

PARAMYOSIN IN INVERTEBRATE MUSCLES

II. Content in Relation to Structure and Function

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ABSTRACT

By quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, paramyosin:myosin heavy chain molecular ratios were calculated for three molluscan muscles: *Aequipecten* striated adductor, *Mercenaria* opaque adductor, and *Mytilus* anterior byssus retractor; and four arthropodan muscles: *Limulus* telson, *Homarus* slow claw, *Balanus* scutal depressor, and *Lethocerus* air tube retractor. These ratios correlate positively with both thick filament dimensions and maximum active tension development in these tissues. The role of paramyosin in these muscles is discussed with respect to the following characteristics: force development, "catch," and extreme reversible changes in length.

The paramyosin content of molluscan muscle has been observed to vary (*a*) with the structural organization of fibers, and (*b*) with the dimensions of the thick filaments. A paramyosin:myosin ratio greater than 1:1 has been reported for lamellibranch smooth adductors (30, 32). Obliquely and cross-striated adductors have been reported to contain proportionally less (1:2 and 1:3, respectively) of this protein (30, 32). In such molluscan smooth "catch" muscles as lamellibranch opaque adductors (6-8, 10-12, 20, 27) and *Mytilus* anterior byssus retractor muscle (ABRM) (18, 20, 21, 26, 28, 32), thick filaments range from 500 to 1,500 Å in diameter and from 10 to 40 μm in length. They are oriented parallel to the cells, but are not organized into identifiable ordered, repeating sarcomeric units (33). The cross-striated adductors of the lamellibranch *Aequipecten*, on the other hand, resemble vertebrate striated muscle with respect to both filament dimensions and sarcomere organization (24).

In previous papers (1, 5, 19) we and others reported the identification of paramyosin as a component of striated arthropod muscles, by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunodiffusion. We also showed that the arthropodan and molluscan paramyosins in homogenates of glycerinated muscle have identical chain weights, and further, that this protein is similar enough in these different muscles to cross-react immunologically across phyletic lines.

Here we report the paramyosin:myosin heavy chain ratios in the following arthropodan muscles: *Limulus* telson levator, *Homarus* slow claw muscle, *Balanus* scutal depressor, and *Lethocerus* air tube retractor; as well as in the following molluscan muscles: *Aequipecten* striated adductor, *Mytilus* ABRM, and *Mercenaria* opaque adductor. The correlation between paramyosin content, filament dimensions, and maximum active tension development is discussed in relation to the functional role of paramyosin in these muscles.

MATERIALS AND METHODS

SDS-Polyacrylamide Gel Electrophoresis

SAMPLE PREPARATION: Homogenates were prepared for SDS-polyacrylamide gel electrophoresis from glycerinated muscles as previously described (5), up to the point of dilution of the denatured muscle proteins with the tracking dye mixture. Aliquots of each preparation, adjusted to 2 mg protein/ml, were diluted first with buffer (5) and then with tracking dye-sucrose, to final protein concentrations of 1.0 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 0.125 mg/ml. Purified *Limulus* paramyosin and myosin, donated by Dr. George de Villafranca (Smith College, Northampton, Mass.), were also denatured and each was brought to a protein concentration of 0.5 mg/ml with buffer and tracking dye-sucrose. All samples were stored at -18°C . They were reboiled for 3 min, then cooled before use.

