and suggest that such muscle bundles may represent functional units. This finding supported the conclusion drawn from physiologic studies against a syncytial arrangement and in favor of individually innervated and activated smooth muscle cells.[21]

While the evidence of morphologic studies does not negate the possibility of dual innervation, it is far from having provided a definitive proof for sympathetic innervation of the ciliary muscle. Functional evidence for dual innervation or, more specifically, for the

sympathetic innervation of the ciliary muscle is also of long standing.[22] However, it was forcefully revived in the middle 'thirties in studies of the effect of removal or stimulation of the sympathetic system on accommodation in man.[23] Removal of the sympathetic innervation, as in Horner's syndrome, results in an increase in amplitude of accommodation whereas stimulation of the sympathetic by epinephrine hydrochloride reduced significantly the amplitude of accommodation. The reduction in amplitude was found to affect mainly the near point.[24] A series of studies in the rabbit, cat, and man demonstrated an increase in hyperopia and flattening of the lens on sudden excitement or on stimulation of the superior cervical ganglion.[25-31] The humoral effect was shown to act on an innervated receptor by demonstrating that, a week after superior cervical sympathectomy, intravenous injection of epinephrine hydrochloride produced a greater effect on the operated side.[27] In demonstrating sensitization, this experiment showed that that portion of accommodation was normally innervated by the sympathetics. In man, this observation was made earlier:[32] following resection of the superior cervical ganglion, topical epinephrine hydrochloride decreased accommodation significantly on the denervated side without affecting the eye on the normal side. Thus, the sympathetic effect on accommodation was shown to be mediated by an innervated receptor. One could not discern, however, whether this was an effect on blood vessels or on the ciliary muscle. A reduction in blood volume could easily lead to an enlargement of the ciliary circle and increased tension in the zonular ligament with resulting flattening of the lens and reduction in its power.

Pharmacologic studies of the excised iris and ciliary muscle demonstrated significant differences between the iris sphincter and the ciliary muscle and pointed out species differences.[33] In the cat and rabbit, the iris sphincter has mainly beta receptors and a few alpha receptors, while in the monkey the iris sphincter has mainly alpha receptors and a few beta receptors. The ciliary muscle, on the other hand, has mainly beta receptors in the cat and rabbit and exclusively alpha receptors in the monkey. However, the functional demonstration of these receptors in the muscle does not necessarily imply that they are innervated receptors. Yet, stimulation of the sympathetic nerve of the excised eye was shown to produce in the cat a contraction of the ciliary muscle and a deformation pattern which was different from that produced by parasympathetic stimulation.[34] This indicated that the ciliary muscle, and not blood volume in this case, was responding to sympathetic

stimulation. However, the non-specific nature which might accompany strong electrical stimulation reduces the finality of this piece of evidence. At the same time, one can hardly afford to dismiss it completely as artefactual.

Of great significance at this point is an understanding of the structural organization of the ciliary muscle. A meridional section of a human eye shows that the fibers of the ciliary muscle aggregate in three main configurations: the outer portion is composed of compact longitudinal fibers; the intermediate, of oblique or radial fibers; and the inner, of circular fibers. The simplified concept of the functional counterpart of this organization is that the circular fibers contract to reduce the ciliary circle and thus the tension on the zonular fibers, and increase the lens power necessary in accommodation; the radial fibers act to enlarge the ciliary circle and thus increase the tension on the zonular fibers, flatten the lens, and reduce its power; the function of the longitudinal fibers, which are attached anteriorly to the scleral spur and posteriorly to the anterior tip of the choroid, is not clear. While some insisted that the ciliary muscle acted like two muscles each with a different result on accommodation,[35] the presence of true circular fibers was negated at the beginning of the century.[36] This finding was lated confirmed by careful dissection and histologic studies.[37,38] The concept of functional unity of all parts of the muscle was proposed.

Careful dissections demonstrated a complex quadriceps organization for the ciliary muscle in man.[38] Four sets of fibers arise from the ciliary tendon which merges with the scleral spur anteriorly. These fibers are arranged in bundles that follow the configuration of the letter "V" and interlace freely in the substance of the muscle. The first set of fibers is the most superficial one and is made up of three layers. The opening of the "V" is directed anteriorly and attaches to the ciliary tendon while its apex ends in the choroid tip. The second set is made up of a similar "V" arrangement and the apex of the "V" attaches to the posterior end of the ciliary process. The third, which is deeper, attaches with the apex of the "V" to the anterior tip of the ciliary process. The fourth set attaches to the head of the ciliary process and to the iris root relating intimately to the dilator muscle. The fourth set is the only one in which the opening of the "V" has a posterior direction. This arrangement of muscle fibers is very different from that of the conventional simplified description. It emphasizes the concept that the ciliary muscle is a complex structure in which free

interlacing of the different bundles takes place and that it is not a simple stratification of functionally and anatomically distinct components. This unitary concept, together with the uniform distribution of innervation, seriously complicates attempts at explaining the anatomical counterpart of dual action. Indeed, an explanation that takes into account the findings of the more recent studies has yet to be proposed.

The functional domain of the ciliary muscle is complex and important, for, in addition to its cardinal role in accommodation, it plays a major role in modifying resistance to aqueous outflow at the trabecular level[39,40] as well as that of the newly discovered uveal or posterior outflow channels.[41–43] Furthermore, the artery supplying the anterior uveal segment and the entire iris traverses the substance of the ciliary muscle, thus providing it with a means of controlling this portion of the intraocular circulation. Hence, accommodation, aqueous fluid dynamics, and circulation to the anterior segment constitute definitive areas that are subject to the modifying action of the ciliary muscle.

Stimulation or resection of the ciliary ganglion has been employed in a wide variety of investigations.[44–52] Pharmacologic studies established the development of sensitization of the iris sphincter to pilocarpine[48,50] and to acetylcholine[49] following ganglionectomy, and investigated the time course of this sensitization; others differentiated between parasympathomimetic action due to inhibition of cholinesterase and that due to direct action on the smooth muscle.[51] Neurophysiologic studies investigated the representation of the ganglion in the central nervous system as well as the phenomenon of regeneration.[10] Others investigated the effect on aqueous dynamics and on the morphology of the anterior segment.[39,44–46,52] None of these studies explored the effect on the histology of the ciliary muscle or verified the assumption that the structural integrity of the iris sphincter was not modified by denervation. Even the long-term studies of the time course of pupil sensitization to pilocarpine, a year or two following ganglionectomy, failed to test the assumption that the number of the fibers of the iris sphincter remain unchanged. This obviously reflects the degree of acceptance of the notion that autonomic denervation is not followed by any drastic structural changes in the smooth muscle.

The investigation which is to be reported in this paper arose as an off-shoot of long-term studies of the effect of ciliary ganglion resection on ocular fluid dynamics, accommodation, and pupil size, and of the reactivity of these parameters to various physiologic and pharmaco-

logic agents. Over the course of five years it became apparent that the results varied markedly with the time lapse following ganglionectomy. It became crucial for their interpretation to know whether or not ganglionectomy produces any change in the number of previously innervated elements, in this case, the smooth muscle cell of the ciliary muscle and of the iris sphincter. If it does not, then the observed changes in results with duration of ganglionectomy are only functional in nature and may be simply interpreted. On the other hand, if ganglionectomy does produce a reduction in the number of these elements, then simple interpretation will no longer be tenable. Histologic examination became mandatory. It revealed that in the monkey the assumption that only functional changes are produced in the smooth muscle by ciliary ganglionectomy cannot be made, for marked degenerative changes ending in loss of smooth muscle elements were found.

