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Some Histological and Physiological Aspects of the Posterior Adductor of Quadrula quadrula

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SOME HISTOLOGICAL AND PHYSIOLOGICAL ASPECTS OF THE POSTERIOR ADDUCTOR OF QUADRULA QUADRULA

(TITLE)

BY

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THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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SOME HISTOLOGICAL AND PHYSIOLOGICAL ASPECTS OF THE POSTERIOR ADDUCTOR OF *QUADRULA QUADRULA*

presented by

George G. Poulos,

a candidate for the degree of Master of Science and hereby certify that in their opinion it is acceptable.

Signatures

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TABLE OF CONTENTS

INTRODUCTION AND LITERATURE REVIEW

One of the many questions in muscle physiology still left unanswered concerns so-called "smooth muscle" of molluscans. Most pelecypods have an adductor or adductors that can contract for prolonged periods of time without apparent signs of fatigue. This is a paradoxical situation when the relatively quick contractions of this muscle are considered. The aim of this study is to investigate some histological and physiological characteristics of the adductor muscle of a fresh water pelecypod, Quadrula quadrula.

Pavlov (1885) excised the visceral ganglion of Anodonta and stimulated the cut end of the nerve of the adductor. He found that weak faradic stimulation quickly relaxed a tonically contracted adductor. This suggested the existence of some kind of inhibitory mechanism. Bayless et. al. (1930) found a similar situation when they investigated the function of the tonic portion of the adductor of Pecten. They also found that from a relaxed state, very slow twitches fused into a powerful tetanus. Benson et. al. (1942) clearly demonstrated separate excitatory and inhibitory nerves of the adductor.

The "sliding filament hypothesis" proposed by Huxley and Hanson (1954) stimulated renewed interest in muscle histology and physiology. Barnes (1955) while studying the intact animal observed reflex actions and spontaneous slow and fast rhythms of the adductor. Hoyle and Lowy (1955) presented a new interpretation regarding molluscan muscle. They elaborated on some previous work and proposed the existence of a "catch mechanism" to explain prolonged contraction.

The adductor muscles of pelecypods seem to be histologically and

functionally similar, but it has been found that several variations exist. Kawaguti and Ikemoto (1957, 1958, 1960a, 1960b, 1960c, 1961, 1964) worked with numerous genera of Pelecypoda. They observed in all but one genus two portions to the adductors. One portion was described as tonic and the other as phasic. The tonic portion, as the name implies, is mainly responsible for the tone of the adductor that keeps the valves only slightly gaped. This portion is probably partially contracted at all times, accounting for this behavior. The phasic portion is mainly responsible for the relatively quick contractions on the adductor, followed closely by quick relaxations. In the species that had one portion, only a phasic contractile behavior was observed. Histologically, there were variations ranging from a completely smooth muscle to a typically striated muscle. Kawaguti and Ikemoto (1961) refer to Uexkull, T.V. (1912) whose study of Pecten correlated similarly with their study of Spondylus.

Hanson and Lowy (1961) did a very thorough study of the muscle fibers of the translucent portion of the adductor of Crassostrea angulata. This portion was described as having more phasic than tonic properties, but that it had both types of contractile behavior was of significant interest. The translucent portion had double oblique striations under the light microscope. A possible mechanism of contraction was suggested; this muscle probably contracts by sliding of discontinuous filaments over one another. They extended their conclusions to include the "catch mechanism" hypothesis, but concluded that it was unnecessarily complicated and offered an alternative explanation which they called the "linkage hypothesis." The "catch

mechanism" hypothesis, described by Johnson and Szent-Gyorgyi (1959), involved the actual crystallization, thus allowing the muscle to remain contracted without any expenditure of energy. The "linkage hypothesis" of Hanson and Lowy (1961) is an elaboration of the basic sliding filament hypothesis. They believed that the linkages formed during contraction broke at a very slow rate, thus accounting for the low energy expenditure and prolonged contraction.

A study of the adductor of Anodonta by G.M. Forte (1964) also revealed two portions; a phasic portion that appeared double obliquely striated in an overcontracted condition, and a smooth tonic portion.

Elliot (1964) studied the opaque part of the adductor of Crassostrea angulata and noted the absence of striations and the existence of two types of filaments; thin actin and thick paramyosin. Zs Nagy and Salanki (1964b), attempting to determine whether striations did exist in the adductor muscles of pelecypods, demonstrated that in Anodonta's adductor, the presence or absence of striations depended upon the degree or state of contraction.

Twarogg (1954), Johnson and Twarogg (1959) and Lowy and Millman (1963) have all demonstrated the excitatory effect of acetylcholine, the inhibitory effect of 5-hydroxytryptamine and the effect of various types of electrical stimulation on the anterior byssus retractor muscle (a.b.r.m.) of Mytilus. Using their work as a guideline, the present investigation is to relate some of these physiological characteristics of the anterior byssus retractor muscle to the adductor of Quadrula sp.

MATERIALS AND METHODS

Thirty-eight Quadrula quadrula (identified by author using Parmalee, 1967) were collected at a depth of 7 or 8 feet, in Lake Charleston, Coles County, Illinois, between June and August of 1968. The clams were maintained for a week or two in five, eight-gallon aquariums, no more than eight clams to an aquarium. The water was kept at room temperature (23°C), unfiltered and well aerated by an air pump. Within the first two weeks, 30 clams were fixed for histological study. The remaining eight were used in physiological experiments.

Histological techniques employed were generally in accord with Humason (1967). To insure rapid fixation, the shells were trepanned with an electric drill. Two, one-quarter inch holes were made in each valve, and care was taken not to damage the soft parts.

The posterior adductor muscle was fixed in five different stages of contraction: 1) relaxed muscle, fixed at an extended length after being in a warm water bath (45-50°C) for 30 minutes; 2) isometric contraction, fixed immediately after excitation; 3) isometric contraction, fixed 30 minutes after excitation; 4) isotonic contraction, fixed immediately after excitation; 5) isotonic contraction, fixed 30 minutes after excitation.

