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A Study of the Mechanisms of Pupillary Dilatation

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A STUDY OF THE MECHANISMS OF PUPILLARY DILATATION

by

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LIFE

Beatrice Ann Berteau was born in Cleveland, Ohio, March 23, 1926.

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This research was undertaken in order to study the exact nature of the phenomena concerning dilatation of the pupil. The theories regarding the mechanisms involved in movements of the pupil, are the muscular theory and the vascular theory. The former was widely accepted when smooth musculature, composed of a sphincter pupillae and a dilator pupillae, was found in the iris of the eye. The sphincter muscle has been established both anatomically and physiologically, but the presence of the dilator muscle has been the subject of much controversy. From a functional point of view, the need for a dilator muscle is evident, but it has not been demonstrated adequately by histological methods. It was for this reason and the fact that the iris is abundantly supplied with blood vessels, that variances in the calibre of these vessels was considered by many investigators to be effective in changing the size of the pupil.

An attempt to elucidate the problem of the vascular mechanism affecting pupillary dimensions, was done by an interruption of the blood supply to the eyeball. In this

way, one could observe any pupillary movements, that resulted from stimulation of the sympathetic supply to the iris, exclusive of any vascular influence.

HISTORY

The Autonomic Nervous System in general, is represented throughout the body by its component divisions, a thoraco-lumbar or sympathetic outflow, and fibers emanating from the brain stem and the sacral levels of the spinal cord, designated as the cranial-sacral or parasympathetic division of the system.

The first concept concerning an Autonomic System, was a description of the paired chain of sympathetic ganglia and the connection of these ganglia with the spinal cord, done by Willis (22) in 1664.

The nineteenth century, however, saw a great advance in the study of this system. Claude Bernard (2) in 1851 and Brown-Sequard (3) in 1852 discovered the existence of vasomotor fibers. Budge and Waller (4), also in 1852, described the autonomic innervation of the eye. The classical studies of Gaskell (7) and Langley (14) form the basis of our modern interpretation of autonomic control. By the use of the osmic acid technique, Gaskell investigated the peripheral autonomic plexuses and described the bulbar, thoraco-lumbar

and sacral outflows.

The pupillary mechanism and its relative dependence for innervation from this system is of fundamental importance, because there is no example of a structure in the body in which the antagonism between the two groups of nerve fibers has been more widely accepted than in the iris of the eye.

For purposes of orientation, it may be stated that the sphincter muscle receives its innervation from the oculomotor, the third cranial nerve. The motor nucleus of this nerve is situated in the ventral aspect of the midbrain. Associated with it is the parasympathetic nucleus, known as the Edinger Westphal nucleus. Each iris is innervated by both homolateral and contralateral portions of the nucleus, and consequently, is under the control of both crossed and uncrossed fibers. The fibers from this nucleus after leaving the midbrain, follow an intradural course in the middle cranial fossa, and enter the orbit through the superior orbital fissure. The oculomotor nerve is motor to the levator palpebrae and to all the extra-ocular muscles, with the exception of the two supplied by the trochlear and abducent nerves.

The inferior branch of the oculomotor nerve gains entrance to the orbit between the two heads of the lateral rectus muscle, in company with the abducent and naso-ciliary nerves. A parasympathetic root passes to the ciliary gang-

lion from the nerve to the inferior oblique muscle, synapses occur here, and post-ganglionic fibers leave by way of the twenty or more short ciliary nerves. These pierce the eyeball around the optic nerve and run forward between the scleral and choroidal coats of the eye. The fibers possess a fine myelin sheath upon leaving the ganglion, but this soon disappears upon entering the choroidal coat.

The sympathetic supply to the iris, leaves the cord at the level of the first and second thoracic segments. The fibers traverse the ventral roots and the white communicating rami of the corresponding spinal nerves, which conduct them to the sympathetic chain of ganglia lying on either side of the vertebral column. The fibers pass cranialward in the chain without synapse until they reach the superior cervical ganglion, where synapses occur. The post-ganglionic fibers destined for the iris follow the internal carotid artery into the cranial cavity. These sympathetic fibers join the naso-ciliary branch of the opthalamic nerve in the cavernous sinus. From the naso-ciliary nerve, the sympathetic fibers reach the eyeball through the two long ciliary nerves and also through the long root of the ciliary ganglion and the short ciliary nerves.

Once the basic anatomy of the autonomic innervation of the eye was discovered, there began a practically

continuous series of studies to further elucidate the mechanism of pupillary control. That stimulation of the sympathetic trunk causes pupillary dilatation, and stimulation of the ciliary ganglion or the short ciliary nerves causes constriction, has been demonstrated innumerable times, and is taken for granted in text-books. However, pupillary control is not as simple as this. Some of the researches which have lead to the present concepts are here reviewed.

Whenever painful stimuli are applied anywhere in the body, there is a reflex dilatation of the pupil. This dilatation, produced by either somatic or visceral afferent stimuli, was believed by many to be mediated through sympathetic nerves to a dilator muscle. However, the researches of Bechterew (1) in 1883, introduced the theory that pain was conducive to inhibitory impulses traversing the parasympathetic neurones, thus eliciting a dilatation of the pupil.