METHOD AND PROCEDURE

The experimental animals employed in these studies were the cat, and two monkey species, the rhesus and the cynomolgus (*M. mulatta* and *M. irus*). Adult animals were used, with equal distribution of the sexes.

Under intravenous nembutal anesthesia, a skin incision three cm long was made extending from the external canthus toward the external auditory meatus. The periosteum of the lateral rim of the orbit and the fascia of the temporalis muscle were exposed. The muscle was freed from the bony rim and elevated from the lateral wall of the orbit. These bony structures were then excised. A horizontal incision through the orbital septum and Tenon's capsule was made to expose the eyeball and the interior of the muscle cone. The lateral rectus was detached at its insertion and reflected to improve the exposure, identification, and resection of the ganglion as well as to minimize surgical trauma. The ciliary ganglion could then be seen in the muscle cone intimately related to the optic nerve lying between it and the origin of the lateral rectus muscle. Its pre- and postganglionic connections could be identified. Following this stage one of three courses was selected:

1. The incision was closed and the lateral rectus was reattached. Tenon's capsule and the orbital septum were closed in layers using 6–0 chromic catgut. The skin incision was closed using 6–0 silk.

2. All the preganglionic connections were cut and then the incision was closed.

3. The ganglion and its branches were resected. The ganglion, freed as in Course 2, was lifted forward carrying with it all the postganglionic fibers. The fibers were traced to their scleral entrance into the globe and were severed at that point. The resected ganglion with its branches attached was placed in 10 per cent neutral formalin. The incision was then closed as in Course 1.

At the end of these operations, 200,000 units of penicillin were given intramuscularly. The second side as a rule was kept as control. In some experiments, both sides were operated using different combinations of the three alternatives described above.

Superior cervical ganglionectomy was performed unilaterally or bilaterally with or without combination with one of the above procedures. The ganglion was identified and separated from that of the vagus and then resected.

After varying periods of observation, the eyes were enucleated and placed in 10 per cent neutral formalin prior to their embedding in paraffin. In some cases, 10 mg of pilocarpine hydrochloride was injected intravenously before sacrificing the animal in order to accentuate the difference between the two sides. Histologic sections 8, 10, and 12 μ thick were prepared and stained with hematoxylin-eosin, Masson, Mallory, and colloidal iron stains.[53]

RESULTS

Exposure of the Ciliary Ganglion

The ciliary ganglion with its pre- and postganglionic branches was exposed unilaterally in 16 cats, 6 rhesus monkeys, and 6 cynomogus monkeys leaving the other side as control. After a period of observation that varied from one to three years, both eyes were enucleated and compared histologically. In no instance could a difference be seen between the operated and the non-operated sides in any of these species when the iris sphincter and the ciliary muscle were compared.

Severing the Preganglionic Connections

The procedure of severing the preganglionic roots of the ciliary ganglion and its connection to the nerve to the inferior oblique leaving only the short ciliary branches intact was performed unilaterally in 22 cats, 6 rhesus monkeys, and 6 cynomolgus monkeys leaving the other side as control. These were observed for a period of one to four and one-half years before both eyes were enucleated for histologic studies. No significant difference could be demonstrated between the

two eyes of the same animal in any of the three species when the ciliary muscle and the iris sphincter were compared histologically.

Resection of the Ciliary Ganglion

The ciliary ganglion and its short ciliary nerves were resected unilaterally in 43 cats, 30 rhesus monkeys, and 36 cynomolgus monkeys. The resected ciliary ganglion was always examined histologically to verify its complete excision. Figures 1 and 2 demonstrate in particular the composition of the ciliary ganglion of the adult rhesus monkey.

After a period of follow-up that ranged from sixty days to five years, the eyes were enucleated and prepared for histologic studies. In the cat, no difference between operated and control eyes could be detected when the iris sphincter and the ciliary muscle were compared (Figures 3 and 4). On the other hand, both monkey species showed consistently and in all cases significant histologic changes in the operated eye involving the iris sphincter and the ciliary muscle.

The changes in the iris sphincter one year and four and one-half years after ciliary ganglionectomy appear in Figures 5 and 6, respectively. The marked reduction in the number of smooth muscle cells of the operated side is readily obvious in comparison with the control eye of the same animal. Masson stain and colloidal iron stain failed to demonstrate any increase in connective tissue components or in mucopolysaccharide deposits in the area previously occupied by smooth muscle cells. It looked as if the muscle cells, sarcoplasm and nuclei, had disappeared leaving empty spaces without being replaced by any fibrous proliferation. The connective tissue framework did not differ in the area of smooth muscle fiber loss from that of the adjacent stroma. There was no evidence at this stage of any cellular tissue response. The area of muscle loss seemed to involve the periphery of the sphincter leaving a small muscle bundle close to the pupillary edge. The stroma with its melanophores, the anterior surface of iris, and the pigment epithelium showed no significant change.

In both monkey species, the ciliary muscle demonstrated characteristic changes following ciliary ganglion resection. Briefly, these changes consisted of the disappearance of smooth muscle fibers, atrophy of the remaining muscle fibers, absence of any evidence of reactive or reparative process, and absence of any change in the composition of the connective tissue framework. As a result, the ciliary muscle of the operated side looked in histologic sections more lacy, rarefied, and moth-eaten. These changes involved predominantly, but not exclusively, the circular and oblique fiber bundles and

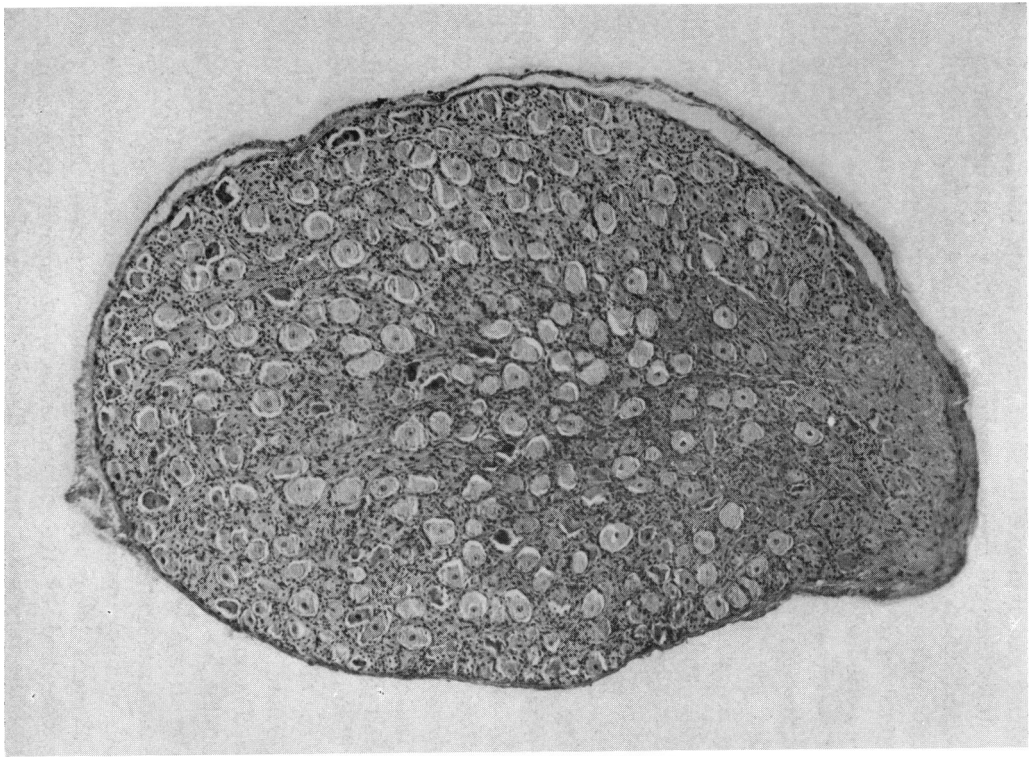

FIGURE 1

Ciliary ganglion of the adult rhesus monkey. Note the complete encircling capsule and the uniform character of the large neurons. Bodian stain; magnification 70 ×.