Before fixation could occur, the proper state of contraction had to be achieved. All the clams were reimmersed in aquarium water after the trauma of trepanization and all seemed to assume a normal gape after about 30 minutes. Each group of clams was subjected to a specific treatment in order to achieve the proper state of contraction. For the first treatment, six clams were immersed in 45-50°C tap water

for 30 minutes in order to relax them. The clams relaxed in this way continued to respond to external stimuli. They were made to gape their shells (2cm) by gentle prying and a cork was inserted between the valves to prevent them from closing. Rubber bands were used to prevent the shells from gaping more than 2 cm. The clams were then shaken to remove any excess fluid and then they were immersed in 5% formaldehyde fixative buffered to a pH of 7. This fixative was used for all clams in this experiment.

The second treatment involved shaking the excess water from six clams and immediately immersing them in the fixative. This achieved an isometric state of contraction. The fixation occurred shortly after the onset of contraction.

The third treatment involved exciting six clams by handling them for 30 minutes in order to achieve an isometric state of contraction with fixation occuring 30 minutes after excitation.

The fourth treatment involved cutting the end of the posterior adductor away from one shell at the hypostracum by inserting a scalpel between the valves. The muscle was then not limited by the shell and could contract isotonically, or as much as it could possibly contract. In this fourth treatment, the clams were immediately immersed in the fixative after the adductor was cut. The fifth treatment was identical to the fourth except the six clams were handled for 30 minutes before they were immersed into the fixative.

After four full days of being in the fixative, the clams were rinsed with running tap water and prepared for opening. The isometrically contracted posterior adductor muscles and the relaxed posterior muscles were cut away from their shells to prevent tearing the muscle. This was done by inserting a scalpel between the valves and cutting the

posterior adductor muscle at the hypostracum. The isotonically contracted adductor muscles had already been cut and the only thing holding the valves closed was the anterior adductor. With a sufficient amount of pressure, the anterior adductor tore loose and exposed the posterior adductor which was then removed with a scalpel and then sliced into 2mm cross sections with a razor blade. The muscle was immersed in aqueous Bouin's fixative for 48 hours. This served as a secondary fixative. The tissue was then washed in tap water overnight. The opaque and translucent portions were then separated with the unaided eye by a simple teasing technique. Dehydration followed this procedure. A standard ethanol series, except for 100% methanol instead of ethanol, was used for one half of the tissue. Pure ethanol (100%) is not used by the Zoology Department of Eastern Illinois University and only 100% methanol was available. The second half of the tissue was dehydrated in "cellosolve" and cleared in methyl benzoate. Benzene was the final clearing agent in both cases. The reason for the second technique was for one of convenience and the possibility that it was more gentle on the tissue. The tissue was embedded in "Tissuemat" at 58-59°C. The blocks were longitudinally sectioned at 9u using "Gillette Valet" razor blades. These blades are not hollow ground and are therefore suitable for sectioning. The sections were affixed to slides with albumin. Heldenhain's iron hematoxylin was the only stain used, because of its ability to stain striations exceedingly well. All observations were made using an American Optical light microscope at either 430X or 1000X. All measurements were made using an ocular micrometer.

Photomicrographs were made using a Bausch and Lomb light microscope with a Honeywell Pentax Spotmatic camera using Kodak Pantomic-x film. The light source was a tungsten light bulb of a 500 watt capacity with a blue filter.

Five physiological experiments were conducted on five individual clams. The experiments were devised to demonstrate the tonicity of the posterior adductor muscle and its reaction to electrical and chemical stimulation once it had been stripped of all nervous and epithelial tissue.

One requirement of each physiological experiment was to leave the muscle attached to the shell and to leave as much of the shell intact as possible in order to maintain normal muscle length. This was achieved by removing the anterior half of the right valve, along with the anterior adductor, leaving the posterior adductor attached to the entire left valve and to the remaining posterior half of the right valve. The right valve was divided into two by drilling consecutive small holes along a mid dorsal-ventral line. This, in essence, "scored" the right shell making it crack in half. The anterior half was then pulled away from the clam and all of the soft parts were removed except for the posterior adductor. The specimen was then immediately immersed in 0.16% physiological solution (Forte 1964) and allowed to repolarize for about 30 minutes. The specimen was then placed in a 1.5 liter "finger bowl" with enough physiological solution, at room temperature, to cover the muscle. The intact left valve was in contact with the bottom of the bowl and restrained by a rod attached to a ring stand; the total weight holding the shell down being approximately 5 lbs. The free, right, upper half of the shell was attached to an E&M myograph "A", by means of three

hooks and a strong nylon thread. The connection was direct; no pulleys or levers were necessary since the total muscle travel did not exceed 5 to 10 mm.

The myograph was attached to an E&M "Physiograph Four" (E&M Instrument Company, a division of Narco Scientific Company). An initial experiment was conducted to record any spontaneous activity by the muscle, and to determine its response to changes in tension effected by raising and lowering the myograph while it was attached to the right valve; the applied tension ranged from 0g. to 10g. A series of square wave stimuli were then applied to each muscle by means of two stainless steel electrodes inserted into the muscle and connected to the stimulator unit of the physiograph. The electrodes were inserted in various locations of each muscle and the effect of the various positions in which the muscle was stimulated was noted. The range of the voltage used for stimulation was 1 to 100 stimuli per second.

Chemical stimulation was limited to the use of norepinephrine and acetylcholine chloride. Concentrations of 10^{-1}M., 10^{-3}M., and 10^{-5}M. were injected directly into the muscle and the response was recorded by means of the physiograph. Initially, an attempt was made to bathe the muscle in various concentrations of neurohumors, but this proved to be awkward and ineffective. No response could be gotten from the muscle by means of bathing it in one of the various solutions. The reason for this was not apparent.

RESULTS

Histology

The posterior adductor in cross section is divided into two portions; a dorsal, translucent portion and a ventral, opaque portion. The appearance of these two portions was correlated with their functions. The difference in appearance between the two portions is readily observable in a fresh or fixed portion of the posterior adductor muscle by simply holding a cross section of the adductor in front of a light source.

