

# The Myofilament Lattice: Studies on Isolated Fibers

## III. *The effect of myofilament spacing upon tension*

ERNEST W. APRIL and PHILIP W. BRANDT

From the Department of Anatomy, College of Physicians & Surgeons of Columbia University,  
New York 10032

**ABSTRACT** The effects of ionic strength on the generation of tension and upon the interfilament spacing in living intact and skinned single striated muscle fibers from the walking leg of crayfish (*Orconectes*) were determined by isometric contraction studies correlated with low-angle X-ray diffraction. Sarcomere lengths were determined by light diffraction. Tensions were induced in intact fibers by caffeine in the bathing medium and by ionophoretic microinjection of calcium. Tensions were induced in skinned fibers by a buffered calcium-EGTA solution. The interfilament spacing of intact and skinned fibers over the range of ionic strengths investigated were determined by X-ray diffraction and correlated with the physiological data. It is demonstrated that the ionic strength affects the tension-generating capacity of the muscle as it affects the chemo-mechanical transform of excitation-contraction coupling. It is further demonstrated that interfilament spacing changes encountered during shortening and with variation in the osmotic strength have no effect upon the tension-generating capacity of muscle.

### INTRODUCTION

According to the sliding filament model for the shortening of striated muscle (35, 41, 48), active tension (at lengths greater than rest length) is directly proportional to the number of thick filament active sites (cross bridges) which are overlapped by thin filaments. Gordon and coworkers (32-34) demonstrated that the shape of the entire length-tension curve for active muscle could be precisely explained in terms of the amount of myofilament overlap.

As muscle changes length, the myofilament lattice remains isovolumic (1, 5, 15, 25-27, 43, 44, 46), thus the spacing between the myofilaments varies as a function of the square root of the sarcomere length. Several investigators have questioned the role of interfilament distance on the generation of muscle

tension (22–25, 31, 46, 50, 54), as a factor in the determination of the shape of the length-tension diagram (22).

Interfilament spacing can also be varied by osmotic means (1, 6, 16, 49, 50, 63, 70). Edman and Anderson (22) presented evidence based upon experiments accomplished under various osmotic conditions which suggest that variation in the interfilament spacing may not alter the ability of muscle to generate tension.

In addition to the relation with sarcomere length, muscle tension has been demonstrated to be a function of the tonicity of the medium. Numerous investigators (22, 30, 31, 39, 40, 52, among others) have demonstrated that muscle behaves as an osmometer in hypertonic and hypotonic solutions, and that tensions are diminished and enhanced in the respective solutions. It has been suggested (22, 40, 57) and recently demonstrated (7, 31) that it is the change in the internal ionic strength which alters the tension-generating capacity of muscle. Perry (56) and Weber and Herz (69) presented evidence which suggested that myosin ATPase activity is a function of the ionic strength of the medium. Eisenberg and Moos (23) demonstrated that ionic strength affects the dissociation constant of the acto-heavy meromyosin complex, but not the Michaelis constant of the acto-heavy meromyosin ATPase. These *in vivo* and *in vitro* ionic strength effects may be related.

It was reported by Rome (63) that ionic strength can alter myofilament spacing within glycerinated frog muscle. It has since been demonstrated, however, that ionic strength per se has no discernible effect upon myofilament spacing in living single intact fibers of crayfish muscle (5, 6). It also has been shown that the myofilament spacing of the skinned crayfish fiber is only slightly affected by halving or doubling the ionic strength alone. However, myofilament spacing in intact single fibers has been demonstrated to be a function of the osmolarity of the medium (6).

By correlating the effects of tonicity upon the tension-generating capacity with the osmotic behavior of the myofilament lattice, this paper presents evidence that the tension-generating capacity of muscle is independent of myofilament separation. Brief accounts of these results have appeared (2, 8, 9).

#### MATERIALS AND METHODS

The preparation of single muscle fibers from the meropodite of the walking leg of crayfish (*Orconectes*) as well as a description of some of the apparatus and electron microscope procedures have been described in detail in a previous paper (5).

Single fibers were dissected in a modified van Harreveld crustacean saline solution in a Plexiglas chamber fitted with glass sides. The chamber was mounted in an optical diffractometer and the distal tendon was attached to a strain transducer (Model FT.03C, Grass Instrument Co., Quincy, Mass.) which was adjustable through a micrometer mechanism to provide various amounts of stretch to the fiber. Isometric tensions were recorded on an ink pen polygraph (Model 7, Grass Instrument Co., Quincy, Mass.). The balanced recorder was calibrated with a 1000 mg weight after

the muscle fiber had been attached to the strain transducer. Tensions were elicited by perfusing 25 ml of experimental solutions, containing 10 mM caffeine, through the chamber at a flow rate of 1 ml/s. The capacity of the chamber was approximately 2 ml, thus a rapid and complete turnover of solution within a few seconds was ensured. The caffeine-evoked contractures were of the same magnitude as those evoked by a potassium depolarization and were reproducible with less than 5% variation, provided that the muscle had been bathed in caffeine-free solution for 20 min. Electron microscope observations (P. W. Brandt, unpublished observations) indicate that this concentration of caffeine results in no apparent damage to the membranes of muscle fibers fixed either during the peak of tension or after 2 h of continuous exposure to caffeine. Caffeine was chosen as the preferred activator because it by-passes the electrically mediated processes of excitation-contraction coupling.