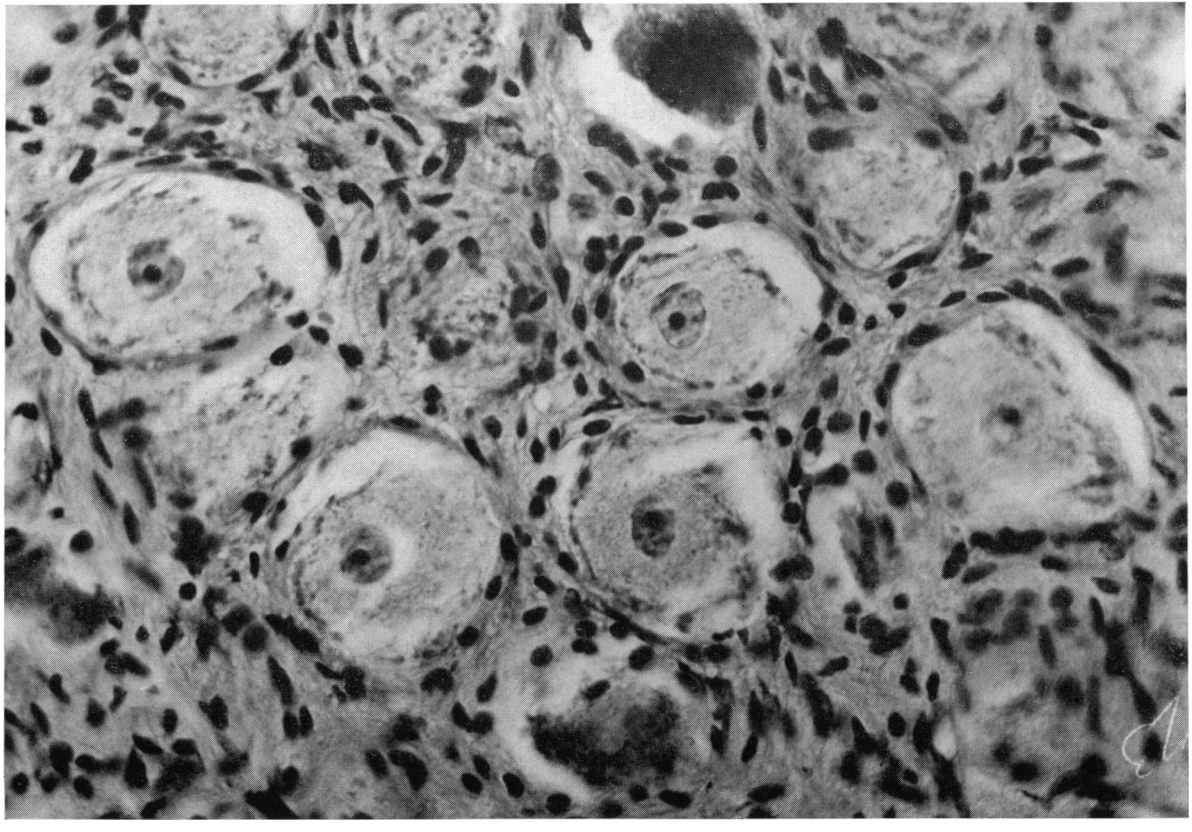

FIGURE 2

Neurons of the ciliary ganglion of the adult rhesus monkey. Note granular cytoplasm and prominent nucleus and nucleolus. Bodian stain; magnification 600 ×.

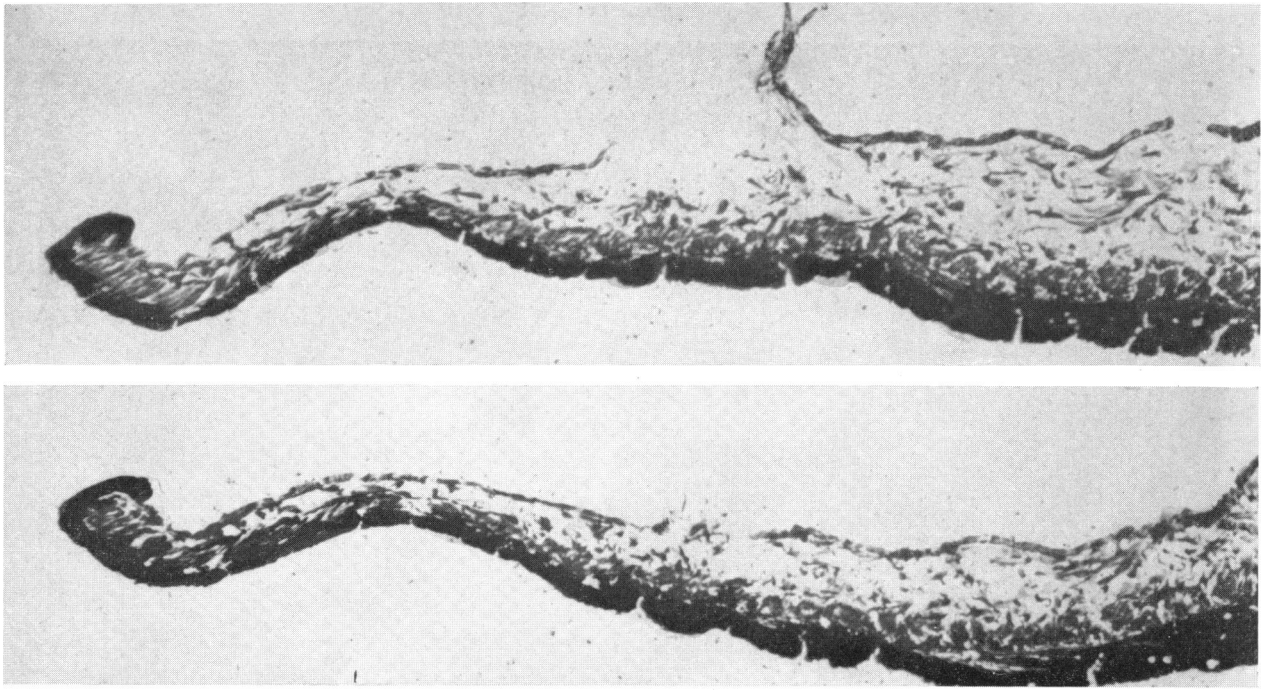

FIGURE 3

The iris sphincter of the cat four and one-half years after ciliary ganglion resection (top) and in the control eye (bottom). Note absence of difference between the two sections with respect to pigment epithelium, sphincter, and stroma. Mallory stain; magnification 150 ×, reduction factor 2.