When the adductors were removed from the clams, a difference in the lengths of the fixed muscle, depending on the state of contraction, was noted. A fully extended, relaxed muscle averaged 2.0 cm in length. An isotonically contracted muscle averaged 1.0 cm in length, and an isometrically contracted muscle averaged 1.5 cm in length. A posterior adductor, in vivo, would never shorten less than 1.5 cm or the isometric length.

Histologically, the translucent and opaque portions were similar but with significant differences occurring when the muscle was contracted isotonically. The fibers in both portions seemed to run the entire length of the muscle.

The opaque portion of the posterior adductor, in longitudinal sections, appeared differently at various fixed stages of contraction. In all cases, a fibrillar structure was apparent. The isometrically contracted adductor muscle fibers fixed immediately after excitation (fig. 2) were straight and without any striations or other patterns. The fibrils appeared to be parallel with the fiber axis and, occasionally, a helical spiral arrangement of fibrils was apparent. The median fiber

diameter was 3.8u but fibers of 2.85u to 5.5u were present. The nuclei were centrally located in all fibers and measured about 2.85u to 3.8u in width and 11.5u to 17.1u in length.

Isometrically contracted muscle fixed 30 minutes after excitation (fig. 4) appeared to be very similar to the isometrically contracted muscle fixed immediately after excitation; however, the spiral arrangement of fibrils was not observed very often. The muscle had the appearance of smooth muscle that had cylindrical fibers instead of spindle-shaped fibers. The nuclei measured about 13.3u to 17.1u long and about 2.85u to 3.8u wide.

Isotonically contracted adductor muscle with immediate fixation (fig. 7 and 8) appeared significantly different from isometrically contracted adductor muscle. Frequent buckling or bending characterized the fibers of this muscle. Fibrils were apparent and seemed more widely spaced in the wider fibers. Fiber diameter varied from about 1.9u to 9.5u, however, the median was 3.8u. No striations or other patterns were apparent and very unusual staining characterized the fibers of this muscle. The thinner fibers tended to stain more darkly than wider fibers. In an oblique section, light and dark areas become apparent suggesting a pattern of contraction bands. Occasionally in longitudinal section, there would be wide fibers with alternating light and dark areas; these fibers measured about 5.5u in diameter and had a wavy appearance. The nuclear dimensions varied much more than in the previous states of contraction; their diameter varied from 1.9u to 3.8u and their lengths varied from 3.8u to 17.1u.

Isotonically contracted muscle fixed 30 minutes after excitation

(fig. 11) exhibited an even greater degree of buckling or bending of fibers. Fiber diameter again ranged from 1.9u to 9.5u with most of the fibers at about 3.8u. The nuclei measured the same as those of isotonically contracted adductor muscle with immediate fixation. The nuclear diameters ranged from 1.9u to 3.8u and their lengths from about 3.6u to about 11.4u.

Relaxed muscle fibers (fig. 13 and 14) were attenuated and straight. They varied in thickness from 1.9u to 5.7u with a median of 1.9u. No striations or any other patterns were observed. The nuclei were generally as wide as the fiber they were in and sometimes were over 21u long. A certain amount of uneven staining was of some interest but this may be due to staining techniques.

The translucent portion of the posterior adductor differed significantly in appearance from the opaque portion in the state of isotonic contraction. In the translucent portion, fibrils were apparent, as they were in the opaque portion, and again seemed parallel to the fiber axis.

In an isometric state of contraction, fixed immediately after excitation (fig. 1), the translucent portion exhibited no striations or other patterns. The fibers were straight and the fiber thickness was fairly uniform, 3.8u being the median fiber diameter. The fibrillar structure was apparent but the helical arrangement was not. The nuclei were very similar in appearance to those found in the opaque portion; about 3.8u in diameter and about 11.5u long, and centrally located.

Isometrically contracted adductor muscle fixed 30 minutes after excitation (fig. 3) was very similar to isometrically contracted

adductor muscle fixed immediately after excitation. The fibers appeared to be of a more uniform width, about 3.8u. No striations or patterns were observed and no buckling or folding of the fibers was present. Nuclei appeared very similar to the other nuclei and measured 3.8u wide and the length varied from 13.3u to 17.1u.

Isotonically contracted muscle fixed immediately after excitation (fig. 5 and 6) exhibited a significant change in appearance. Buckling or folding of the fibers was very characteristic of this muscle in longitudinal section. Many nuclei were oval, measuring 3.8u in width and 5.5u in length. Some nuclei were actually round at 3.8u in diameter, whereas some were long, measuring 13.5u. The wider, apparently more contracted fibers had the more rounded nuclei, whereas the elongated nuclei were found in thin, apparently not fully contracted, fibers. No definite striations were apparent but some faint patterns were present. The most definite pattern was the "checkerboard" or braided fibers.

Isotonically contracted muscle fixed 30 minutes after excitation (fig. 9 and 10) was different in appearance from adductor muscle in other stages of contraction; it had occasional definite striations. The striations were in fairly wide fibers of about 5u. They did not occur frequently and were atypical when compared to striations of vertebrate striated muscle. The sarcomeres of this translucent portion averaged 3.8u long whereas the sarcomeres of vertebrate muscle are about 2.5u long. The borders of the A and I bands were not well defined and often appeared serrated. No other bands could be seen, and the striations did not always seem to be at right angles to the fiber axis. Buckling

or folding of fibers in this muscle predominated, but not in portions of the fibers having striations. As before, there were darker staining thin fibers and lighter staining wide fibers. Nuclei were mostly round and about 3.8u in diameter. Fiber diameter varied from 1.9u to about 9.5u with 3.8u being the median. Translucent fibers of the relaxed posterior adductor (fig. 12) seemed to be similar to those of the opaque portion of the relaxed muscle. The fibers were again attenuated and straight and measured from .9u to 3.8u in diameter. The nuclei varied from .9u to 3.8u in diameter and 3.8u to 20.0u in length. The staining seemed fairly uniform with no visible striations or patterns. Fibrils with a spiral arrangement did appear occasionally.