GEL ELECTROPHORESIS: 6% polyacrylamide gels containing 0.1% SDS were prepared as previously described (5). Using the purified *Limulus* paramyosin and myosin, we determined the range of protein loadings over which Beer's law holds, as well as the differences in dye binding for the proteins. Gels were loaded with increasing amounts of each protein, between 0.5 and 20.0 μg , respectively. At least four gels were run of each protein at each loading. After electrophoresis the gels were stained and destained as before (5). The gels were scanned at 550 nm on an EC model 910 densitometer (E-C Apparatus Corp., St. Petersburg, Fla.). The scans were traced on a Corning model 840 integrating recorder (Corning Scientific Instruments, Medfield, Mass.) with both scanning and integrating circuits operating simultaneously. The base lines were set to zero at the background of the gels. Staining intensity, as obtained from the integration record, was plotted against protein loading for each protein (myosin loadings were corrected to give the values for heavy chains only). Linear regression analyses were performed to determine the slopes. The slopes calculated for each protein, where dye binding was a linear function of loading, are shown in Fig. 1. A factor for converting staining intensity ratios to molecular ratios was obtained from these slopes.

Gels were always loaded with 20.0 μl of each muscle preparation, respectively. A minimum of five gels each, at loadings of 2.5 μg , 5.0 μg , 10.0 μg , and 20.0 μg of total muscle protein, were run on each muscle preparation. The gels were stained, destained, and scanned as previously described (5). The staining intensities of the myosin heavy chain and paramyosin chain bands were obtained from the integration records. The mean and standard deviation of paramyosin:myosin staining intensity (uncorrected) ratios were calculated for each muscle preparation. This was done for each protein loading between 2.5 and 20.0 μg . These ratios were compared at different loadings, for each muscle type.

Staining intensity ratios were converted to paramyosin:myosin heavy chain molecular ratios, with the

factor obtained from the slopes in the dye binding experiments.

RESULTS

SDS-Gel Electrophoresis

Fig. 1 shows the relationship between staining intensity and protein loading for both purified *Limulus* paramyosin and myosin. Dye binding is seen to be a linear function of protein loading up to 10.0 μg of paramyosin and 16.0 μg of myosin (thus, 14.5 μg of myosin heavy chains). We determined that paramyosin chains stain 1.1 times more intensely with Coomassie brilliant blue than do myosin heavy chains, giving a correction factor: paramyosin/myosin heavy chain = 0.9.

Gels loaded with between 2.5 and 20.0 μg of total muscle homogenate gave remarkably consistent paramyosin:myosin heavy chain "uncorrected" ratios within each muscle type. This indicates that within the protein loadings we used, Coomassie brilliant blue binding to these proteins was linearly related to the protein content of these bands. The "uncorrected" values were converted to molecular ratios (Table I). We observed considerable variation in paramyosin:myosin heavy chain ratios among the different muscles studied.

Morphological and Physiological Correlations

Examination of the paramyosin:myosin molecular ratios we obtained reveals that these ratios fall into three groups which correspond to the three

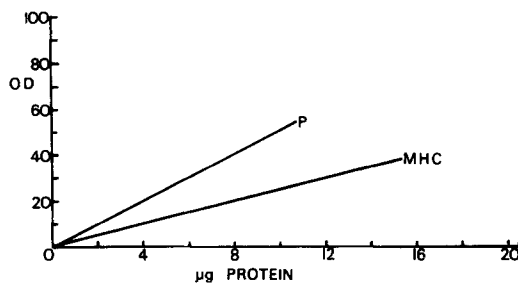


FIGURE 1 Dye-binding curve showing Coomassie brilliant blue binding to paramyosin chains and myosin heavy chains on SDS-6% polyacrylamide gels. OD in arbitrary units, read at 550 nm. P = paramyosin chains (slope = 5.1); MHC = myosin heavy chains (slope = 2.5). Slopes were calculated by linear regression analysis of four separate loadings of each protein at points between 0.5 μg protein and that loading at which the OD was no longer a linear function of protein present.