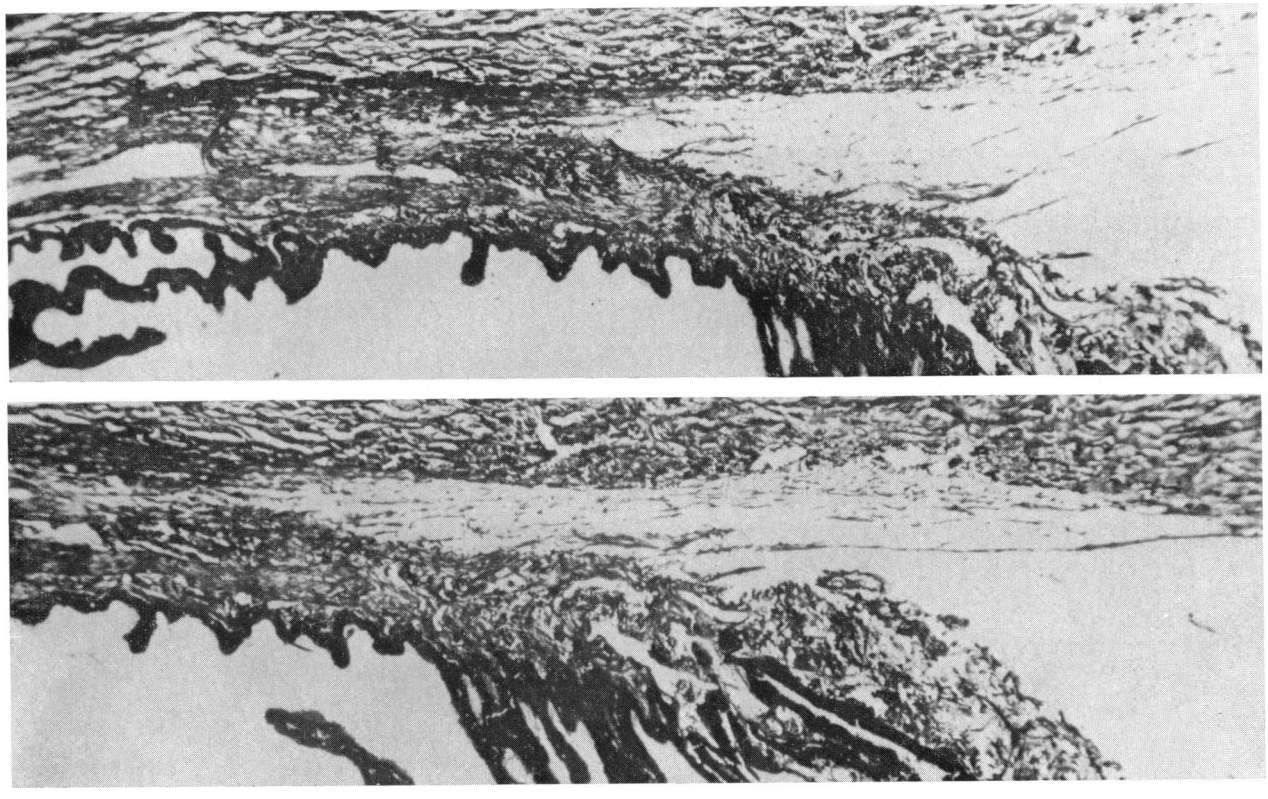

FIGURE 4

The ciliary body of the cat four and one-half years after ciliary ganglion resection (top) and in the control eye (bottom). Anterior chamber to the right, posterior chamber below and to the left sclera superiorly. No difference in muscle size or cellularity can be seen on comparison. Mallory stain; magnification 150 ×, reduction factor 2.

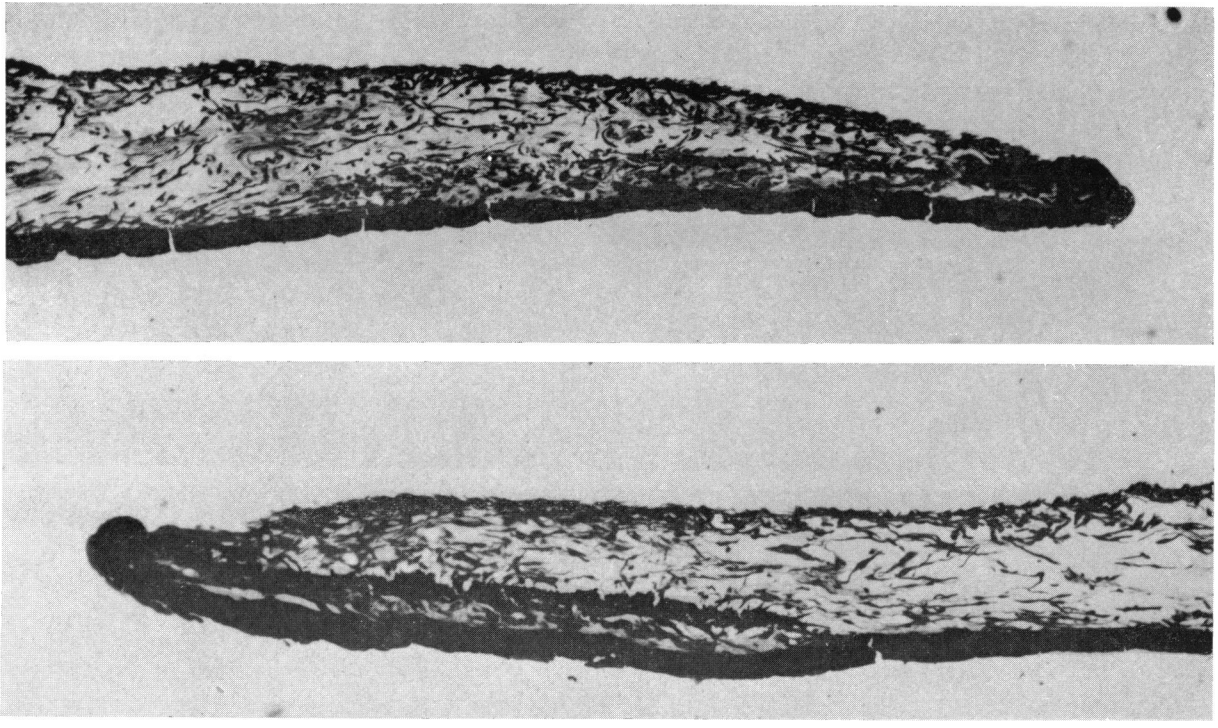

FIGURE 5

Iris sphincter of the rhesus monkey one year after ciliary ganglion resection (top) and in the control eye (bottom). Note the normal density of the sphincter in the control eye as compared with only a central remnant of the sphincter in the ganglionectomized eye. Few muscle fibers remain in the outer two-thirds of the sphincter. Note the similarity of the stroma of the two sections. Mallory stain; magnification 150 ×, reduction factor 2.