An unusual condition in both the opaque and the translucent portions existed at the hypostracum (fig. 3 and 12). Many nuclei were lined up along the hypostracum; such nuclear alignment occurred at no other place within the fibers of the adductor.

Physiology

The five experiments described in this report deserve only tentative and preliminary consideration. The results expressed are meant to suggest possible behavioral characteristics of the posterior adductor as a whole.

The adductor muscle preparations revealed some spontaneous activity. Under some tension, the muscle slowly accommodated by lengthening; if small amounts of tension were applied to the muscle it would increase its rate of lengthening. On the other hand, if the tension was decreased, the muscle would shorten. This activity was reversed for 20

to 30 seconds by direct current stimulation (fig. 15). No response was obtained below 50V, regardless of the frequency. Varying the stimulus frequency had little effect. Very low frequencies evoked little or no response whereas stimuli applied at a rate of one or more per second did produce a response.

The rate of shortening and lengthening of the muscle was very slow except when acetylcholine was administered. The muscle continued to shorten very slowly for many hours if allowed, or lengthen for many hours if sufficient tension was maintained.

Changing the position of the two electrodes in the muscle produced little variation in response to electrical stimulation. Generally, when they were close together, less response was obtained than when they were farther apart.

The effect of adrenalin or epinephrine was minimal or negative; no definite response could be noted at any concentration.

At certain concentrations, acetylcholine chloride did produce a positive response (fig. 15). When 1 cc of 10^{-5}M. solution was administered, no response was noted; the muscle was washed in balanced salt solution and 1 cc of 10^{-3}M. solution was administered. The 10^{-3}M. solution did evoke a definite contractile response. The 1 cc of 10^{-1}M. solution also produced contractions, but in one case, not as strong a response as evoked by the 10^{-3}M. solution.

RESULTS

The results of the histological examination of the posterior adductor of Quadrula sp. are listed in Table I and Table II.

TABLE I: The histological characteristics of the translucent portion of the posterior adductor muscle of Quadrula sp. at different stages of contraction.

STATE OF CONTRACTION	FIBER DIAMETER	FIBER PATTERNS & ARRANGEMENT	NUCLEAR DIMENSIONS
Isometrically contracted with immediate fixation	2.85u-5.5u median 3.8u	No striations or patterns. Fibers were straight and loosely arranged. Fibrils seemed to form loose spiral.	Diameter: 2.85u-3.8u Length: 11.5u-17.1u
Isometrically contracted with 30 minutes post fixation	3.8u	Similar to isometrically contracted muscle with immediate fixation	Diameter: 3.8u Length: 13.3u-17.1u
Isotonically contracted with immediate fixation	1.9u-5.5u median 3.8u	Many fibers wavy or buckled, alternating with straight fibers. Very few faint striations?	Diameter: 3.6u- Length: 3.6u-13.5u
Isotonically contracted with 30 minutes post fixation	1.9u-9.5u median 3.8u	Much waviness or buckling, oblique "striations" and normal striation occasionally present. Sarcomete length is 3.6u.	Diameter: 3.6u Length: 3.6u-5.5u
Narcotized muscle fixed immediately after 45-50°C water bath for 30 minutes	.9u-3.8u median 1.9u	All fibers were attenuated, and distance between fibers often exceeded fiber diameter	Diameter: .9u-3.8u Length: 3.8u-20.0u

TABLE II: The histological characteristics of the opaque portion of the posterior adductor muscle of Quadrula sp. at different stages of contraction.

STATE OF CONTRACTION	FIBER DIAMETER	FIBER PATTERNS & ARRANGEMENT	NUCLEAR DIMENSIONS
Isometrically contracted with immediate fixation	1.9u-3.8u median 1.9u	Straight fibers, little spacing. No striations or patterns. Some helically arranged fibrils	Diameter: 2.85u-3.6u Length: 11.5u-17.1u
Isometrically contracted with 30 minutes post fixation	1.9u-3.8u median 3.8u	Fibers were relatively straight, no striations or patterns. Some helically arranged fibrils.	Diameter: 2.85u-3.6u Length: 13.3u-17.1u
Isotonically contracted with immediate fixation	1.9u-5.5u median 3.8u	Most of the fibers were buckled or wavy, No striations of patterns were present.	Diameter: 1.9u-3.6u Length: 3.6u-17.1u
Isotonically contracted with 30 minutes post fixation	1.9u-7.2u median 3.8u	About half the fibers are buckled or wavy. No striations or patterns were present.	Diameter: 1.9u-3.6u Length: 3.6u-17.1u
Narcotized muscle fixed immediately after 45-50°C water bath for 30 minutes	1.9u-3.8u median 1.9u	Straight attenuated fibers similar to translucent narcotized muscle. No patterns or striations	Diameter: 1.9u-5.7u Length: 5.5u-21.0u

Fig. 1 Isometrically contracted translucent portion fixed immediately after excitation. 1000x

Fig. 2 Isometrically contracted opaque portion fixed immediately after excitation. 1000x

Fig. 3 Isometrically contracted translucent portion fixed 30 minutes after excitation. Note hypostracum 500x

Fig. 4 Isometrically contracted opaque portion, fixed 30 minutes after excitation. 500x

Fig. 5 Isotonically contracted translucent portion fixed immediately after excitation. 500x

Fig. 6 Isotonically contracted translucent portion fixed immediately after excitation. 1000x

Fig. 7 Isotonically contracted opaque portion fixed immediately after excitation. 1000x

Fig. 8 Isotonically contracted opaque portion fixed immediately after excitation. 1000x