*A. Length-Tension Relation in Response to Caffeine under Conditions of Varied Osmolarity*

The length-tension experiments were conducted in two ways. In both, the fibers were initially adjusted to a selected short sarcomere length (slack length). In one procedure the response of the fiber to caffeine in a particular medium was measured over a series of increasing sarcomere lengths. In the second method the procedure involved the alternation of a caffeine tension in the control solution with a caffeine tension in either a hypertonic or hypotonic solution before the sarcomere length was changed. In the latter procedure each fiber served as its own control.

Fibers near the maximum length frequently broke when subjected to caffeine tensions especially in hypotonic solutions in which the tensions were potentiated. Return to rest length was often impractical due to hysteresis effects (58) and such return along the length-tension curve usually required two consecutive caffeine contractures to obtain the maximum tension at the new shorter sarcomere length.

The amount of muscle stretch was either 0.2 or 0.4 mm, representing increases of approximately 5 and 10% of the muscle length, respectively. If the measurements of sarcomere lengths differed by 10% or more from the proximal to the distal end of the fiber, the experiment was terminated and the results were not used.

The experiments were conducted using the solutions summarized below:

- (a) Control (190 mM NaCl, 13.5 mM CaCl<sub>2</sub>, 10 mM sucrose or caffeine, 5 mM KCl, 2.5 mM NaHCO<sub>3</sub>, pH 7.6).
- (b) Hyposmotic (hypotonic) to control by deletion of 95 mM NaCl.
- (c) Hyposmotic (hypotonic) to control by deletion of 47.5 mM NaCl.
- (d) Hyperosmotic (hypertonic) to control by addition of 190 mM NaCl.
- (e) Isosmotic (hypotonic) to control by substitution of 47.5 mM KCl for an equivalent of NaCl.
- (f) Isosmotic (hypotonic) to control by substitution of 95 mM KCl for an equivalent of NaCl.

*B. Tension Determination in Intact and Skinned Single Fibers under Conditions of Varied Osmolarity*

The experiments designed to determine the effect of ionic strength upon tension in the intact and skinned fibers were undertaken near the rest length. The sarcomere

length was determined by light diffraction and was not further changed. Tensions were evoked with 10 mM caffeine in control saline solutions. After relaxation the fibers were washed with one of a series of hyposmotic solutions (containing 130 mM NaCl, 150 mM NaCl, 170 mM NaCl), equilibrated for 30 min at each step and the tensions evoked by caffeine. The same procedure was continued through a series of hyperosmotic solutions (containing 270 mM NaCl, 350 mM NaCl, 430 mM NaCl, 510 mM NaCl, 570 mM NaCl, 650 mM NaCl) after which the control was repeated.

Single muscle fibers with the sarcolemma removed were prepared according to the procedure described by Reuben et al. (59, 60). After dissection in control saline solution the fibers were equilibrated for 30 min in a solution containing 200 mM potassium propionate, 5 mM MgCl<sub>2</sub>, 9 mM K<sub>2</sub>-EGTA, 1 mM calcium propionate, 1 mM ATP and buffered to pH 7 with 20 mM Tris. The sarcolemma was then stripped back along most of the fiber length and the fiber attached to the strain transducer. The ionic strength was varied by adjusting the concentration of potassium propionate in the medium. In another series of experiments, increasing concentrations of sucrose were added to the media with constant potassium propionate concentrations. The skinned muscle fibers were photographed through a microscope to monitor the volume of the fiber during the experimental procedures. Tensions were evoked in the skinned fibers by replacing the bathing medium with one containing 9 mM calcium propionate and 10 mM K<sub>2</sub>-EGTA, thereby providing a pCa of 5.7 (59). The activating solution was washed out of the chamber when the tension had reached a maximum.

## RESULTS

### A. *The Length-Tension Relation as a Function of Osmolarity*

The relation between sarcomere length and tension for six single muscle fibers from crayfish is presented in Fig. 1. The tension generated by single fibers in response to caffeine (10 mM) in control saline solution increases as the fiber is stretched from a shortened sarcomere length of 6.5  $\mu\text{m}$  to approximately 7.0  $\mu\text{m}$  where the tension response reaches a plateau of maximum tension. No further change in the magnitude of tension is reached until the fiber is stretched to a sarcomere length of approximately 8.2  $\mu\text{m}$ . The sarcomere lengths which define the plateau of maximum tension are called the upper and lower limits of optimum tension (34), thereby circumventing the often ambiguous term "rest length." From this point tension response to caffeine stimulation diminishes as the sarcomere length is further increased. Tension is about 10% of maximum at a sarcomere length of 13.0  $\mu\text{m}$  and by extrapolation it appears that the tension would be zero at approximately 13.5  $\mu\text{m}$ .

Similar results were obtained when reversal along the length-tension curve was accomplished or when the length-tension curve was obtained by commencing with long sarcomere lengths.