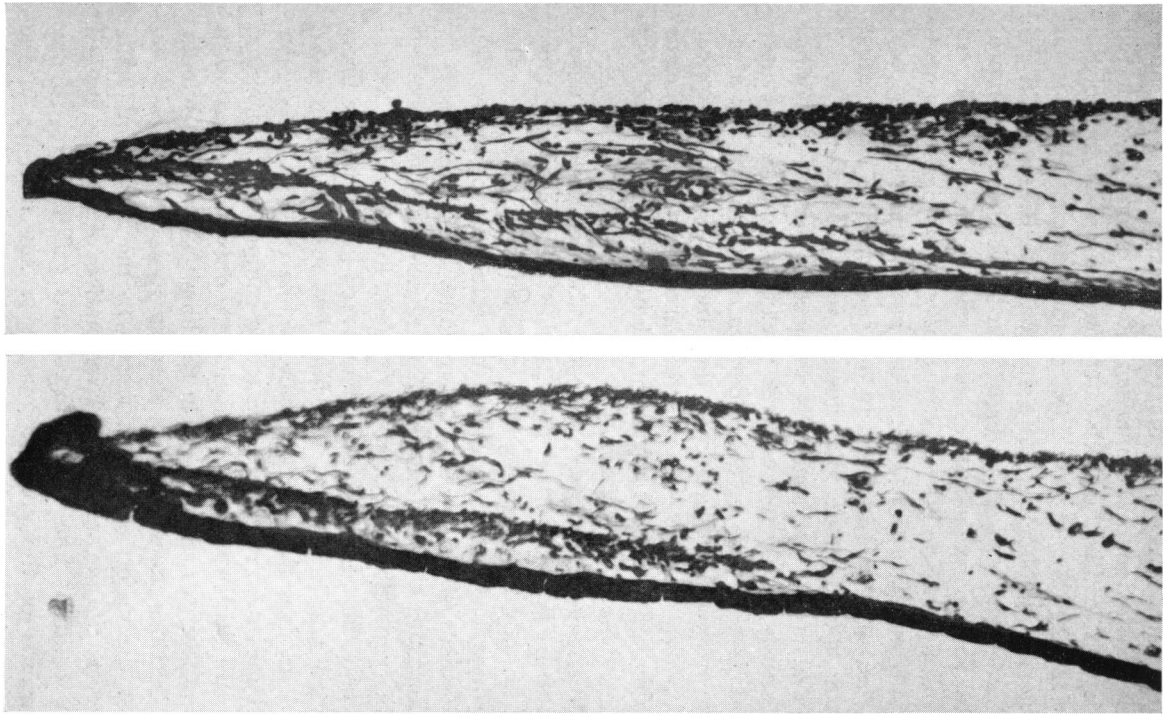

FIGURE 6

Iris sphincter of the rhesus monkey four and one-half years after ciliary ganglion resection (top) and in the control eye (bottom). Note disappearance of smooth muscle cells from the outer three-fourths of the sphincter and the persistence of a small central bundle in the ganglionectomized eye as compared with that of the control. Note similarity of stromal elements in the region of the degenerated sphincter to those in the immediate neighborhood indicating absence of reaction. Mallory stain; magnification 150 ×, reduction factor 2.

were therefore more impressive in the anterior and inferior portion of the muscle where these bundles are normally located.

Figure 7 illustrates changes in the ciliary muscle one year after ciliary ganglion resection. A comparison of the operated with the control side reveals readily the moth-eaten rarefied appearance of the former especially in the anterior third of the muscle. There is an unquestionable loss of fibers in this portion, which is evident in the figure even though one cannot distinguish in black and white reproductions between the melanophores and smooth muscle cells. In stained sections, the muscle fiber loss was more dramatically appreciated. If we now compare the longitudinal bundles in the two eyes, we find that they also were involved in this process. The longitudinal bundles on the operated side look thinner and are made of fewer muscle cells that are in turn markedly reduced in size; their eosinophilic sarcoplasm is greatly reduced, the nucleus is smaller and more darkly stained. In the more posterior portion of the muscle, in areas where oblique or circular fibers prevail, they are less dense and appear to be made up also of cells with reduced sarcoplasm and a nucleus which is readily differentiated from normal by its smaller size and its more intense basophilic staining.

Thus, while changes indicative of degeneration of smooth muscle cells seemed to be limited to the anterior third of the muscle, those indicative of atrophy of smooth muscle cells could be seen over the entire ciliary muscle. Changes involved the three sets of fibers with greater involvement of the circular and oblique fiber bundles.

Figure 8 illustrates changes in the ciliary muscle four and one-half years after ciliary ganglion resection. Here again, in spite of the limitation of black and white reproduction, the marked loss of muscle fibers on the operated side is obvious. The fiber loss in this case is more extensive and involves the ciliary muscle. While the loss of circular and oblique bundles is readily appreciated, a comparison of operated and control eyes demonstrates with equal certainty the reduction in size and in number of muscle fibers in the longitudinal fiber bundle. The stained sections showed these changes more dramatically by revealing that the major source of density in the black and white reproductions are the melanophores. Examination of sections stained with Masson, colloidal iron, or Mallory stains failed to reveal any cellular infiltration of the involved muscle or any increase in the ground substance, in the cellular or in the fibrous components of the connective tissue. There was no increase in mucopolysaccharide deposits; both operated and control eyes showed sparse delicate deposi-

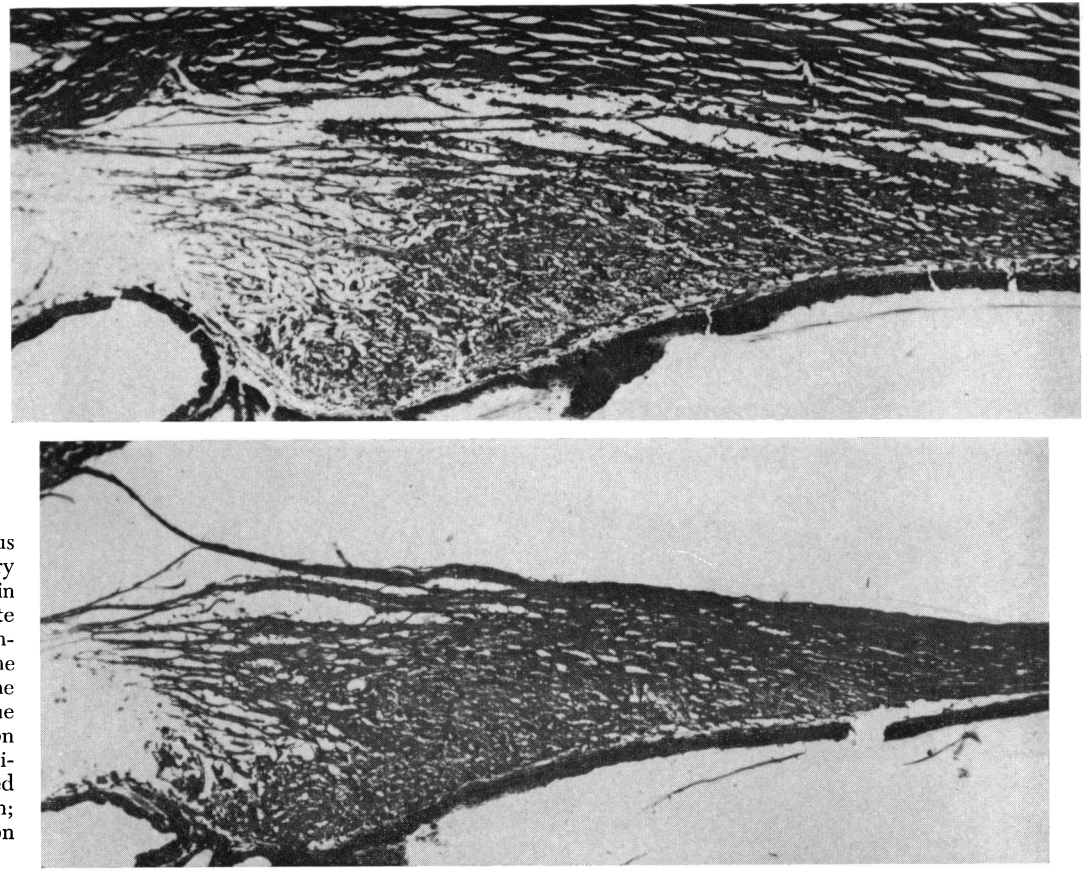

FIGURE 7

The ciliary muscle of the rhesus monkey one year after ciliary ganglion resection (top) and in the control eye (bottom). Note in upper illustration the moth-eaten rarefied appearance of the anterior third of the muscle, the loss of circular and oblique fibers as well as the reduction in size and number of longitudinal fibers when compared with the control. Mallory stain; magnification 70 ×, reduction factor 2.