Fig. 1

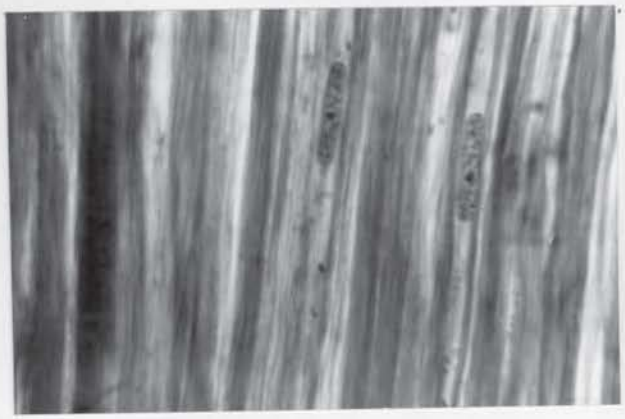


Fig. 2

Fig. 3

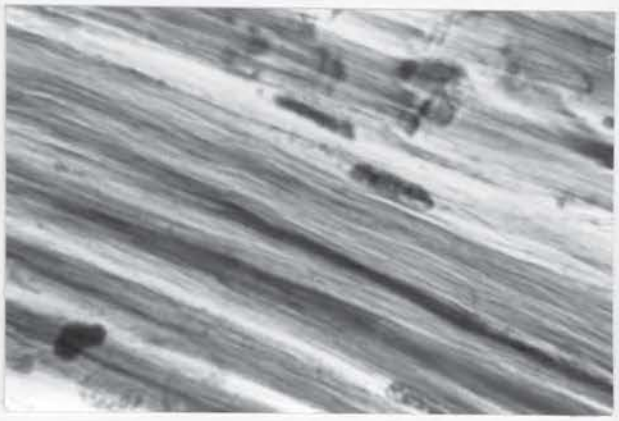


Fig. 4

Fig. 5

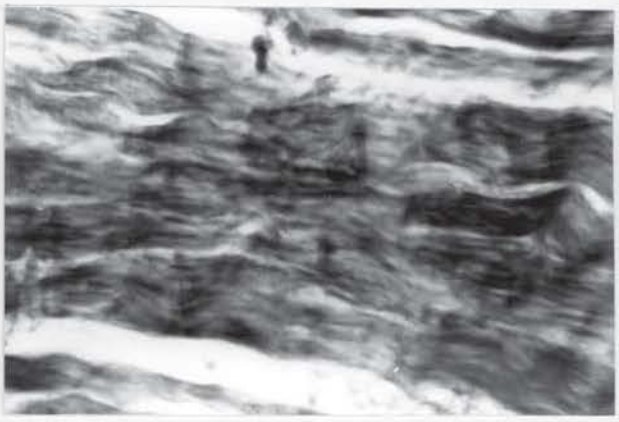


Fig. 6

Fig. 9 Isotonically contracted translucent portion fixed 30 minutes after excitation. 1000x

Fig. 10 Isotonically contracted translucent portion fixed 30 minutes after excitation. 1000x

Fig. 11 Isotonically contracted opaque portion fixed 30 minutes after excitation. 500x

Fig. 12 Relaxed translucent portion, fixed 30 minutes after 45-50°C water bath. 500x

Fig. 13 Relaxed opaque portion, fixed 30 minutes after 45-50°C water bath. 500x

Fig. 14 Relaxed opaque portion, fixed 30 minutes after 45-50°C water bath. 1000x

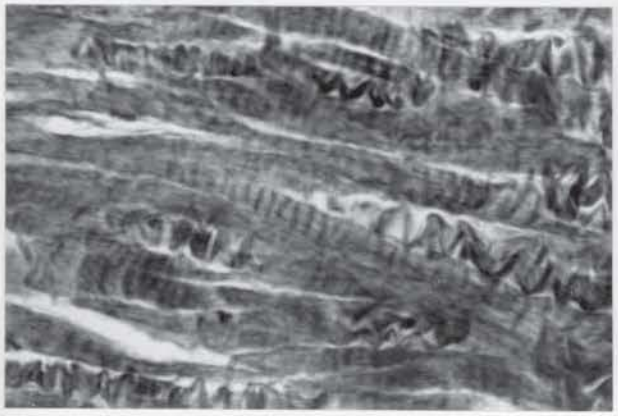


Fig. 9

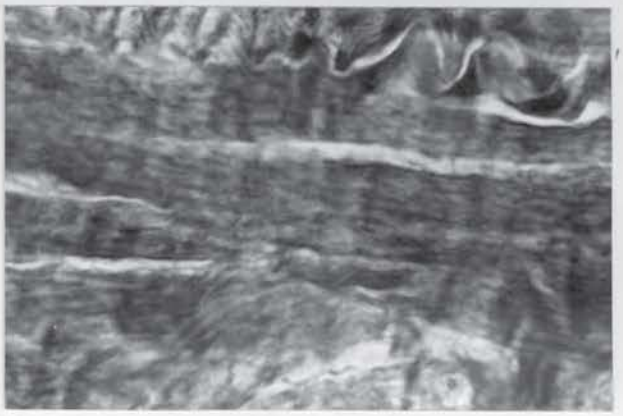
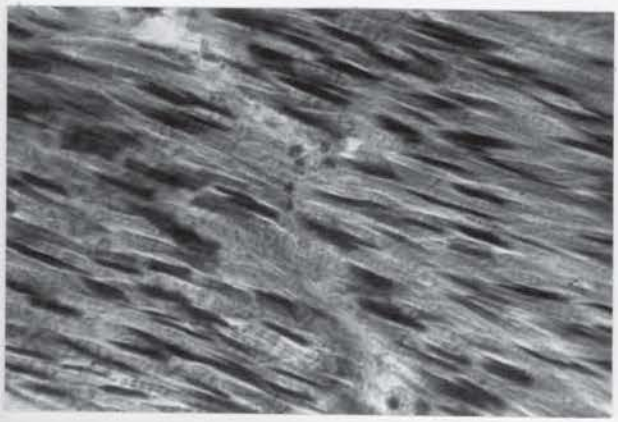