This work provided the stimulus for the subsequent research which emphasized reflex pathways and inhibitory phenomena as determining pupillary size.

Gullberg, Olmstead and Wagman (10) in 1938, used infra-red photography for their studies of the pupil. In this way, they were able to study the pupil in the dark and therefore in the absence of any reflex stimulation.

Dilatation, in their opinion, was caused by three

mechanisms: 1) active contraction of a dilator muscle, 2) the passive stretch of radial elastic fibers which tend to restore the resting configuration, and 3) the overcoming of the residual contraction of the sphincter muscle. These authors found this tonus to be constant for a completely dark-adapted eye, the range being 5.0 - 5.3 mm.. Once this residual tonus was overcome, the dilator muscle was free to act alone, dilating the pupil maximally. A maximally dilated pupil in an animal such as a dog, cat or rabbit, is about 12.0 - 14.0 mm.. The normal pupillary diameters in the same animals, which are the usual experimental animals, are from 3.0 - 5.0 mm..

These three mechanisms were found to be active in the intact eye, but the contraction of the dilator muscle was lost when the sympathetic innervation was interrupted. Simultaneously, the rate of dilatation is diminished with sectioning of these fibers, the assumption being that the active contraction of the dilator muscle acts immediately upon reception of the stimulus, proceeds to overcome the residual tone of the sphincter muscle, followed by the passive stretch of the radial elastic fibers.

Ury and Gellhorn (19) in 1939, carried out experiments to determine the role of the sympathetic nervous system in reflex dilatation of the pupil. They observed that

after section of the oculomotor nerve and the local application of eserine, any reflex stimulation which evoked struggle and vocalization elicited no change in the parasympathectomized pupil. It remained unchanged or showed a very slight dilatation of not more than 1.0 mm., whereas the normal pupil would dilate to 7.0 or 8.0 mm.. Their experiments also involved the application of metrazol to the pupil. After sensitization with the drug, the reflex response of the entire pupil increased. However, the dilatation was but a small fraction of that elicited in the normal eye under the same circumstances. It appears that the drug metrazol increases the excitability of the ciliospinal center and that a discharge of impulses along the sympathetic nerves follows.

Ury and Oldberg (20) in 1940, enumerated the factors inducing afferent stimuli and affecting pupillary reactions, either in the direction of constriction or dilatation.

Dilatation occurs when sensory stimuli elicit a state of inhibition and a consequent decrease in the number of stimuli through the parasympathetic fibers. This involves the reflex-dilator phenomenon we have been discussing in the above. Wilson (personal communication) has demonstrated that the ciliary ganglion of a cat can be inhibited by stimulation of the cerebral cortex in Area 8, and thus elicit

dilatation of the pupil. Dilatation can be positively brought about by direct stimulation of the superior cervical ganglion or the sympathetic nerve fibers.

Constrictor effects, meanwhile, may result from the stimulation of the visual cortex, located in the occipital lobes of the cerebrum. The light reflex and the accomodation reflex are the types of constrictor reflexes occurring normally in the intact animal. The former is mediated through the pre-tectile area of the brain stem, but the accomodation reflex requires the presence of Area 17, the visual cortex.

These authors also mention a constrictor tonus, which differs from the above, in that it is not dependent on reflex stimuli. These rhythmic impulses represent the "basal tonus" of the Edinger Westphal nucleus and are present when the phenomena of excitation and inhibition are at a minimum or completely in the inactive state. This suppressive state probably exists in the sleeping, or in comatose individuals, in which the pupils are constricted in the absence of light.

Seybold and Moore (18) in 1940, likewise inquired into the relations existing between the parasympathetic innervation and the reflex dilator mechanism. Cats were used in

their study. Oculomotor nerve sections were completed intracranially, through a temporal approach. This operation resulted in immediate pupillary dilatation, the transverse diameter attaining a maximum of 13 mm.. The sympathetic supply was also interrupted in a few animals at a later date. Following this second manipulation, the pupillary aperature was constricted to a diameter of about 8 mm.. The miotic, eserine, was applied to the eye after parasympathetic denervation in order to constrict the pupil so that any dilator effects would be readily observable. The pupil on the side of the third nerve section remained 1.0 - 2.0 mm. larger than the normal pupil likewise constricted by eserine. When the sympathetic pathway was interrupted, the pupil was smaller on the sympathectomized side.

The authors in their attempt to evaluate the effect of sympathetic and parasympathetic fibers in pupillo-dilatation employed the following three stimuli: 1) light adaptation, 2) painful stimuli, and 3) emotional stimuli, such as restraining the animal on its back, or subjecting the animal to the barking of a dog.

The normal eye dilated immediately upon reception of the above three stimuli, as was to be expected. In the parasympathectomized dog, dilatation was not evident, although the pupil was well constricted by eserine.

Their experimental results were confirmed by applying the same three stimuli to animals with their sympathetic fibers interrupted. The dilatation was prompt, but decreased in magnitude in all of the cases studied.