In order to determine the effects of myofilament spacing upon the generation of tension, fiber volumes and hence myofilament lattice volumes were

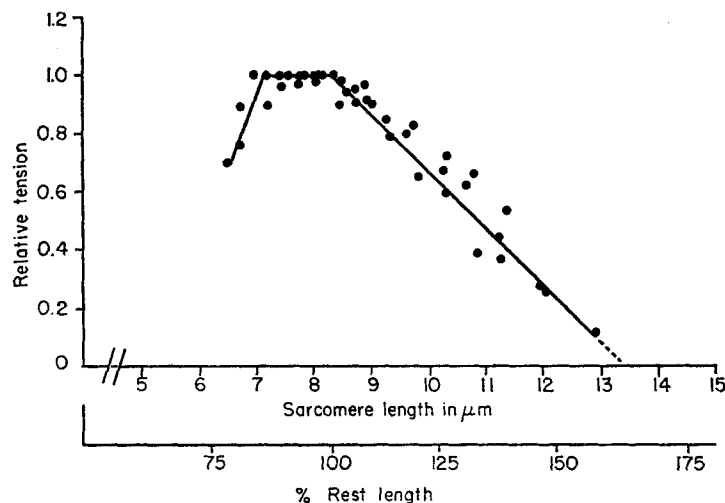


FIGURE 1. Control length-tension relation. The percentage of maximum tension (relative tension) generated by six single crayfish muscle fibers is plotted against the relative and actual sarcomere length. The upper limit of optimum length ("rest length") is at approximately  $8.2 \mu\text{m}$  and the lower limit of optimum length ("slack length") is at approximately  $7.0 \mu\text{m}$ . The solid lines are fitted to the experimental points by the method of least squares. Caffeine stimulation.

adjusted osmotically. It has been previously demonstrated (6) that the volume of the myofilament lattice is a function of the volume of the whole fiber. The conditions of the fiber and myofilament lattice under the various osmotic conditions are summarized in Table I. Length-tension experiments in a medium made hyperosmotic by addition of 190 mM NaCl to the control medium result in a length-tension curve similar to the control, but with diminished amplitude. The maximum tension obtained is 70% of the estimated maximum tension projected from the initial point elicited in control saline solution (Fig. 2). The locations of the points of change in the slopes of the experimental length-tension curve are not different from that of the control curve.

Fig. 3 *a* compares three length-tension curves which were obtained from four self-controlled experiments. In two experiments, single fibers were alternately bathed in hyposmotic (solid circles) and isosmotic (open circles) solutions, while in two other self-controlled experiments, fibers were bathed in hyperosmotic (solid squares) and isosmotic (open squares) solutions. Tension in the hyposmotic conditions (made by the deletion of 95 mM NaCl from the medium) is an average 29% greater than the control (upper curve in Fig. 3 *a*). The shape and dimensions of the hyposmotic curve are not as precise as the control and hyperosmotic curves because single fibers frequently broke under the potentiated contractures of the hyposmotic conditions. In the self-controlled experiments, tension in hyperosmotic media is an average of 22% below the control tension levels (lower curve in Fig. 3 *a*). Otherwise,

TABLE I  
RELATION BETWEEN FIBER VOLUME AND LATTICE VOLUME

	Time	Relative volume	
		Fiber	Lattice
	<i>min</i>		
1. Control	30	No change	No change
2. Hyperosmotic NaCl	30	Decrease	Decrease
3. Hyposmotic NaCl	30	Increase	Increase
4. Hyperosmotic KCl	4	Decrease	Decrease
5. Hyperosmotic KCl	30	Return	Return
6. Isosmotic (hypotonic) KCl	30	Increase	Increase

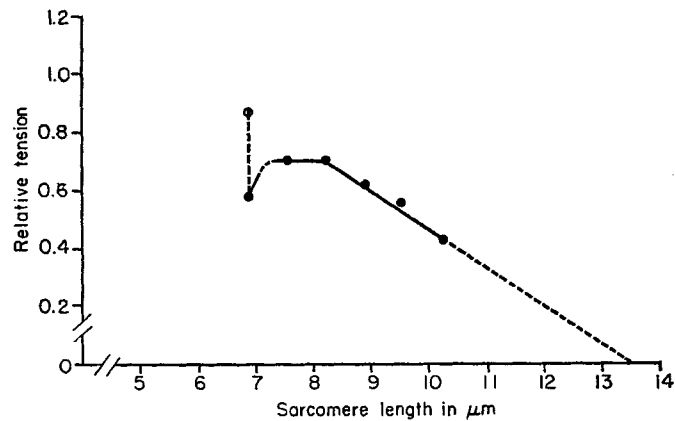


FIGURE 2. Hyperosmotic length-tension relation. The relative tension in a single muscle fiber bathed in a medium made hyperosmotic by addition of 190 mM NaCl is plotted against the sarcomere length. The first point (open circle) is the tension obtained in control saline before the medium was made hyperosmotic. This point provides a control point from which the maximum control tension may be projected. Caffeine stimulation

the shape of the curve and the locations of the changes in slopes in hypertonic media are similar to those of the control curve with no indication of any lateral shift of the upper or lower limits of optimum tension. The hyperosmotic, control, and hyposmotic curves of this series of experiments each extrapolate to zero tension in the vicinity of 14.6  $\mu\text{m}$ . The intercept of the abscissa in Fig. 3 *a* is higher than in Fig. 1. The difference is probably due in part to the extremely prolonged experimental procedures and the frequent changes in volume which may have resulted in increased skewing of the sarcomeres at the longer sarcomere lengths.