Fig. 10



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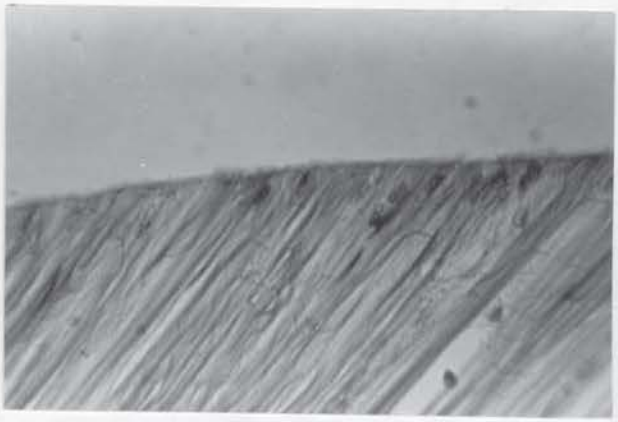


Fig. 12

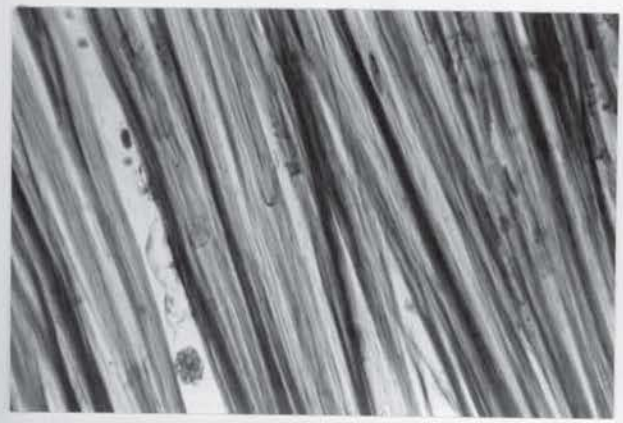


Fig. 13

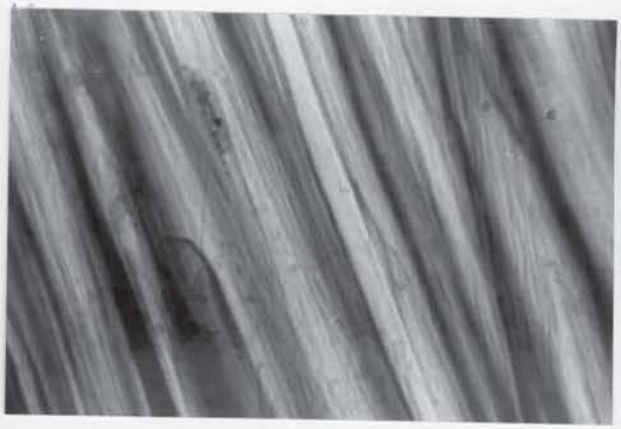
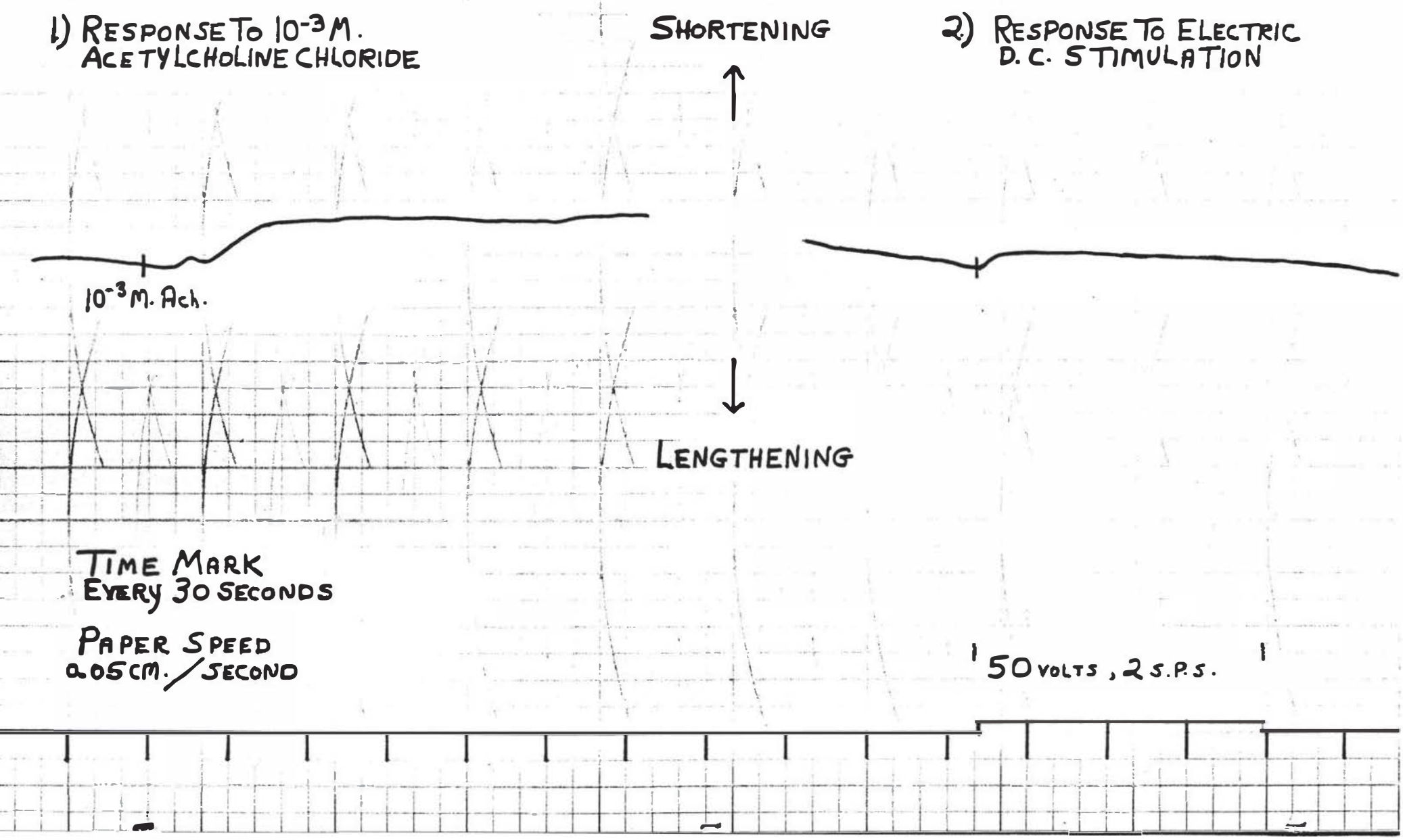