In 1944, Harris and Magoun (11) observed that pupillary dilatation was evoked by stimulation of the ends of the sciatic, splanchnic and trigeminal nerves in slightly anesthetized cats. Studies were made of the response following sections of parts of the spinal cord and the brain stem. These author's results demonstrate that if the spinal cord is sectioned at any place below the level of the oculomotor nucleus in the midbrain, dilatation will not be elicited. Any destruction of parts of the brain stem above the oculomotor nucleus does not impair the effect.

The common pathway from the sources mentioned, ascends through the lateral funiculus, distinct from the lateral spino-thalamic tract, traverses the reticular formation of the medulla and ascends through the midbrain in or near the ventral grey of the aqueduct.

Although most experimental evidence indicates pupillary dilatation as being due to oculomotor nerve inhibition, Ward and Reed (21) at the University of Illinois in 1946, observed dilatation elicited by electrical stimulation of Area 8 in the cerebral cortex of a monkey. The response was

abolished by section of the cervical sympathetic chain. The authors believe that the active dilator component travels over pathways involving the hypothalamus. They observed a greater increase in pupillary diameter from stimulation of these dilator foci in the cerebral cortex, than from parasympathetic inhibition.

Extensive experimental analyses of the reflex pupillo-dilator mechanism were carried out in 1946 by Kuntz and Richins (13). Their results afforded conclusive evidence that the parasympathetic nerves play the major role in dilatation of the pupil in response to peripheral pain producing stimulation and other emotional stimuli.

These authors undertook a series of operations on cats and dogs to determine the exact nature of this inhibitory phenomenon. They obtained a slight constriction of the pupil when the oculomotor nerve was stimulated intracranially, the sympathetic fibers being intact. This was immediately followed by a dilatation when the manipulation was completed. H owever, no dilatation was observed upon oculomotor stimulation, when the sympathetic effect was precluded by section of the sympathetic trunk. A definite pupillary constriction was easily elicited by mild faradic stimulation. They found that if they sufficiently and completely atropinized the eye, that faradic stimulation of the third nerve produced a

pupil of a diameter of 13.0 mm.. If however, atropinization was incomplete, oculomotor stimulation resulted in a smaller pupil.

The presence of a dilator apparatus is evident, since complete removal of the parasympathetic fibers to the eye does not result in a maximal dilatation of the pupil. The existence of a muscular mechanism must be assumed to obtain the maximal reaction.

Kuntz and Richins emphasize the fact that in their experiments in which pupillary dilatation was brought about by painful stimulation, the reaction was not equal in magnitude to dilatation obtained after the parasympathetic fibers were extirpated. The former reflex response seems to involve a very integrated mechanism. These authors believe that pupillary dilatation mediated through the Edinger Westphal nucleus, similiar to the constrictor effect, concerns efferent impulses reaching the sphincter pupillae through the oculomotor nerve. Thus, in an animal in which the cholinergic fibers are rendered ineffective by the application of atropine, stimulation of the third nerve and subsequent pupillary dilatation is explainable only on the assumption that the circular iris muscle is the recipient of inhibitory impulses reaching it through adrenergic components of the short ciliary nerves. When ergotoxin phos-

phate was administered to the animal, the adrenergic response was prevented, and a very weak pupillo-dilatation was seen following peripheral pain producing stimulation. This further emphasizes the presence of efferent adrenergic fibers in the short ciliary nerves.

They contend that both cholinergic and adrenergic fibers are incorporated in the parasympathetic innervation, and the light reflex is via the former, and pupillary dilatation by peripheral stimulation is concerned with inhibitory impulses through adrenergic neurones. Thus, the inhibition of the sphincter muscle is due to activation and not inhibition of the parasympathetic center.

These results are most significant in that they not only demonstrate the conditions prevailing in the reflex dilator mechanism, but show clearly the pattern of the component fibers in autonomic nerves. They also afford some evidence regarding central inhibition.

The foregoing experiments are based on a mechanism composed of constrictor and dilator muscles under nervous control. What is the evidence regarding the role of the vascular structure in regulating the size of the pupil?

Orthello Langworthy and Luis Ortega (15) in 1943, at Johns Hopkins University, conducted experiments in which the circulatory system of laboratory animals was

perfused with India ink. It was observed that when the blood vessels supplying the iris were distended, the pupils were small. Conversely, when the vessels were constricted, the pupils were dilated. These experimental findings indicated that the blood vessels calibre contributed a significant and quite determining effect on pupillary size.

Information concerning the opinions of early anatomists and physiologists on the controlling effect of blood vessels on pupillary size was obtained, according to Langworthy, from a thesis by A. Magitot (16), titled L'Iris, published in Paris, in 1921. It provides a review of the earlier literature related to the subject.