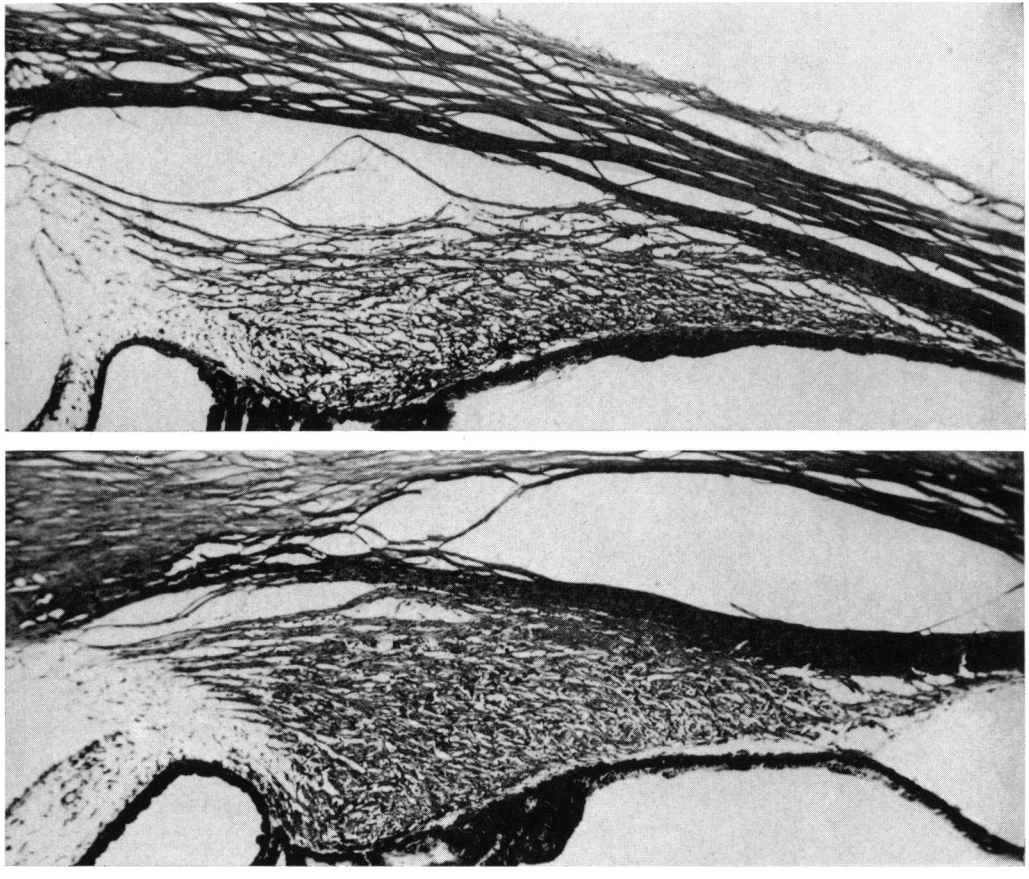

FIGURE 8

The ciliary muscle of the rhesus monkey four and one-half years after ciliary ganglion resection (top) and in the control eye (bottom). Note the lacy rarefied appearance of the denervated muscle as compared to that of the control eye. Note the almost total loss of circular and oblique fibers throughout the entire muscle and the partial survival of slender longitudinal bundles. The lighter muscle bundles may be distinguished from the darker-staining chromatophores. Mallory stain; magnification 70 ×, reduction factor 2.

tion of this material. Thus, no evidence of reactive or reparative process could be seen at this stage.

Figure 9 emphasizes the variability of the extent of degeneration following similar durations of ganglionectomy. In Figure 9, a year after ciliary ganglion resection, the changes are far more extensive than those seen in Figure 7 and are equal in extent to those seen in Figure 8 four and one-half years after ganglionectomy. Again one can readily verify the loss of muscle fibers in the oblique, circular, and

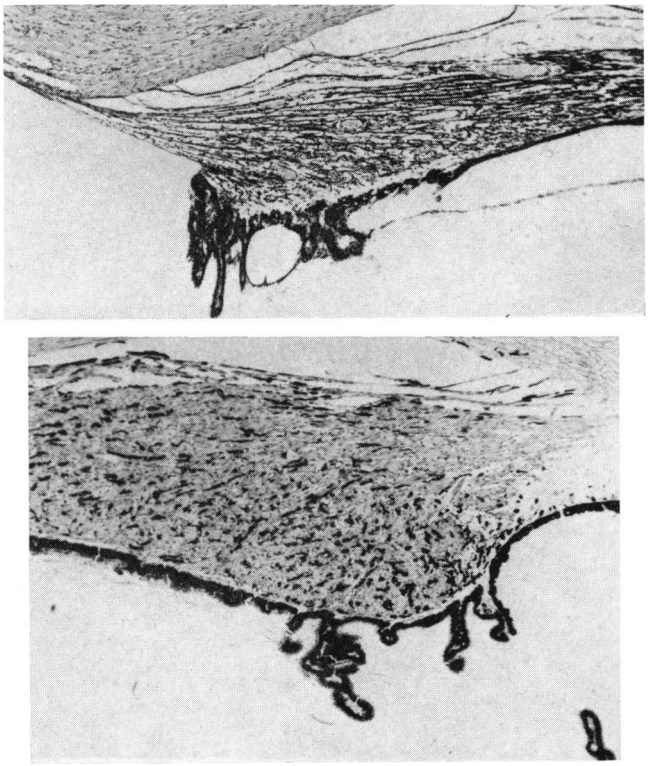

FIGURE 9

The ciliary muscle of the cynomolgus monkey one year after ciliary ganglion resection (top) and in the control eye (bottom). Note the lacy appearance and the reduction in size of the ciliary muscle on the right. There is uniform loss of circular and oblique fiber bundles on the right and survival of slender longitudinal bundles made up of fewer cells that are thinner containing less sarcoplasm with darkly staining nucleus as compared with the control. Note abundance and uniform distribution of chromatophores in the control eye. Hematoxylin-eosin stain; magnification 70 ×, reduction factor 3.