Fig. 14

P.-18a-

FIG. 15

RESPONSE OF THE POSTERIOR ADDUCTOR OF *QUADRULA* SP. TO 1) 10^{-3} M. ACETYLCHOLINE AND 2) ELECTRIC D.C. STIMULATION



DISCUSSION

Histology

Almost every pelecypod adductor described since 1954 by investigators is made up of two portions. These two portions are described in various ways; yellow and white, red and white, and translucent and opaque. Quadrula's posterior adductor fits the description used by Hanson and Lowy (1961) for the adductor of Crassostrea angulata. Upon macroscopic examination, the two portions appear translucent and opaque. The significance of this distinction between the two portions of the adductors is not certain. Histologically, adductor of Crassostrea, Anodonta (Forte 1964), and Quadrula occasionally exhibit "patterns" when allowed to contract far enough. The adductors of these bivalves have what is called by Hanson and Lowy (1961), "diamond-lattice" appearing fibrils or what this author calls "braided". The translucent portion of the adductorssof Anodonta and Quadrula also have fibers with striations when the fibers are allowed to contract isotonically. The opaque portion of all three species seems to be smooth; no patterns are described for any of the opaque adductor muscle fibers.

The description of each portion of the adductors of Crassostrea, Anodonta, and Quadrula, becomes more meaningful when one considers them in relation to the contractile behavior of Pelecypod adductor muscles. Most pelecypod adductors have two major types of contractile behavior. Crassostrea's translucent portion is said to be phasic (Hanson and Lowy, 1957), and the opaque portion is said to be tonic (Elliott 1964, Hanson and Lowy 1961). This same situation exists for four out of six adductors described by Kawaguti and Ikemoto

(1957, 1960a, 1960b, 1964), as shown in Table III.

The paradox of certain molluscan muscle, including the adductor muscle, is that it can contract quickly and for prolonged periods of time without apparent signs of fatigue (Hoyle and Lowy 1955). The specialization of the adductor into two portions is not, therefore, unreasonable and may very well have been selected for during the course of evolution. The need for tight and prolonged valve closure becomes apparent for a mussel or oyster when a predator, such as a raccoon, starfish or octopus, decides to feed upon the bivalve.

The division of labor or specialization of the adductor of bivalves has necessitated a designation or assignment of the duties of prolonged contraction. This is not as simple as might first appear. Either portion, phasic or tonic, may exhibit prolonged contractile capabilities. The presence of paramyosin in both portions (Hanson and Lowy 1961) has been related to the ability of an adductor or anterior byssal retractor muscle to undergo durable or prolonged contraction. This situation needs to be investigated further.

The question of whether or not an adductor muscle of a pelecypod is striated or smooth has been answered inconsistently. According to Zs Nagy and Salanki (1964b) many authors refer to adductor muscles as either striated or smooth; the question is partly answered by Zs Nagy and Salanki, in their use of the term polymorphic.

Now that it is known that there are two portions of the adductor of most species, and that the portions differ to some extent, histologically, labels like "striated" and "smooth" should be reassessed. The term, striated, is used especially (and originally) to describe vertebrate skeletal muscle.

The term, smooth, most often used to describe visceral muscle, has been used indiscriminately to identify any muscle not having striations. These descriptive terms are not really exact enough, and more appropriate labels should be used. Hanson and Lowy (1961) attempt to correct this misusage by using the phrase "contraction bands" instead of striations. They still, however, use "smooth" to describe the tonic portion. A fair argument for not using "smooth" might be the fact that the opaque portion's fibers are cylindrical, not spindle shaped. The adductor of Crassostrea angulata (Hanson and Lowy, 1957) has tonic and phasic contractile behavior, but they are not isolated or limited to the separate portions of the adductor. It may be true that the opaque portion is mainly tonic and the translucent portion is mainly phasic but in each case the division of labor is not complete. The tonic portion may behave sometimes in a phasic way and the phasic portion is sometimes tonic (Hanson and Lowy, 1961).

The history of confusion regarding the histology of adductor muscles of pelecypods is understandable when attention is given to the fact that each individual portion of the adductors, in many species, appears differently at various fixed stages of contraction. As previously stated, Zs Nagy and Salanki (1964) appropriately defined the adductor of Anodonta as polymorphic. This term, in my opinion, is appropriate for describing the adductor muscle of Quadrula.

The translucency and opaqueness of the two portions of the adductors of Anodonta and Crassostrea has been explained in two different ways. Zs Nagy and Salanki (1964) mention that the only

difference between the two portions is the undqual distribution of connective tissue. The opaque or white portion is described as having more connective tissue than the translucent or yellowish portion. Henson and Lowy (1957) and Kawaguti and Ikemoto (1957) considered the possibility of connective tissue being the reason for the difference in appearance between the two portions, but finally concluded that the difference is more likely to be a result of ultramicroscopic regularity, and its effect on light. The regularity is related to or a result of the alignment of fibrils and filaments within the fibers. The translucent portion is translucent because its ultrastructure interfers with light less than the ultrastructure of the opaque portion. The presence of striations in the translucent portion of the adductor of Anodonta and Quadrula supports this hypothesis, even though they occur only in an isotonic state of contraction; striations are definitely an indication of ultrastructure regularity.

Light microscopy has limited the information obtainable during this study of the posterior adductor of Quadrula. An electron microscope study is needed in order to determine the filament structure at different stages of contraction, and possibly determine what contractile proteins are present. The light microscope study may be made more complete by a more refined examination. Serial sections of an entire portion of the adductor might help clarify the peculiar alignment of fiber nuclei at the hypostracum, and quantitatively determine the occurrence of various patterns such as striations and diamond lattice or braided-appearing fibrils. The occasional appear-

ance of striations during isotonic contraction, 30 minutes after excitation has begun, is of special interest. Do only certain fibers become striated or do all fibers have this potential? Maybe with a faster penetrating fixative that will not dissolve out, a more substantial result could be obtained. The protein bound picrates of Bouin's fixation are water soluble, and during any one of the washes, they could have dissolved out too completely and disrupted the protein structure of the fibers.