TABLE I
Relationships of Paramyosin:Myosin Heavy Chain Molecular Ratios to Both Filament Dimensions and Development of Maximum Active Tension in All Muscles Studied

Muscle source	No. of gels	PM:MHC molecular ratios \pm SD	Filament dimensions		Maximum active tension (kg/cm^2)
			length μm	diameter* nm	
Class I					
<i>Aequipecten</i> striated adductor	33	0.065 ± 0.02	1.8	21	1.2‡
<i>Lethocerus</i> flight	34	0.13 ± 0.02	2.4	18 (27)	0.3 (14)
Class II					
<i>Lethocerus</i> ATR	24	0.32 ± 0.14	~3.0	~18	1.25 (34)
<i>Limulus</i> telson	70	0.48 ± 0.12	4.9	22 (4)	3-5§
<i>Homarus</i> claw	40	0.51 ± 0.13	6.0	18 (13)	2.8 (23)
<i>Balanus</i> scutal depressor	56	0.47 ± 0.19	5.0	20 (14)	5.2 (15)
Class III					
<i>Mytilus</i> ABRM	52	2.88 ± 0.72	25.0	65-70 (20)	10-14 (21, 22)
<i>Mercenaria</i> opaque adductor	42	5.50 ± 1.43	40.0	>100 (12)	12.8 (22)

* All diameters, except for *Aequipecten*, were measured in sectioned material.

‡ Millman, Gibson, and Overy, manuscript in preparation.

§ Our unpublished data.

|| Slow muscle, not claw.

Numbers in parentheses denote references.

classes of muscle fiber types described in the previous paper (5). Class I muscles, structurally most similar to vertebrate striated muscle, have the lowest ratios (less than 0.2). Class II muscles, intermediate in structural type between Class I and smooth catch muscles (Class III), have higher ratios (0.3-0.6). Class III muscles have the highest ratios (greater than 2.0) (Table I).

We find a much lower paramyosin:myosin ratio in *Aequipecten* striated adductor (Class I) and a much higher ratio in *Mercenaria* opaque adductor (Class III) than those quoted by Squire (30). Szent-Györgyi et al. (32) reported ratios, in agreement with Squire's, which were obtained from isolated filaments. In such preparations, considerable loss of protein can occur, and this may seriously affect the ratios obtained. Our experiments were performed on glycerinated, otherwise intact tissue, in which such protein loss is minimized (5).

There is a marked correlation between the paramyosin:myosin heavy chain molecular ratios and both our own and published values of thick filament length (Fig. 2). The figure shows a linear relationship between these parameters. The filament diameters of Class I and II muscles are about the same, and are less than those of Class III muscles (Table I).

The values for tension which the muscles can

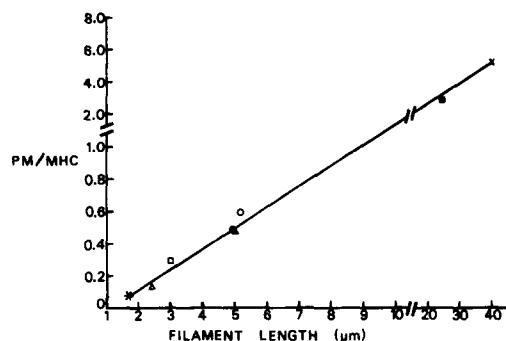


FIGURE 2 Linear relationship between thick filament lengths (abscissa) and paramyosin:myosin heavy chain molecular ratios (ordinate) for all the muscles studied here. Class I: *—*Aequipecten* striated adductor, Δ —*Lethocerus* asynchronous flight muscle; Class II: \square —*Lethocerus* air tube retractor, \blacktriangle —*Balanus* scutal depressor, \bullet —*Limulus* telson levator, \circ —*Homarus* slow claw muscle; Class III: \blacksquare —*Mytilus* ABRM, \times —*Mercenaria* opaque adductor.

develop can be roughly grouped according to our class designations (Table I). Maximum active tension development is lowest (0.3-1.2 kg/cm^2) in Class I muscles. These values are lower than those reported for vertebrate skeletal muscles (2.3 kg/cm^2 ; reference 22). In Class II muscles, the lowest value for maximum active tension is seen in *Letho-*

cerus air tube retractor (1.25 kg/cm²), which has the shortest thick filament length in this class. The maximum active tension developed by the other members of Class II ranges between 2.8 and 5.2 kg/cm². Class III muscles, which have the highest paramyosin:myosin ratios and the greatest thick filament lengths and diameters, show extremely high values for maximum active tension development (10–14 kg/cm²).