#### B. Length-Tension Relation as a Function of Tonicity

The results of five experiments in which single muscle fibers were swollen in hypotonic, but isosmotic, KCl solutions are presented in Fig. 4. Little or no

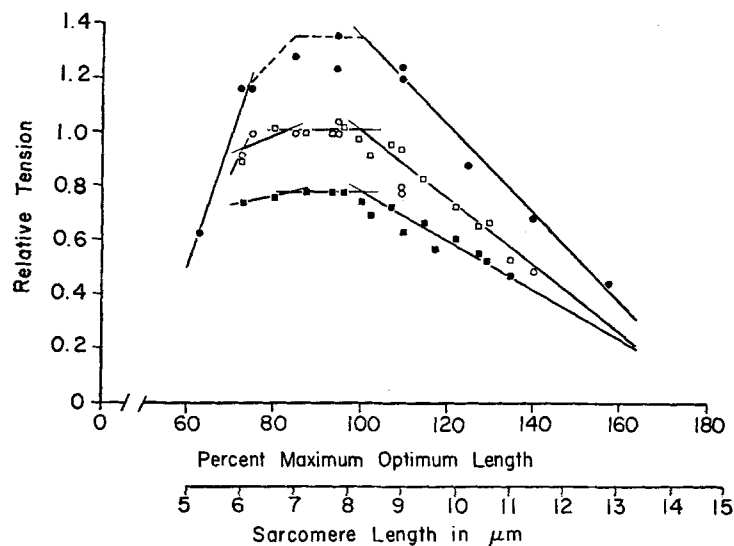


FIGURE 3a

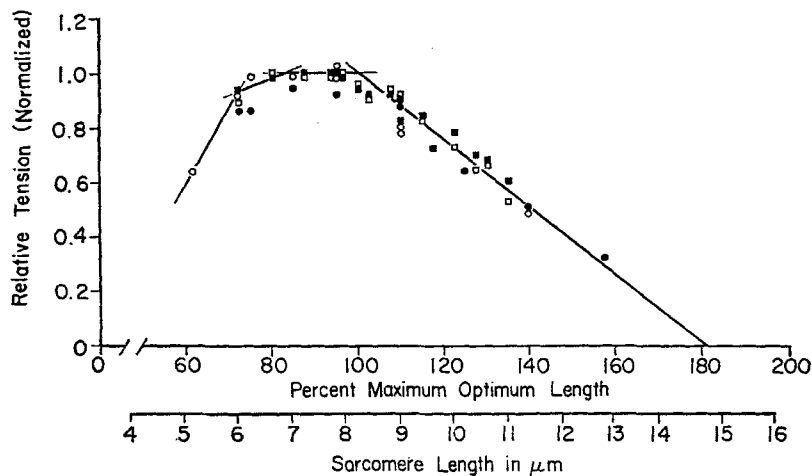


FIGURE 3b

FIGURE 3. (a) Caffeine induced length-tension relations in crayfish single muscle fibers under conditions of varied osmolarity. The tension relative to maximum control tension in hyposmotic (100 mM NaCl) media (solid circles) and in isosmotic (190 mM NaCl) media (open circles) obtained from two self-controlled experiments and the tension relative to maximum control tension in hyperosmotic (380 mM NaCl) media (solid squares) and in isosmotic media (open squares) from two other self-controlled experiments is plotted against the sarcomere length. The solid lines (except those representing the plateaus of maximum tension) are fitted by the method of least squares while the interrupted line suggests a possible shape for the hyposmotic curve where less data could be obtained. (b) Caffeine induced length-tension relations in crayfish single muscle fibers under conditions of varied osmolarity. Data from (a) normalized to maximum tension under each experimental condition. The lines (except for the plateau of maximum tension) are fitted by the method of least squares. Changes in the slopes of the length-tension curve occur at 5.9, 6.8, 8.2, and 14.6  $\mu\text{m}$ .