The variability of fibers in each portion of the adductors raises some interesting questions. Do these fibers not only appear differently but also function differently? The fibers, when in a partially buckled arrangement do not seem to be contracting equally; some fibers seem to have contracted, while others did not contract or contracted much less.

The relationship of fiber thickness to contractile potential is vague. The wider fibers (5.5u), in an isotonic state of contraction fixed 30 minutes after continuous excitation, occasionally had striations whereas thinner, darker staining fibers did not. The differential staining suggests an unequal protein distribution. Heidenhain's iron hemotoxylin is probably a protein stain (Puchtler, 1958); this might explain the light and dark fibers.

If any conclusion can be drawn from the histological examination of the posterior adductor of Quadrula, it is that the portions of the adductors, even though they are divided macroscopically and do differ somewhat histologically, are nevertheless somewhat similar and are therefore incompletely distinct. The appearance of some fibers seems to be the same in both portions and justifies the claim that

the portions are indistinct.

The evidence that molluscan muscle, including Pelecypod adductor muscle, contracts by means of a sliding of discontinuous filaments over one another, is not as plentiful as the evidence to support this theory for vertebrate muscle. The problem has been examined, however, and it is now generally assumed that it does contract in this manner (Hanson and Lowy, 1957, 1959, 1961; Elliott 1964; Kahn and Johnson, 1961).

Physiology

The anterior byssus retractor muscle of Mytilus is the only Pelecypod muscle which has been studied physiologically by recent investigators. It has been used more than any other Pelecypod muscle probably because it is very accessible and has all of the contractile and behavioral characteristics of Molluscan muscle with which physiologists are concerned. The anterior byssus retractor muscle has both tonic and phasic activity, and the ability to contract for prolonged periods of time without apparent signs of fatigue. Therefore, the anterior byssus retractor muscle and most adductors have the same contractile characteristics, and may very well have the same mechanisms of contraction.

Twarog (1954), Jewell (1961), Johnson and Twarog (1959), and Lowy and Millman (1964) have described the effects that various forms of stimulation have on the anterior byssus retractor muscle of Mytilus and have recorded spontaneous activity of the muscle. They have demonstrated the effect of 5-hydroxytryptamine (5-HT), and have concluded that it is an inhibitor, destroying tonic response, and

causing the muscle to relax. Johnson and Twarog (1959) have reason to believe that it is naturally occurring. It may have the same effect on the adductor muscles of Pelecypods, but this is not yet known. All agree that acetylcholine is the excitatory or stimulating neurohumor for the anterior byssus retractor muscle. The experiments on the posterior adductor of Quadrula indicate that acetylcholine evokes a positive response from the adductor as it does from the anterior byssus retractor muscle. The presence of cholinesterase has been demonstrated in the anterior byssus retractor muscle of Mytilus edulis (Lowy and Millman 1963), and in the adductor of Anodonta cygnea (Zs Nagy and Salanki, 1964a). This needs to be determined for the posterior adductor of Quadrula.

The negative effect of electrical stimulation during tonic contraction, except at unusually high voltage, suggests that depolarization depends significantly upon the presence of neurohumors. The tonic behavior was very difficult to disrupt except when acerylcholine chloride was administered.

The presence of an excitatory and inhibitory mechanism probably necessitates the need for dual innervation. This has been demonstrated by Pavlov (1885), Benson et al (1942), and Salanki, J. and Labos, E. (1963), who found that weak faradic stimulation of the cut end of the otherwise intact nerve to the adductor caused the muscle to relax. Benson demonstrated how this inhibitory effect abolished tonic contraction, thus creating a purely phasic muscle.

TABLE III: The relationship between structure and contractile behavior of various adductors of certain bivalve genera: (after Kawaguti and Ikemoto, 1961).

	ACTIVITY	DURABLE CLOSURE	YELLOW OR TRANSLUCENT PORTION	WHITE OR OPAQUE PORTION
Pecten	fast cont. fast relax.	yes	cross striated	smooth
Lima	fast cont. fast relax.	no	cross striated	none
Spondylus	frequent fast cont. rather slow relax.	no	cross oblique striations	smooth
Ostrea	occasional fast cont. slow relax.	yes	smooth	smooth
Meretrix	occasional fast cont.	yes	smooth	smooth
Pinna & Atrina	occasional fast cont. slow relax.	yes	smooth	smooth
Quadrula	occasional fast cont. slow relax.	yes	occasional cross and oblique striations	smooth

cont.= contraction
relax.= relaxation

SUMMARY AND CONCLUSION

In conclusion, these experiments have revealed six important points about the posterior add ctor of Quadrula:

1. The posterior adductor is divided into two portions.

2. One portion is transi cent and is probably phasic muscle. Some of its fibers are patterned with striations on a diamond-lattice like network.

3. The other portion is opaque and is probably tonic muscle. It has no striations or any other patterns and may be referred to as smooth muscle.

4. The characteristic ability of molluscan so-called "smooth" muscle to undergo prolonged contraction, may not belong to any one portion of the adducter, but possibly both.

5. The evidence derived from the experiments on the anterior byssus retractor muscle of Mytilus may explain the contractile mechanism of the posterior add ctor of Quadrula. There seemed to be separate excitatory and inhibitory mechanisms controlling these muscles.

6. 5-HT is the inhibitory neurohumor of the anterior byssus retractor muscle and might be for the adductor.

7. Acetylcholine is the excitatory neurohumor of the anterior byssus retractor muscle and, from experimental evidence, is probably the excitatory neurohumor for the posterior adductor of Quadrula.

8. The existence of separate excitatory and inhibitory systems would necessitate the existence of dual innervation.

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