DISCUSSION

Effect of Paramyosin on Thick Filament Dimensions

On the one hand, the length (approximately 1.6 μ m) and diameter (approximately 16 nm) of thick filaments in different vertebrate striated muscles (which do not contain paramyosin) are remarkably similar. These dimensions probably are determined by the manner in which the constituent myosin molecules aggregate (25). On the other hand, thick filaments containing paramyosin are always longer and of greater diameter than those lacking this core protein, and vary greatly among different muscles (24, 32).

FILAMENT LENGTH: Filament length, in particular, is influenced by the presence of paramyosin. Ikemoto and Kawaguti (17), using only myosin isolated from horseshoe crab (*Tachypleus tridentatus*) muscle, reported that the lengths of reconstituted thick filaments never exceeded those of vertebrate skeletal muscle. Addition of paramyosin, however, resulted in the formation of considerably longer thick filaments. Thus, paramyosin may provide for an assembly of proteins resulting in structurally stable filaments of lengths unlikely or impossible with myosin alone.

We show that there is a linear correlation between the paramyosin:myosin heavy chain molecular ratios and the lengths of the thick filaments in all of the muscles studied here (Fig. 2). Thus, it appears that the amount of paramyosin present determines thick filament length. In order to establish this relationship conclusively, we are reconstituting thick filaments with varying molar ratios of paramyosin:myosin.

FILAMENT DIAMETER: While the thick filaments of paramyosin-containing invertebrate muscles are always of greater diameter than those of vertebrate striated muscle, no clear relationship exists between paramyosin content and filament diameter. The diameters of the thick filaments of Class I and Class II muscles are very similar,

despite the 5–10-fold greater paramyosin content of the latter (Table I). Class III muscles, however, which have a paramyosin content at least six times greater than that of Class II muscles (and 30–60 times greater than that of Class I muscles), do have thick filaments with unusually large diameters (Table I). It should be noted that these thick filaments are also of unusually great length (Table I).

Relationship Between Filament Dimensions and Tension

FILAMENT LENGTH: Increased thick filament length allows for an increased region of overlap between a thick filament and its surrounding thin filaments. Lowy et al. (22) suggested that individual thick filaments of greater length, having greater numbers of sites available for interaction with surrounding thin filaments, can produce greater tensions than individual shorter thick filaments. This suggestion was based on a comparison among frog sartorius, oyster oblique adductor, and *Mytilus* ABRM with respect to filament length and maximum active tension developed by each of these muscles.

Our results indicate that thick filament length can be correlated with maximum active tension development, when the three "Classes" of invertebrate muscles are compared with one another. This correlation does not hold, however, when Class I muscles are compared with vertebrate skeletal muscle (2.3 kg/cm²; reference 22), or when muscles within Class II are compared with one another (Table I). It is not clear, moreover, that increased filament length would necessarily be solely responsible for increased tension development by a whole fiber. It is conceivable that a larger number of shorter thick filaments, serially arranged in adjacent shorter sarcomeres, could provide an equivalent number of crossbridges along an equivalent length of muscle.

FILAMENT DIAMETER: There is no relationship apparent between filament diameter and tension development when Class I, Class II (Table I), oyster obliquely striated (~30 nm; 5 kg/cm²; reference 22), and vertebrate striated muscles are compared with one another. Class III muscles, having unusually large thick filaments, develop unusually high tensions (Table I). Their enormously increased filament diameters may provide greater filament strength (22). This might be required to support the increased load borne by each

thick filament of Class III muscles, which interact with a very large number of surrounding thin filaments via numerous crossbridges (6, 29, 34). Other parameters, however, also must be considered in determining a structural basis for tension development.