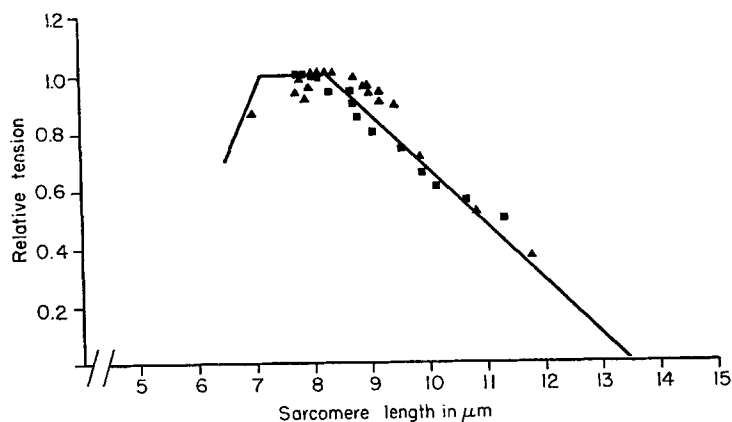


FIGURE 4. Length-tension relation under conditions of constant intracellular ionic strength, but increased muscle volume. The relative caffeine-induced tension of three swollen single muscle fibers and two swollen single muscle fibers bathed in respective solutions modified by the substitution of 47.5 mM KCl (triangles) and 95 mM KCl (squares) for equivalent amounts of NaCl is plotted against the sarcomere length. The solid line represents the control length-tension obtained by applying the least squares method to the results (not plotted) of six single control fibers. The slight variation between sets of data is not of statistical significance ( $P > 0.1$ ).

TABLE II  
RELATION BETWEEN IONIC STRENGTH AND TENSION

	Equilibrium ionic strength (estimated)	Equilibrium tension
1. Hyperosmotic NaCl	Increase	Decrease
2. Hyposmotic NaCl	Decrease	Increase
3. Hyperosmotic KCl	Increase	Decrease
4. Isosmotic (hypotonic) KCl	No change	No change

change in the intracellular ionic strength is anticipated under conditions in which the KCl concentration is altered isosmotically (13). In these experiments control tensions were measured before the volumes of the fibers were adjusted. In order to compare the results from numerous fibers of different sizes, initial tensions in control medium were normalized to that percentage of maximum tension which is expected at the particular sarcomere length. Three experiments were conducted in a saline solution in which there was a 25% replacement of the NaCl by an equivalent amount of KCl (triangles). The slope of that portion of the length-tension diagram beyond the upper limit of optimum length is  $-18.93$ . Two experiments were conducted in a medium in which there was a 50% replacement of the NaCl by KCl (squares). The slope of the same portion of the length-tension diagram in this instance is  $-16.60$ . The control slope is  $-19.16$  and the slope of all of the grouped



experimental points for this portion of the curve is  $-18.69 (\pm 1.4 \text{ SD})$ . The individual slopes of all of the experimental data in this series of experiments were subjected to analysis of variance which indicated that there is no significant difference between any of the slopes ( $P > 0.1$ ). In these experiments the tension in response to caffeine stimulation is not changed even though the fiber and the myofilament lattice are swollen by as much as 130% of the control. Thus, increasing the fiber volume and the lattice volume at constant ionic strength is accompanied by neither a significant change in the tension nor a change in the shape of the curve. The relationship between tension and ionic strength is summarized in Table II which can be compared to the volume changes summarized in Table I.

C. *Tension Response of Single Fibers to Caffeine and Calcium under Conditions of Varied Osmolarity*

The relative tension produced by caffeine stimulation in intact single fibers is a function of the tonicity of the medium. The relative caffeine-induced

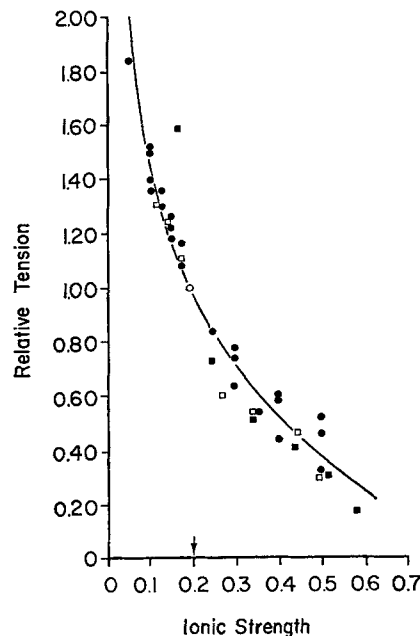


FIGURE 5. Tension as a function of ionic strength. The relative tensions induced in the intact crayfish fiber by calcium microinjection ( $2 \times 10^{-4} \text{ M/s}$ ; solid squares, replotted from April et al., 7) and by caffeine (10 mM; open squares) and in three skinned fibers by a buffered Ca-EGTA solution (pCa 5.7; solid circles) are plotted against the ionic strength of the medium which was controlled by the NaCl concentration for the intact fibers and by the potassium propionate concentration for the skinned fibers. The open circle represents the control point for all of the experiments. The line is fitted by eye. Regardless of the differences in myofilament spacing (cf. Fig. 6) the tensions remain similar.

tensions are plotted (open squares) against the ionic strength of the medium in Fig. 5. The relative tensions resultant from caffeine stimulation are enhanced in media made hypotonic by deletion of NaCl and tensions progressively diminish as the solution is made hypertonic by addition of NaCl. Tension is abolished at 3–3½ times the control NaCl concentration.