Gellhorn, Darrow, and Yesnik (8) in 1940, conducted experiments concerning the effect of blood pressure on the Autonomic Nervous System. They state that an inverse relationship exists between blood pressure and pupillary diameter. Pentothal anesthetized cats were used and a rise in blood pressure was obtained by the injection of adrenalin. This manipulation resulted in a pupillary constriction of both the normal and the sympathectomized eye. If the blood pressure were lowered by the injection of amyl-nitrite, a dilatation of the pupil occurred. This effect was also produced when the adrenal glands were removed and the cervical sympathetic trunk excised. The conclusion was drawn that

the increase in blood pressure incurs a decreased sympathetic and an increased parasympathetic tone.

The authors state in the substance of their article, "that the only other reference in the literature to this phenomenon of which we are aware is a brief remark by Koch (12), that endosinusal pressure and pupillary diameter are indirectly related."

It is felt that the early work of Magitot and these later experiments by Gellhorn, Darrow, and Yesnik at the University of Illinois, and Langworthy at Johns H opkins University, are the only ones concerned with the idea that vascular variances influence the pupillary aperature.

PROCEDURE

We have seen that most of the literature is based on the interpretation that the size of the pupil is dependent upon the active contraction and the tonus of smooth muscle under direct autonomic control. On the other hand, the idea that the size of the pupil is governed indirectly by autonomic control of the blood vessels of the iris has been advanced. To test this hypothesis an experiment was designed, in which the sympathetic innervation to the iris was stimulated, following the

interruption of the blood vessels to the eyeball.

A routine approach to the vago-sympathetic trunk was carried out. The trunk was then freed from its position and a loose ligature placed around it for further identification.

The operative procedure to expose and ligate the opthalamic artery involved many anatomical considerations. Dogs and cats were the experimental animals used. A 4 cm. incision was made at approximately a 60° angle from the lateral canthus to the ear. The relatively large masseter muscle was detached from the zygomatic arch and the anterior portion of the zygoma was removed. The temporalis muscle was detached from the skull and the coronoid process of the mandible removed.

When the posterior orbital area was exposed completely, further dissection was carried out with the utmost precision, due to the relative minuteness of the area and the proximity of the vessels and nerves. The extrinsic muscles of the eye were all ligated at two points and transected, in order to interrupt the arteries entering the bulb through them. The posterior aspect of the eyeball was dissected free from the surrounding tissue, leaving the eyeball relatively free in the orbit, save for the attachment of the optic nerve, with its surrounding nerves and

blood vessels.

The opthalamic artery enters the orbit through the optic foramen, below and lateral to the optic nerve. For all practical purposes, it is firmly incorporated with the latter, and perforates the sheath of the dura mater that is prolonged through the optic foramen over both the artery and the nerve. It then runs in a gentle curve with a lateral convexity between the optic nerve and the lateral rectus muscle, being crossed here by the naso-ciliary nerve. Turning forward and upward, it passes over the optic nerve to its medial side and ends by dividing into frontal and dorsal nasal branches. Ligation of the opthalamic artery at any point between its origin from the internal carotid artery and where it gives off its first branch, a rather short distance, would entirely remove the blood supply to the eyeball and the immediately adjacent areas. The devascularized structures in which we were particularly interested for our experiments, were the iris and the ciliary bodies. To insure complete interruption of the blood flow to the eyeball, a bull-dog clamp was applied to the optic nerve.

The vago-sympathetic trunk which had been previously exposed in the neck, was now stimulated. Stimuli were applied at five minute intervals and observations

were made for a period of fifty to sixty minutes.

The results from the reactions of twelve adult animals were recorded. The animals, dogs and cats, were all in good condition and had normal pupillary responses.

In all cases under observation, an active dilatation of the pupil resulted when the vago-sympathetic trunk was stimulated by an electric current, following a complete interruption of the blood flow to the eyeball. The dilatation was immediate upon reception of the stimulus and the range of the diameters obtained was between 7.0 and 9.4 mm. for the twelve animals recorded (Table 1).

These figures were considered to be indicative of a moderate and not a maximal dilatation. The stimulus was applied to the vago-sympathetic trunk until no further dilatation was observable. The amplitude of the dilator effect appeared somewhat diminished in the final fifteen minutes of the operation. This was not considered unusual, since all bodily structures depend on their blood supply for their nutritive needs, and cannot respond effeciently in its absence.

During the early stages of the experiment the pupil would constrict to the size of the opposite pupil, the range being 3.0 - 4.5 mm., upon removal of the stimulus. But gradually the constriction became less and less.

After about thirty minutes, the pupil usually remained dilated. Stimulation of the short ciliary nerves or of the back of the eyeball resulted in a constriction of the pupil at this time.

The dilator effect was tested before devascularizing the area, by electrical stimulation of the vago-sympathetic trunk. In all cases, the diameter of the dilated pupil was similiar both before and after the ligation. Differences ranged between a plus or minus 1.0 mm..

Insurance was made that the blood supply was completely and adequately removed by sectioning through the optic nerve and the opthalamic artery, distal to the bull-dog clamp at the close of the experiment. In all of the cases studied, no bleeding occurred from the cut edge. Hence, one may safely conclude that no blood was allowed to flow past the clamp, thus indicating that the sympathetic dilator response elicited on stimulation of the vago-sympathetic trunk, was a pure motor response and not the result of a modification in the calibre of the blood vessels.