The Roles of Paramyosin in Muscle Function

“CATCH”: The phenomenon of “catch” is seen only in Class III muscles, having unusually high paramyosin:myosin ratios and thick filaments of unusually large length and diameter. The role of paramyosin, if any, in either initiating, maintaining, or effecting the release from catch in these muscles, however, is obscure. Different investigators have suggested that this protein affects each of these parameters (2, 9, 18, 32). The mechanisms invoked have involved shifts either in paramyosin-paramyosin interactions within the filament core (2, 32), or between filaments (18) or in paramyosin interactions with cortical myosin molecules (2, 9, 32). In any case, it appears that a critical mass of paramyosin is necessary for the type of actin-myosin interaction characterizing catch to occur. The pharmacology of neurotransmitters involved in both establishing and releasing catch is known (3, 33), and the changes in intracellular calcium after sarcolemmal binding of these transmitters is being studied (B. M. Twarog, personal communication). It would be of interest, therefore, to study the effects of such changes in the ionic environment on isolated filaments.

THICK FILAMENT SHORTENING: We have documented changes in length of *Limulus* telson muscle A bands and thick filaments (*a*) as sarcomeres shorten below $\sim 7.0 \mu\text{m}$ (4), or (*b*) after activation of intact fibers.¹ This phenomenon may occur in other Class II muscles as well. In *Balanus* scutal depressor, Hoyle et al. (16) reported sarcomeres as long as $20.3 \mu\text{m}$ and as short as $3.9 \mu\text{m}$, having A bands of $14.3 \mu\text{m}$ and $2.2 \mu\text{m}$, respectively. Similar changes in both sarcomere and A-band lengths are seen in *Homarus* slow abdominal muscle (our unpublished observations).

A combination of (*a*) long, thick filaments with longer regions available for actin-myosin interaction, and (*b*) shortening of these filaments endows Class II muscles, so equipped, with the ability to

undergo extreme, reversible length changes. Indeed, *in situ* changes of 100% or greater in sarcomere length have been observed in living *Limulus* telson, *Homarus* slow abdominal, *Lethocerus* air tube retractor, and *Balanus* scutal depressor muscles.

In vertebrate striated muscle and in Class I muscles (24, 27) which have no or little paramyosin, thick filament lengths remain constant during contraction. These muscles do not undergo great changes in sarcomere length. We are currently investigating whether or not the thick filaments of Class III muscles change length under various conditions.

The phenomenon of filament shortening, like catch, may be produced by a shift in the interactions between myosin and paramyosin. Again, a critical paramyosin content appears to be required for such behavior. Such an alteration may result in changes in molecular packing in the filament cores. Most paramyosin-containing filaments in noncatch muscles (Classes I and II) have cores which appear electron lucent (“hollow”) in electron micrographs of cross-sectioned tissue. In contrast, filaments containing larger amounts of paramyosin (Class III muscles) appear solid in cross section (33). It is not known in the first case whether the hollow core (*a*) is present along the entire length of the filaments, or (*b*) represents (the location of) paramyosin molecules, although the latter has been assumed (30). It is possible that the electron-lucent regions of Class II thick filaments are indeed empty of protein molecules, and represent sites which can be occupied by internalized protein during filament shortening. A change in cross-sectional appearance of these filaments may thus accompany a change in filament length. We have seen both hollow and solid profiles of thick filaments in *Limulus* telson muscle (4), but at this time we cannot determine whether these differences are due to variations in molecular packing (i.e. the presence or absence of paramyosin) normally occurring along the length of the filaments, or changes in filament organization occurring during shortening. A detailed analysis of the behavior of these paramyosin filaments *in vitro* and a systematic study of their subunit structure in cross-sectioned material, at different sarcomere lengths, is underway. Information gained from these investigations, together with the paramyosin:myosin molecular ratios reported here, should (*a*) elucidate the molecular organizations of these filaments, (*b*) allow comparison with

¹ Dewey, M. M., B. Walcott, D. E. Colflesh, H. Terry, and R. J. C. Levine. Submitted for publication.