In four experiments, single muscle fibers with the sarcolemma removed were bathed in media of different ionic strengths. The relative tensions induced by raising the pCa of the medium to 5.7 are plotted in Fig. 5 (solid circles) against the ionic strength of the medium. The relative tension in the skinned fiber in response to calcium in the bathing media is nearly identical, over the range of ionic strength studied, to the response in the intact fiber induced by caffeine. Both curves are nearly identical to that obtained by ionophoretic microinjection of calcium (solid squares, Fig. 5, replotted from April et al., 7). In all of these experiments the relative tensions are enhanced or diminished in media which result in respectively lower or higher intracellular ionic strengths.

#### DISCUSSION

##### *The Effect of Tonicity on Tension and the Length-Tension Relation*

Changes in the composition of the bathing medium affect the threshold and mechanical response of the muscle fiber to electrical stimulation (18, 31). Therefore, to obtain reliable tension in single muscle fibers, methods were selected which were independent of the electrical excitation process. Either caffeine was used as a stimulus or, after the sarcolemma had been removed, calcium was applied directly. In previous studies, calcium was microinjected ionophoretically into the myoplasm of single crayfish fibers (7).

Caffeine is capable of activating the contractile mechanism even when the membrane is depolarized (10, 19–21) or when the fiber is in hypertonic solutions (18). Korey (51) and Hasselbach (36) have shown that caffeine has no effect upon the actomyosin system. Bianchi (11), employing radiokinetic techniques, demonstrated that caffeine quickly penetrates the muscle fiber and Caldwell and Walster (17) evoked tension by microinjection of the alkaloid. Herz and Weber (37) and Weber (68) suggest that caffeine causes an increase in the cytoplasmic calcium concentrations through inhibition of calcium uptake by the sarcoplasmic reticulum. Since a multitude of studies indicate that calcium is the final contractile activator (12, 64), it appears that caffeine either mimics or initiates the final step of the electrical excitation process, i.e., the increase of cytoplasmic calcium concentration (64).

When single muscle fibers from the crayfish are suspended at approximately maximum optimum length in media of various tonicities, caffeine-evoked contractures and those evoked in the skinned fiber by changing the

pCa from 8 to 5.7 are potentiated in hyposmotic media while diminished in hyperosmotic media (cf. Fig. 5). Both in these and in our previous experiments (7) tensions are not detectable when the NaCl concentration of the medium is increased by 3 times to 600 mM.

The slight variation in data in these three types of experiments can be attributed to the different techniques used. One major difference between the whole fiber stimulation involving caffeine and calcium from that of the ionophoretic microinjections of calcium previously reported (7) is related to the uniformity with which the contraction occurs along the muscle fiber. Caffeine stimulation in the intact fiber and calcium stimulation in the skinned fiber under isometric conditions involves the whole length of the fiber. However, it is possible that there may be an alternating, out of phase shortening and stretching of the sarcomeres. In the instance of localized ionophoretic microinjection of calcium, the sarcomeres within the contraction spheres appear very uniform with a supercontracted sarcomere length of about  $3\ \mu\text{m}$  (7, 14). Even though the fiber is held at constant length, there is extreme shortening of sarcomeres in the stimulated volume with a concomitant stretching of sarcomeres in the nonstimulated volume of the muscle. Yet, the relative tension curve is similar to those obtained by other methods.

Since the tension response curve of the intact fibers is essentially identical to that of the skinned fiber under the same conditions of ionic strength, but since the intact and skinned fibers have markedly different lattice spacings, it is clear that the tension-generating capacity of muscle is strongly dependent upon the ionic strength of the medium and not the fiber volume per se.

It has been demonstrated that muscle volume is a function of the osmotic pressure of the medium (13, 53) and that this relation held for single muscle fibers of the frog (62) and crayfish single fibers (61). The results discussed thus far and summarized in Table II as well as those presented in a previous paper (7) indicate that, at constant sarcomere length, tension in the intact crayfish fiber is a function of the volume-associated changes in intracellular ionic strength and that in the skinned fiber tension is directly dependent upon ionic strength.

The results obtained in crayfish fibers with caffeine stimulation are in conflict with those reported by Caputo (18) who found that hypertonic solutions ( $2\times$ ) potentiate the caffeine contracture in frog single fibers. Podolsky and Sugi (57), however, show a 10-fold reduction in the rate of shortening for frog fibers activated with caffeine in a solution which was hypertonic ( $3\times$ ). Since the osmotic pressure of the frog physiological saline solution is one-half that of crayfish physiological saline solution, this may account for the incomplete blockage of tension in the frog at tonicities of twice or three times the control. The results discussed in this report for intact crayfish fibers by caffeine stimulation are in substantial agreement with the results obtained by calcium

microinjection and by direct stimulation from calcium in the skinned fiber. The minor differences between the crayfish and frog may be due to species differences.