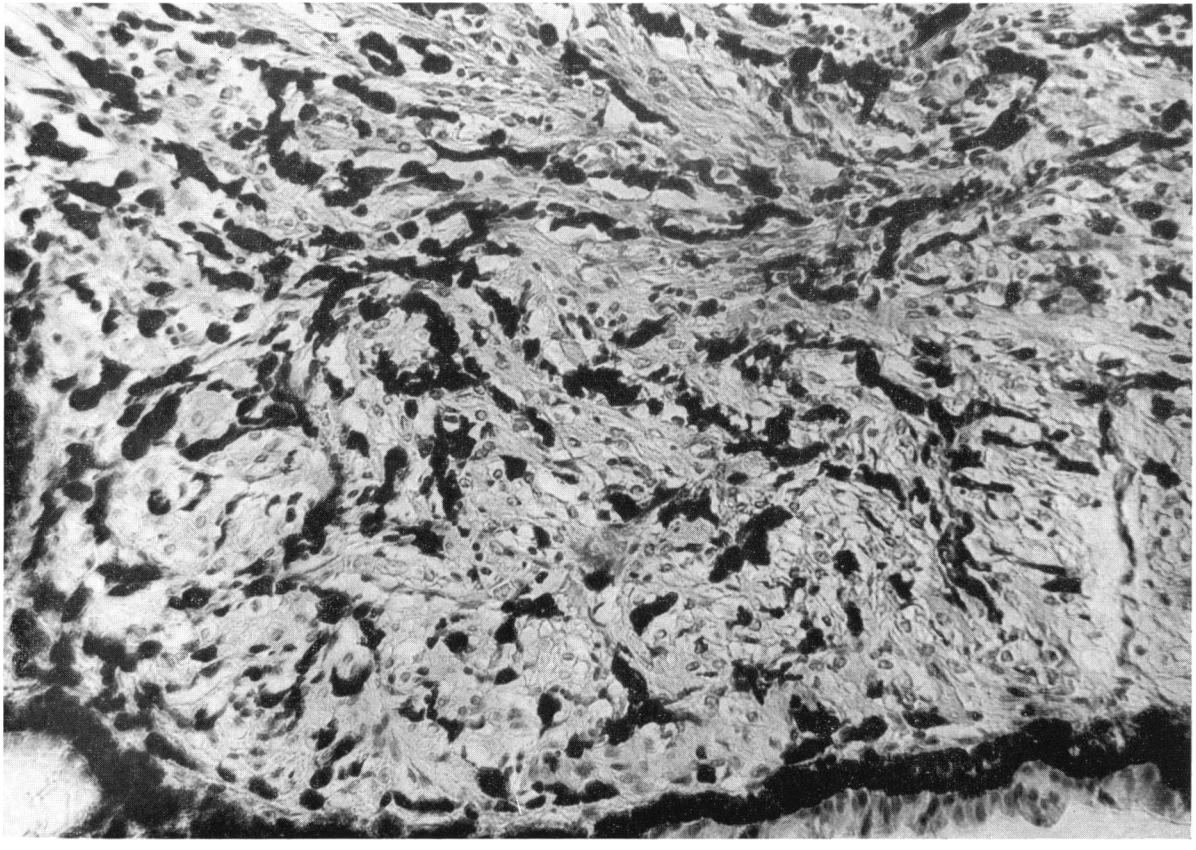

FIGURE 10

Circular fibers of the ciliary muscle of the cynomolgus monkey sixty days after ciliary ganglion resection. Note the reduced density of sarcoplasmic staining in the circular bundles in the lower and right portion of the section as compared with the more healthy looking fibers in the upper portion. Note abundance of chromatophores. Hematoxylin-eosin stain; magnification 300 ×.

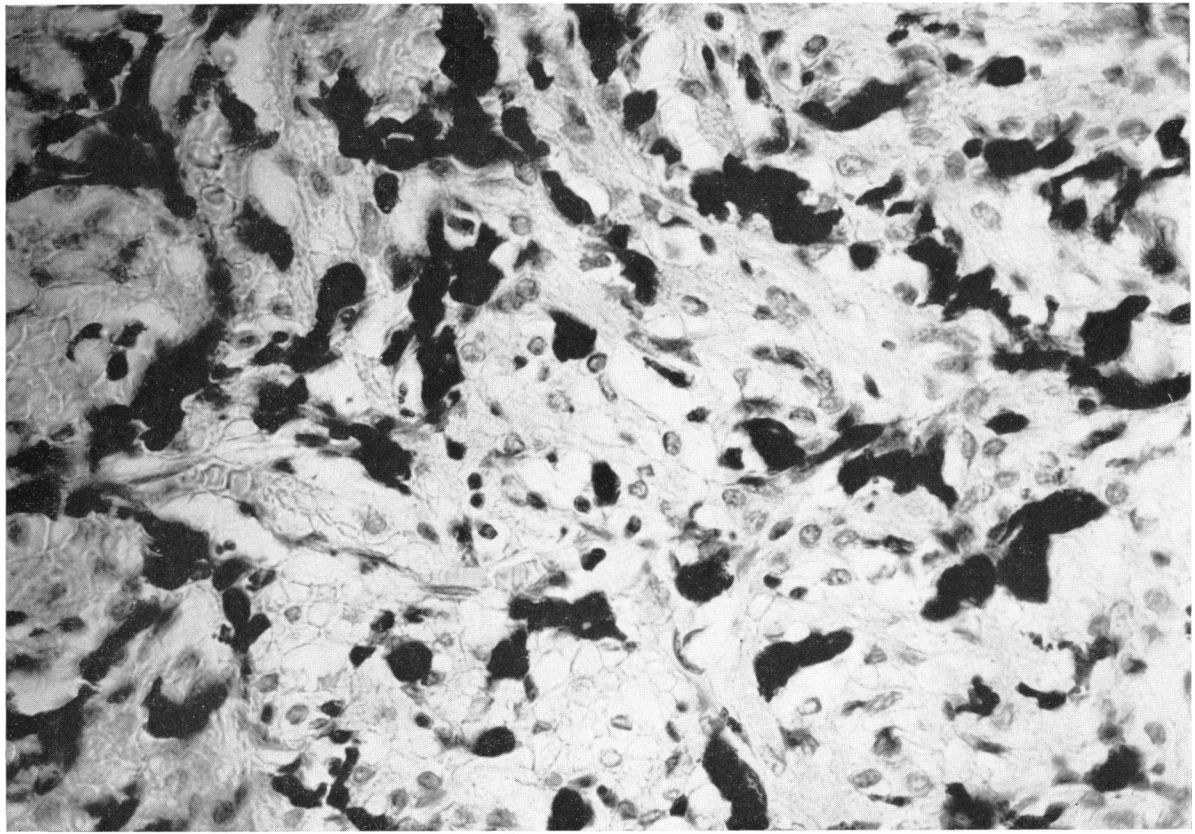

FIGURE 11
Circular fibers of the ciliary muscle of the cynomolgus monkey sixty days after ciliary ganglion resection. Note absence of staining of the sarcoplasm in the circular bundles in the lower right portion as compared with those in the left upper portion demonstrating uniform eosinophilic sarcoplasm and normal nuclei. The involved fibers consist mainly of the cell membrane surrounding an optically empty space or one lined with a thin remnant of sarcoplasm. Hematoxylin-eosin stain; magnification 600 ×.

longitudinal bundles. In this figure, the contrast successfully demonstrates the abundance of melanophores and their diffuse distribution in the ciliary muscle. Note the marked reduction in the over-all size and thickness of the ciliary muscle in the operated eye.

Figures 10 and 11 show that changes in the circular fibers are already apparent sixty days after ciliary ganglion resection. Figure 10 shows the difference between the affected circular fibers and the unaffected longitudinal fibers. The circular bundle shows vacuolation of the individual cells and, therefore, less intensive staining than the uniform eosinophilic staining of the unaffected sarcoplasm. In Figure 11, one can contrast the normal smooth uniform sarcoplasm and nucleus in the uninvolved circular fibers with the varying degrees of change in the involved fibers. These changes consist of granularity and irregular staining of the sarcoplasm proceeding to varying degrees of vacuolation and of non-staining of the sarcoplasm until only the cell membrane is seen intact and the interior of the cell non-staining and empty.