Squire's models (30, 31) for paramyosin-containing filaments, and (c) provide a broader basis for understanding their behavior.

The authors wish to thank the following students who were involved with this project: Sandra Ahlum, Oliver Brooks, John Handal, Sondra King, Mary Kohut, and Maria Mendez. We appreciate the technical expertise of David E. Colflesh, Janice Sowinski, and Harriet Stout and the secretarial assistance of Barbara Buzby.

This project was supported, in part, by United States Public Health Service grants GM 20628, GM 21956, and HL 15835 to the Pennsylvania Muscle Institute. Rhea Levine is the recipient of a Research Career Development Award (NS 70476).

Received for publication 10 March 1976, and in revised form 9 June 1976.

REFERENCES

1. BULLARD, B., B. LUKE, and L. WINKELMAN. 1973. The paramyosin of insect flight muscle. *J. Mol. Biol.* **75**:359-367.
2. COHEN, C., A. G. SZENT-GYÖRGYI, and J. KENDRICK-JONES. 1971. Paramyosin and the filaments of molluscan "catch" muscles. I. Paramyosin: structure and assembly. *J. Mol. Biol.* **56**:223-237.
3. COLE, R. A., and B. M. TWAROG. 1972. Relaxation of catch in a molluscan smooth muscle. I. Effects of drugs which act on the adenyl cyclase system. *Comp. Biochem. Physiol.* **43A**:321-330.
4. DEWEY, M. M., R. J. C. LEVINE, and D. E. COLFLESH. 1973. Structure of *Limulus* striated muscle: the contractile apparatus at various sarcomere lengths. *J. Cell Biol.* **58**:574-593.
5. ELFVIN, M., R. J. C. LEVINE, and M. M. DEWEY. 1976. Paramyosin in invertebrate muscles: I. Identification and localization. *J. Cell Biol.* **71**:261-272.
6. ELLIOTT, A. 1974. The arrangement of myosin on the surface of paramyosin filaments in the white adductor muscle of *Crassostrea angulata*. *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **186**:53-66.
7. ELLIOTT, G. F. 1964. Electron microscope studies of the structure of the filaments in the opaque adductor of the oyster *Crassostrea angulata*. *J. Mol. Biol.* **10**:89-104.
8. ELLIOTT, G. F., J. HANSON, and J. LOWY. 1957. Paramyosin elements in lamellibranch muscles. *Nature (Lond.)* **180**:1291-1292.
9. EPSTEIN, H. F., B. J. ARONOW, and H. E. HARRIS. 1975. Interaction of myosin and paramyosin. *J. Supramol. Struct.* **3**:354-360.
10. HALL, C. E., M. A. JAKUS, and F. O. SCHMITT. 1945. The structure of certain muscle fibrils as revealed by the use of electron stains. *J. Appl. Phys.* **16**:459-465.
11. HALL, C. E., M. A. JAKUS, and F. O. SCHMITT. 1946. An investigation of cross-striations and myosin filaments in muscle. *Biol. Bull. (Woods Hole)*. **90**:32-50.
12. HANSON, J., and J. LOWY. 1960. Structure and function of the contractile apparatus in the muscles of invertebrate animals. In *Structure and Function of Muscle*. G. H. Bourne, editor. Academic Press, Inc., New York. 1st edition. **1**:265-336.
13. HAYES, D., M. HUANG, and C. R. ZOBEL. 1971. Electron microscope observations on thick filaments in striated muscle from the lobster, *Homarus*. *J. Ultrastruct. Res.* **37**:17-30.
14. HOYLE, G. 1969. Comparative aspects of muscle. *Annu. Rev. Physiol.* **31**:43-84.
15. HOYLE, G., and B. C. ABBOTT. 1967. Dynamic properties of giant muscle fibers of the barnacle. *Am. Zool.* **7**:611-614.
16. HOYLE, G., P. A. MCNEIL, and A. I. SELVERSTON. 1973. Ultrastructure of barnacle giant muscle fibers. *J. Cell Biol.* **56**:74-91.
17. IKEMOTO, N., and S. KAWAGUTI. 1967. Elongating effect of tropomyosin A on the thick filaments of the long sarcomere muscle of the horseshoe crab. *Proc. Jpn. Acad.* **43**:974-979.
18. JOHNSON, W. H., and D. E. PHILPOTT. 1959. Paramyosin periodicity in contracted molluscan muscle. *Proc. Biophys. Soc. Pittsburgh, Pa.* C-3.
19. LEVINE, R. J. C., M. M. DEWEY, M. ELFVIN, and B. WALCOTT. 1974. *Lethocerus* flight muscle paramyosin: antibody localization and electrophoretic studies. *Am. J. Anat.* **141**:453-458.
20. LOWY, J., and J. HANSON. 1962. Ultrastructure of invertebrate smooth muscle. *Physiol. Rev.* **42** (Suppl. 5):34-47.
21. LOWY, J., and B. M. MILLMAN. 1963. The contractile mechanism of the anterior byssus retractor muscle of *Mytilus edulis*. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **246**:105-148.
22. LOWY, J., B. M. MILLMAN, and J. HANSON. 1964. Structure and function in smooth tonic muscles of lamellibranch molluscs. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **160**:525-563.
23. MENDELSON, M. J. 1969. Properties of a very fast lobster muscle. *J. Cell Biol.* **42**:548-563.
24. MILLMAN, B. M., and P. M. BENNETT. 1976. The structure of a cross-striated molluscan muscle: the adductor-muscle of the scallop. *J. Mol. Biol.* In press.
25. PEPE, F. A. 1967. The myosin filament. I. Structural organization from antibody staining observed in electron microscopy. *J. Mol. Biol.* **27**:203-226.
26. PHILPOTT, D. E., M. KAULBROCK, and A. G. SZENT-GYÖRGYI. 1960. Filamentous organization of molluscan muscles. *J. Ultrastruct. Res.* **3**:254-269.
27. REEDY, M. K. 1967. Cross-bridges and periods in insect flight muscle. *Am. Zool.* **7**:465-481.
28. SCHMITT, F. O., R. S. BEAR, C. E. HALL, and M. A. JAKUS. 1947. Electron microscope and x-ray