Histological Investigation.

The histological investigation was undertaken in an attempt to demonstrate, if possible, dilator fibers in the posterior layer of the iris tissue and secondly, to

determine the relative abundance of sympathetic terminations in the smooth muscle of the iris, by the use of a special stain for autonomic fibers.

For the study of dilator fibers, sections of dog and cat irises were cut and stained by the Masson-Goldner method (9). This is a general stain for smooth muscle, connective tissue and cell nuclei. Sections of the tissue were cut in three planes. Transverse sections were obtained by cutting the tissue at right angles to the radius of the iris. Unfortunately, such sections are strictly transverse at only a single point, owing to the radial course of the iridial structures. The farther one proceeds from this point, the more oblique are the elements encountered. Sections cut on a plane parallel to the surface of the iris, would seem to be ideal for study of the posterior layer of the iris. However, this proves to be the least instructive section due to the uneveness of the iridial layers. The sections that were cut in a meridional or radial direction, were found to be the most desirable. A view of the iris was obtainable from anterior to posterior surfaces, as well as from the pupillary to the ciliary borders.

Smooth muscle fibers stained red by the Masson-Goldner method, were observed pursuing a radial course adjacent to the pigment layer, in the posterior iris region.

(Plate 1). The muscle cells were spindle shaped with central nuclei. They form a less dense mass than do the sphincter muscle fibers. A transition from the thicker band of sphincter fibers around the pupillary margin to the dilator fibers stretching to the ciliary border, is readily seen in a meridional section through the iris (Plate 2).

The meridional section demonstrates well the iris crypts outlined in epithelium. Pigment cells or chromatophores are abundantly scattered throughout the iris stroma, as well as in the anterior border layer. The large amount of pigmentation in these cells usually masks the nuclei, so that the cells appear only as dark, stellate cells. Numerous myelinated nerve fibers lie in relation to muscle tissue and blood vessels. These appear as golden-yellow fibers with the Masson-Goldner stain. Autonomic fibers, in which we were primarily interested, are not demonstrable by the use of this stain.

In order to demonstrate autonomic fibers in the iridial musculature, a special silver stain devised by Nonidez (17) in 1939, was used. The stain was applied to both the irises of control and experimental animals. Cats and dogs were again the experimental animals employed. Both the control and experimental irises were used in order to determine: 1) the relative abundance of autonomic nerve

fibers in the iris, both sympathetic and parasympathetic, and (2) to determine the relative number of sympathetic fibers alone. The latter procedure was accomplished by the removal of the parasympathetic nerves to the iris, leaving the sympathetic supply intact.

The control sections were cut in the same three planes as described above, fixed in chloral hydrate solution, subsequent to staining by the Nonidez method. The technique is considered to be advantageous in demonstrating fibers of the Autonomic Nervous System in shades of brown to black. The post-ganglionic sympathetic fibers appear lighter than the other autonomic fibers.

The use of this stain on the iridial tissue was experimental in itself, since the stain has been employed successfully, according to the literature, only on cardiac tissue in young, healthy, dogs.

The experimental group of animals was subjected to a ciliary ganglionectomy, in which the parasympathetic supply to the intrinsic muscles of the eye was removed. The ciliary ganglion of four animals, all cats, was removed under aseptic conditions. Unfortunately, one animal succumbed before the proper time alloted for degeneration of the nerve fibers had passed. In the other three cases, evidence of the success of the ganglionectomy was the ex-

tremely dilated pupil, which persisted even in the presence of light. Histological studies were made of the ganglion to confirm their identity as such, but usually the prompt dilatation which appeared immediately upon removal of the ganglion was sufficient proof.

The method employed to remove the ciliary ganglion in one animal was the same as the operation described for the ligation of the opthalamic artery. In this animal, however, regeneration of the nerve fibers occurred, since the pupil returned to its normal size and responded by constriction to a light stimulus.

The ganglionectomy in the second approach was done via the buccal cavity. This procedure was less time consuming and involved fewer relationships.

A horizontal incision was carried laterally from the mid-line at the junction of the hard and soft palate. The lateral pterygoid muscle was encountered and cut across with little difficulty. A wing-like projection of the sphenoid bone, known as the alisphenoid in the cat, was removed and the area cleared of the intervening tissue. The infraorbital nerve now appeared in the operative field. This was retracted with care due to a sizable artery running in conjunction with the nerve. Excessive hemorrhage is especially avoided due to the minuteness of the area. Tenon's

capsule was incised in a horizontal direction and transections of the inferior oblique and the lateral rectus muscles were carried out. The ciliary ganglion was now apparent in the angle between the cut muscle and the optic nerve, and excised with little difficulty.

The ciliary or lenticular ganglion, lies posteriorly in the orbital cavity on the lateral side of the optic nerve, between the nerve and the lateral rectus muscle. It is a very small, reddish, quadrangular body, measuring about 2.0 mm.. Christensen (5) believes that in the cat, no sympathetic or sensory fibers pass through the ganglion. A meeting of the sympathetic and parasympathetic fibers occurs a short distance from the ganglion, but in such a manner, that a separation of the parasympathetic fibers can be accomplished without damage to the sympathetic component of the iris.