The basic shape of the length-tension curves as explained by the sliding filament model for muscle shortening would not be expected to vary with interfilament spacing (except in magnitude as a function of ionic concentration) if filament overlap were the sole parameter determining the amount of tension. Since the myofilament lattice shortens isovolumically with the interfilament spacing varying inversely as the square root of the sarcomere length, the possible effect of interfilament spacing upon tension must be considered. When crayfish striated muscle shortens from 8.2 to 5.7  $\mu\text{m}$  (the apparent physiological range), the center to center spacing between the thick myofilament increases by a factor of 1.12 and the thick to thin myofilament spacing increases by the same factor (5). The interfilament distance increases by a factor of 1.89 over the entire range of shortening (11.2–3  $\mu\text{m}$ ). The distance over which the contractile forces between the thick and thin filaments must operate increases as the square root of the amount of shortening. It was conceivable that this change in interfilament spacing might alter the amount of tension which is capable of being generated at a specific sarcomere length.

The filaments not only increase their lateral spacing during normal shortening, but filament spacing of crayfish myofibrils can be made to vary by osmotic means as well (5, 6, 8, 9). A fiber with a control thick filament spacing of 548  $\text{\AA}$  at a sarcomere length of 9.6  $\mu\text{m}$  corresponds to 596  $\text{\AA}$  in  $\frac{1}{2}\times$  hypotonic solution and 486  $\text{\AA}$  in  $2\times$  hypertonic media at the same sarcomere length. These two interfilament distances are equivalent, respectively, to shortening a control fiber to 8.05  $\mu\text{m}$  and stretching the fiber to 12.1  $\mu\text{m}$  in the control saline solution. If change in lateral interfilament spacing, due to swelling or shrinking, affects the tension-generating capacity of the muscle, an alteration in the magnitude or a skewing of the shape of the length-tension curve would be evident. This follows because the lattice spacing of the shrunken fiber, for example, is equivalent to that of a control fiber which has been stretched to a longer sarcomere length.

The effects of volume changes on tension were studied over a series of different sarcomere lengths (7, 8). From the length tension diagrams for single fibers bathed in hypertonic media (cf. Fig. 2) it would appear that, at various sarcomere lengths, tension might be indirectly dependent upon the volume of the muscle fiber. However, the locations of the points of change in the slopes of the length-tension diagram remain the same (cf. Fig. 3 *a*). When the tensions of the experimental curves are normalized to the maximum tension of the control and the relative data are plotted together, there is no significant difference among the curves (cf. Fig. 3 *b*). These results are in agreement with the report by Edman and Anderson (22) that electrical stimulation of

shrunk or swollen single fibers from the frog provide length-tension curves which are nearly identical when plotted as superimposed relative tensions.

Length-tension curves were also determined in solutions in which various amounts of KCl were substituted for NaCl (cf. Fig. 4). There is no significant change in the amount of absolute tension or in the shape of the length-tension curve when fibers are swollen in this isosmotic, but hypotonic, KCl solution (6, 7). This is what might be expected because there is no change in the intercellular ionic strength (13) even though the fiber swells considerably. If, however, a change in interfilament spacing were to affect the tension, then a change in the shape or magnitude of the length-tension diagram would have been predicted. Analysis of variance of the data from these experiments indicates that there is no change in the tension and no significant change in the shape of the length-tension curves even though the amount of swelling surpassed (by 12%) that of the hypotonic NaCl experiments.

In all of the length-tension experiments, the superposition of the relative curves indicates that the sarcomere length (filament overlap) parameter alone determines the shape of the length-tension curve and that the shape and magnitude of the curve is independent of the distance between the filaments over which the contractile forces are presumed to operate. The shape of the length-tension curve evidently bears very little relation to the actual width of the myofilament lattice.

While it has been demonstrated that ionic strength affects maximum tension response ( $P_0$ ), the mechanism of action is not clear. Supposedly,  $P_0$  represents a state of contraction in which the statistical maximal number of cross bridges capable of interacting are so doing (34, 45). Ionic strength has been reported to affect actomyosin ATPase activity (56, 69). It has been demonstrated, however, by Eisenberg and Moos (23) that ionic strength affects specifically the rate of dissociation of heavy meromyosin from actin, not the acto-heavy meromyosin ATPase activity. A similar ionic strength dependency upon the binding of heavy meromyosin to actin would be expected (23). While it is quite plausible that there may be a relation between the dissociation constant of actin-heavy meromyosin and the tension output, there appears to be no model for contraction which relates these factors as to cause and effect. It appears unlikely that decreasing ionic strength would increase the number of functional cross bridges, unmask additional active sites on the actin filaments or change the force generated by a single cross bridge. One might speculate, however, that an increased rate of actomyosin association in hypotonic solutions could result in a net decrease in the time necessary for free cross-bridge attachment. If no other change occurs (if duration of cross-bridge attachment remains the same), there could be a statistically greater number of cross-bridges engaged at any instant and, therefore, greater  $P_0$ .