- diffraction studies of muscle structure. *Ann. N.Y. Acad. Sci.* **47**:799-812.
29. SOBIESZEK, A. 1973. The fine structure of the contractile apparatus of the anterior byssus retractor muscle of *Mytilus edulis*. *J. Ultrastruct. Res.* **43**:313-343.
30. SQUIRE, J. M. 1971. General model for the structure of all myosin-containing filaments. *Nature (Lond.)*. **223**:457-462.
31. SQUIRE, J. M. 1973. General model of myosin filament structure. III. Molecular packing arrangements in myosin filaments. *J. Mol. Biol.* **77**:291-323.
32. SZENT-GYÖRGYI, A. G., C. COHEN, and J. KENDRICK-JONES. 1971. Paramyosin and the filaments of molluscan "catch" muscles. II. Native filaments: isolation and characterization. *J. Mol. Biol.* **56**:239-258.
33. TWAROG, B. M., M. M. DEWEY, and T. HIDAKA. 1973. The structure of *Mytilus* smooth muscle and the electrical constants of the resting muscle. *J. Gen. Physiol.* **61**:207-221.
34. WALCOTT, B., and M. BURROWS. 1969. The ultrastructure and physiology of the abdominal air-guide retractor muscles in the giant waterbug, *Lethocerus*. *J. Insect Physiol.* **15**:1855-1872.