Following the ganglionectomy, a week to ten days was allowed for degeneration of the nerve fibers. The experimental irises were removed and fixed in chloral hydrate, and likewise stained by the Nonidez staining method.

The histological sections stained by the Nonidez silver nitrate method, unfortunately, were not as conclusive as those by the Masson-Goldner method of staining. In the former, the relationships between the nerve fibers and the

muscle fibers was not well identified. The presence of the abundant pigmentation undoubtedly masked the specific nerve terminations. The tissue appeared to absorb the stain in such a manner, that outlines of the individual structures were not demonstrable.

DISCUSSION

The extensive vascularity which prevails in a small structure such as the iris of the eye, could logically lead to the conclusion that variances in this vascular pattern could produce a change in the size of the pupil. Early anatomists and physiologists resolved the idea into the theory that changes in the size of the pupils were dependent upon constriction and dilatation of the blood vessels of the iris.

The identification of specific, smooth muscle, sphincter fibers, however, altered the significance of this theory, and the musculature was considered the dominant factor in pupillary control. The vascular theory became less prominent and was subjected to relatively little investigation until the experimental work of Gellhorn, Darrow, and Yesnik in 1940. These authors observed that an increase in blood pressure brought on by adrenalin administration, was followed by a decrease in the pupillary diameter.

Intensive study of the complexity of the vascular pattern of the iris was made by Langworthy and Ortega in 1943. The arrangement of the blood vessels was well demonstrated when they were injected with India ink. In some preparations, the superior cervical ganglion was removed before the injection was completed. In these cases, the vessels appeared more dilated and the pupil smaller. All injections were made at a pressure approximating the systolic blood pressure of the animal. In some instances, in order to make the injection complete, the pressure was increased or the outflow through the venous channel was decreased sufficiently to cause a distension of the vessels. At such times, the pupillary diameter was freely altered, depending on the distension of the vessels at the particular time studied. When the pressure in the vessels was increased, the pupil was small, and conversely, if decreased, the pupil was large.

The authors observed that the shape of the pupil was often altered during the injection procedure. Certain sectors of the iris received the dye first, and under these circumstances, the injected portion was wider, thus causing the pupillary aperature to present an irregular outline. At times, however, after complete injection this pupillary irregularity persisted. In the normal eye, however, the

pupillary border is smooth and regular in outline, in both the dilated and the constricted state. This even pupillary contour postulates the existence of a well-integrated, neuro-muscular mechanism, which reacts as a unit to either direct or reflex stimulation.

Furthermore, from the injection studies by Langworthy, it was well demonstrated that the blood vessels of the iris and the ciliary body are extremely curved and have the appearance of coiled springs. Langworthy states that this tortuous structure of the blood vessels is evidence that they are capable of effecting a change in the size of the pupil, when their position in the iris is changed. Thus, when the blood vessels are maximally coiled, the iridial membrane is constricted and the pupil is larger. Likewise, if the vessels are dilated and loosely arranged, the iridial membrane will be expanded and the pupil will then be small.

The above condition undoubtedly exists and can be demonstrated anatomically. From a physiological viewpoint, however, the question arises as to whether the variation in the blood vessel calibre is the prime factor in determining the width of the iris at a particular time, or whether the variation in blood vessel calibre is consequential to muscular movement under nervous control.

This author feels that the latter condition pre-

vails. The iris of the eye is affected by direct or reflex stimuli and responds to these by neuro-muscular activity. The blood vessels then respond secondarily to the demands of the musculature. From the experimental results obtained in this laboratory, the existence of a neuro-muscular mechanism controlling pupillary size has been substantiated physiologically. Thus, the pupil will dilate when adequately stimulated even in the absence of its vascular supply. Furthermore, the presence of dilator muscle fibers has been demonstrated histologically.

Since the iris of the eye functions primarily as a protective mechanism for the retina, reflex stimuli such as light and accomodation, which almost continually play on the retina, must be buffered properly. This so-called buffering effect is the prompt constriction of the pupil when light strikes the eye or when an object is brought close to the eye. In the former condition, excessive light is prevented from striking the retina and damaging the cells therein, and in the accomodation reflex, the lens is adjusted to the optimal thickness by contraction of the ciliary muscle, and the object is focused more clearly on the retina.

It is obvious from the above, that there must be a prompt, harmonious, response to these stimuli. However,

the blood vessels of the iris are little affected by these reflex phenomena, so undoubtedly they are not the causative agents in narrowing or widening the pupil. This further validates the conclusion that the mechanism controlling the size of the pupil is a neuro-muscular one.

CONCLUSIONS

Our operative results indicate that a specific, sympathetically controlled dilator apparatus is present in the iris of the eye. An active dilatation of the pupil was obtained in the complete absence of a blood supply, when the vago-sympathetic trunk was stimulated. The blood vessel layer, which forms the main mass of the iris tissue, may passively affect the size of the pupil by a variation in the calibre of its walls. However, essentially it is not significant, it is only secondary in effect to the neuro-muscular mechanism.