DISCUSSION

The difference between the cat and monkey is indeed difficult to explain and emphasizes species differences, for, in spite of the difference in composition of the ciliary ganglion, similar components were removed in the different species. The ganglionectomy included resection of the short ciliary nerves and, therefore, in each case parasympathetic neurons as well as postganglionic sympathetic and sensory fibers were removed.

Severing all connections to the ganglion other than the short ciliary nerves would mean in the monkey severing the sympathetic and sensory fibers as adequately as would have been achieved in ganglionectomy. However, this procedure as well as unilateral or bilateral superior cervical ganglionectomy failed to produce any histologic changes in the iris sphincter or in the ciliary muscle. Thus, removal of the sympathetic component alone or together with the sensory component was not the cause of the histologic changes encountered when the ciliary ganglion was resected. Therefore, removal of the postganglionic parasympathetic component is necessary to obtain the observed degeneration and atrophy of the iris sphincter and of the ciliary muscle. Atrophy of the circular bundles of the ciliary muscle and of the pupillary edge of the sphincter was reported in two patients associated with congenital oculomotor paralysis and with acquired

oculomotor paralysis of long duration. However, there was no information regarding the integrity of the ciliary ganglion and its connections which would permit the definitive conclusion that central or preganglionic oculomotor paralysis was responsible for these changes.[54]

It should be pointed out that one cannot definitively conclude that removal of the parasympathetic component alone was sufficient to produce these changes, for, in these studies, whenever the parasympathetic component was removed by ganglionectomy, the sympathetic and the sensory components were removed as well. It is conceivable that parasympathectomy produces these changes only when the sympathetic and the sensory components are simultaneously removed. This possibility is currently being pursued using botulinum toxin to destroy the parasympathetic component alone. Nevertheless, the findings that preganglionic parasympathectomy was not capable of producing the trophic effect, that destroying the final parasympathetic neuron was necessary to produce these changes, that the changes were limited to the muscles innervated predominantly by the parasympathetics together with the absence of such changes when only sympathectomy and sensory denervation were done are very strong evidence for implicating the parasympathetic denervation in this effect. Be that as it may, denervation led to loss of structure, atrophy, and degeneration of the smooth muscle.

Trophic changes similar in nature to those described in this presentation are known to occur in skeletal muscles following motor denervation. The ciliary muscle and iris sphincter have been shown to exhibit ultrastructural characteristics resembling those of skeletal muscle and differentiating them sharply from other smooth muscles. Electron microscopy shows them to possess an intracellular network of myofibrils arranged regularly and longitudinally.[17,20] This myofibrillar network, though unstriated and exhibiting no banding, constitutes nevertheless an ultrastructural resemblance to cardiac and to skeletal muscle. The finding in this study of a trophic effect on motor denervation of the iris sphincter and ciliary muscle points out another common feature between them and skeletal muscle. It should be noted that in birds the iris sphincter and the ciliary muscle are indeed made up of striated skeletal muscle.

In spite of the complete removal of the ciliary ganglion and the short ciliary nerves and with them the accessory ciliary ganglia, some muscle fibers continue to survive in the ciliary muscle and in the iris

sphincter. They still existed four and one-half years after denervation. To explain their continued presence, one may consider the following alternatives:

1. Incomplete denervation. This may be due to incomplete removal of the extraocular portion of the parasympathetic neurons or it may mean the presence of intraocular parasympathetic neurons that innervate and are therefore responsible for the structural integrity of the fibers that survive ciliary ganglionectomy. Parasympathetic cholinergic neurons have been demonstrated in the uvea and ciliary body.[55] However, it is not readily obvious why intraocularly located neurons should always innervate only the longitudinal fibers of the ciliary muscle or the central end of the iris sphincter.

2. The presence of a different or of additional innervation. In this case one assumes that the surviving fibers are innervated by the sympathetics alone or in addition to the parasympathetics, so that, when the parasympathetics are removed, the sympathetics remain or are not affected. The findings of electron microscopy and of fluorescence staining are not consistent with this restricted distribution of adrenergic innervation. Furthermore, sympathectomy did not influence the survival of these fibers. In addition, one has to explain why some of the longitudinal fibers were in reality lost and some remained. Electron microscopic study and fluorescence staining techniques are being employed in studying the effect of ciliary ganglionectomy in order to elucidate this question.

Finally, the difference in magnitude of degeneration and atrophy that followed similar procedures of denervation remains unexplained. Figures 7 and 9 demonstrate this difference clearly. Whether this reflects a difference in magnitude of the innervational components that are responsible for the survival of the residual fibers or a difference in the rate of the degenerative process is not clear at this stage. One is tempted to conclude from comparing Figures 8 and 9 that the degenerative process is completed shortly after the first year of denervation and that further degeneration does not occur with time. The extent of degeneration was not clearly related to the duration of denervation once that exceeded one year. However, the one eye with shorter duration (Figures 9 and 10) showed that after sixty days the degenerative process has not attained its maximum effect.

Tissue reaction, cellular or otherwise, was notably absent in all the eyes examined. One wonders whether this finding is characteristic of the late stages of this response or applies equally to its entire course. Studies of the early effects of denervation are in progress to elucidate

this point and to help uncover factors that might modify the intensity or rate of progress of this trophic effect. By performing such studies on chronically denervated preparations and correlating functional results with histologic structural changes, the function of the longitudinal fibers of the ciliary muscle and their effect on accommodation and outflow facility can be elucidated. Such studies will also elucidate the effect of parasympathomimetic agents on outflow facility and in so doing clarify the contribution of the various components of the ciliary muscle.

The findings of this study are of obvious relevance to the understanding of aqueous dynamics and to glaucoma. With age there is progressive loss and reduction in neurons including those of autonomic ganglia.[56] In the light of this investigation such loss will mean degeneration and loss of the smooth muscles which they innervate and, in addition, sensitization of the remaining muscle fibers to the action of parasympathomimetic agents. Thus, the findings are immediately relevant to the aging process and to the physiologic and pharmacologic differences that arise with age and with glaucoma. The increased sensitivity to pilocarpine in glaucoma is a well-established clinical impression and may reflect the sensitization that results from destruction of parasympathetic neurons in the ciliary ganglion. These aspects as well as possible disturbance in regulation of intraocular pressure are currently being explored in human subjects.

SUMMARY

This presentation reported the existence of a trophic effect on the ciliary muscle and iris sphincter following resection of the ciliary ganglion and the short ciliary nerves. This effect was present in two monkey species, *M. mulatta* and *M. irus*, but was not present in the cat.

The trophic effect consisted of marked reduction in number and in size of smooth muscle cells without evoking any reactive or reparative process or modifying the melanophores or the connective tissue components. In the ciliary muscle, all fiber bundles were involved with more extensive effects present in the circular and oblique fiber bundles; the anterior portion of the muscle was more intensely affected. In the iris sphincter the effect was more extensive in the peripheral portion, sparing a small central bundle.

The different mechanisms were discussed to explain the findings and to point out their relevance and potential value to our understanding of ocular fluid dynamics, accommodation, ciliary muscle innervation

and action, and of changes that develop with age and in human glaucoma.

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