Smooth muscle fibers were identified in the posterior layer of the iris. It is believed that these fibers represent the radially arranged dilator muscle of the iris. In meridional section, these fibers are readily differentiated from the sphincter muscle fibers surrounding the pupillary border. Post-ganglionic sympathetic fibers were not identified in sections of dog and cat irises, using the

the Nonidez silver-nitrate method. It was felt that this stain is not too well-adapted to the iris tissue, due to the abundant pigmentation.

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F I G U R E S

Plate 1

High power view of the posterior aspect of the iris of a dog. The dark-stained tissue along the edge of the section is the posterior pigmented layer. The layer of tissue lying adjacent and parallel to this pigmented layer, represents the dilator muscle. Masson-Goldner stain. x 450.

pl - pigmented layer
dm - dilator muscle

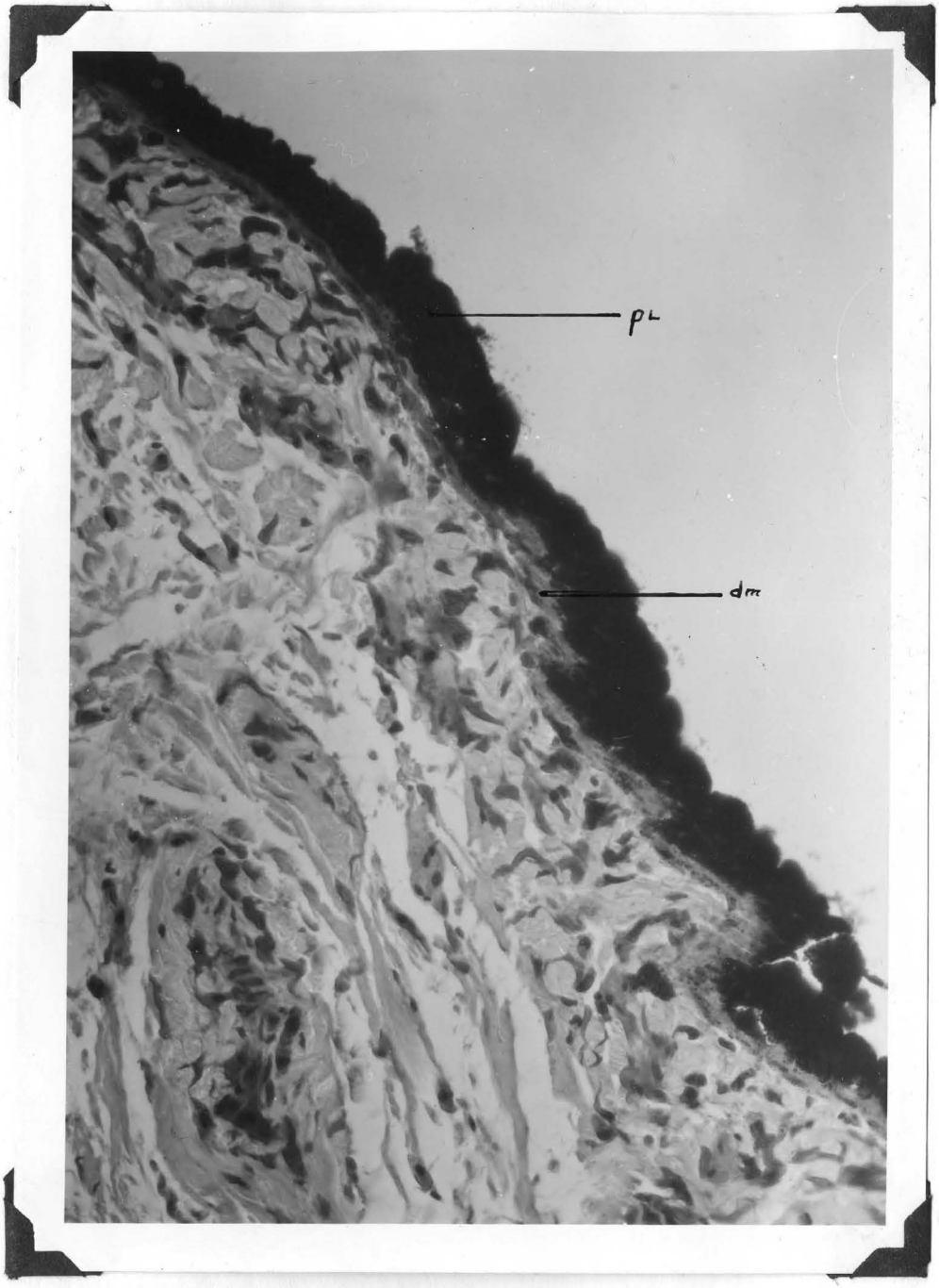

Plate 1

Plate 2

Meridional section of the iris of a dog. The dilator muscle is seen lying adjacent to the pigment layer along the ciliary border. The thicker band of fibers surrounding the pupillary border is the sphincter muscle. Masson-Goldner stain. x 150

abl - anterior border layer
cb - ciliary border
pb - pupillary border
dm - dilator muscle
sm - sphincter muscle

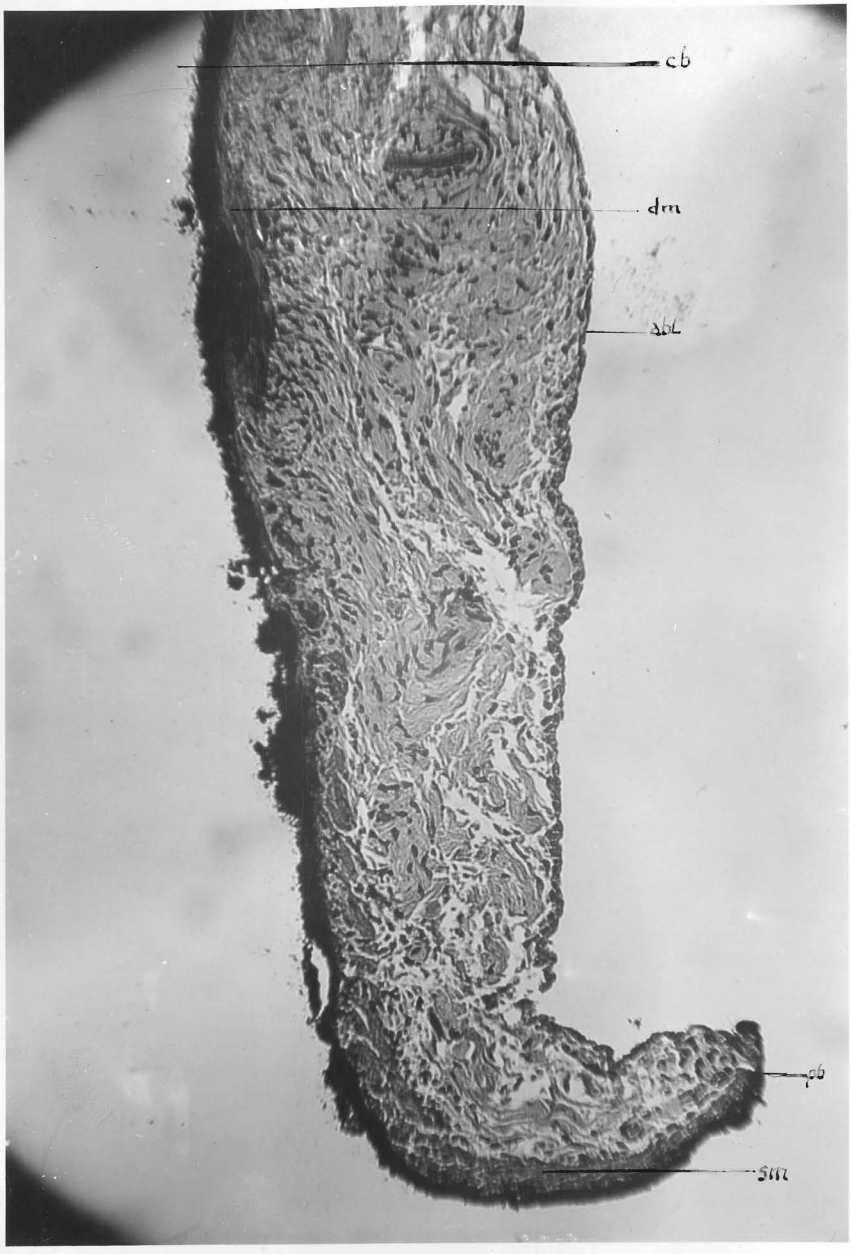

Plate 2

TABLE I

Animal	Manipulation	Pupillary Diameter		Time Observed
		Normal Pupil	Artery Ligated	
		In millimeters		
Cat #1	Vago-sympathetic trunk stimulated.	7.0	7.0	50 min.
Cat #3	"	7.4	7.6	60 min.
Cat #4	"	8.0	8.4	60 min.
Cat #6	"	9.0	8.6	50 min.
Dog #2	"	8.0	7.8	60 min.
Dog #3	"	8.0	8.0	60 min.
Dog #5	"	7.6	7.0	60 min.
Dog #8	"	9.4	9.0	60 min.
Dog #9	"	7.4	7.0	50 min.
Dog #10	"	7.8	8.0	60 min.
Dog #11	"	8.6	8.4	60 min.
Dog #12	"	7.0	7.2	60 min.

Note:

In all of the above cases, the pupillary dilatation was less promptly elicited in the final ten or fifteen minutes of the experiment. Furthermore, when the stimulus was removed, the ciliary nerves had to be stimulated in order to constrict the pupil to its former size.

APPROVAL SHEET

The thesis submitted by Beatrice Ann Berteau has been read and approved by three members of the Department of Anatomy.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Jan 30, 1950
Date

David S. Ham, Ph.D., M.D.
Signature of Adviser