*Correlation between Myofilament Spacing and Tension*

Tension in fibers under conditions of various ionic strengths (cf. Fig. 5) can be correlated with X-ray diffraction measurements of interfilament separation under the same experimental conditions (cf. Fig. 6). This X-ray diffraction

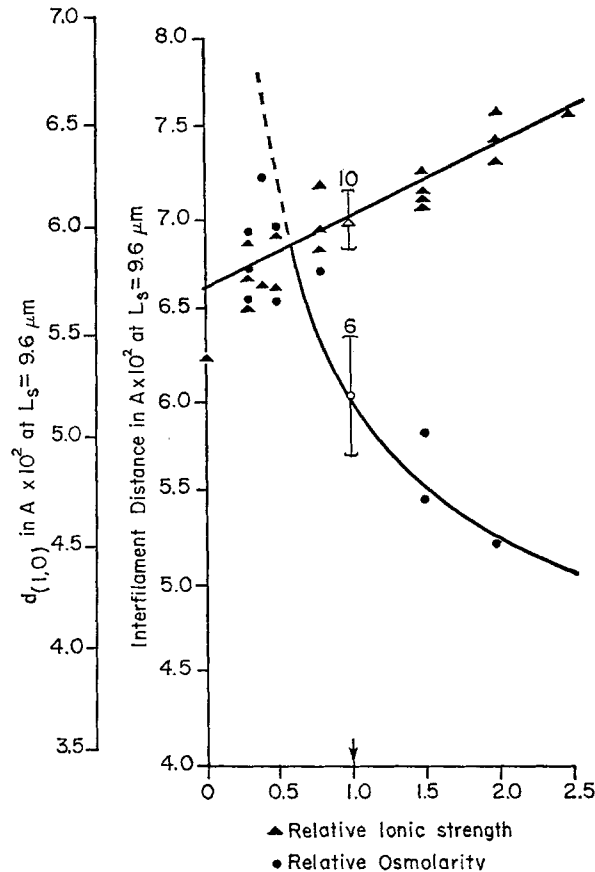


FIGURE 6. Interfilament spacing in crayfish as a function of osmolarity and of ionic strength. The lattice spacing and the interfilament spacing determined by X-ray diffraction for living single fibers (circles) and living fibers from which the sarcolemma has been removed (triangles) are plotted against the same conditions expressed, respectively, as the relative osmolarity and the relative ionic strength of the medium. The curve for the intact fibers is calculated from the Boyle-van't Hoff equation, while that for the skinned fibers is fitted by the method of least squares. The open symbols represent mean values, with  $n$  and the standard deviation from the mean indicated.

data for intact and skinned single fibers in potassium propionate media has been presented in a previous paper (6). It was demonstrated that the myofilament lattice of the intact fiber reflects the osmotic behavior of the whole fiber (Table I) and that the interfilament spacing can be predicted from the Boyle-

van't Hoff relation. After removal of the sarcolemma, the myofilament lattice expands to a new volume and, henceforth, responds markedly less and in the opposite direction to the same osmolar changes, expressed as ionic strength, which greatly modify the lattice volume of the intact fiber. At twice the control ionic concentration the difference in interfilament spacing between the intact and skinned fiber is 222 Å (cf. Fig. 5). Using this method to obtain markedly different myofilament lattice volumes at the same values of ionic strength, the maximum tensions produced by various means under these conditions can be compared.

The absolute values of the tension induced directly by calcium in the skinned fibers is diminished (indicating that the ionic strength within the lattice of the intact fiber may be lower than usually accepted). However, the shape of the tension curve is not significantly different from the relative tension curve produced in the intact single fibers with caffeine or calcium microinjection over the same range of conditions (cf. Fig. 5). It is to be emphasized that at high ionic strengths the myofilament spacing is very much greater in the skinned fiber than in the intact fiber, while at lower ionic strengths the spacing is similar or identical. If tension were a function of the interfilament separation it would be expected that the tension curve of the skinned fiber and that of the intact fiber should diverge as the osmolarity (ionic strength) of the medium is increased. From the data presented in Fig. 6, as the ionic strength or osmolarity of the medium is increased, tension in the skinned fiber might be expected to decrease as a function of the distance between the myofilaments at a much greater rate than tension in the intact fiber because in the former the myofilaments move further apart while in the latter they move closer together. This, obviously, is not the case because it is demonstrated in Fig. 5 that the same amount of relative tension is capable of being generated irrespective of whether the thick filaments are 500 or 750 Å apart. Electron microscopy of fixed muscle and X-ray diffraction evidence from relaxed and contractive muscle indicate that the thin filaments maintain their relative positions in the unit cell of the myofilament lattice. The thin filaments always appear to be equidistant from the nearest thick filaments whether the fiber is shortened or stretched, swollen or shrunken. Thus the range of thick filament spacing just mentioned corresponds to thin-to-thick filament spacing of from 260 to 390 Å in the 6:1 lattice of the crayfish leg muscle. It is evident, therefore, that the tension-generating capacity of muscle is not dependent upon interfilament separation.

#### *Concluding Remarks*

The independence of myofilament separation and tension-generating capacity is of interest in view of the constraints placed upon the various models of muscle contraction. While it is obvious that during shortening the interdigitating

filaments slide past each other, it is not obvious why the tension is unaffected by varying distance between the filaments. With respect to any of the proposed electrostatic (24, 28, 65–67, 71) and electrostatic-hydraulic (29) models of contraction, it stands to reason that tension should vary as an inverse function of the interfilament separation because the electrostatic forces fall off as the 6th power of the distance (24). Consequently, while electrostatic and van der Waal's forces are certainly very important in the maintenance of filament separation and alignment (3, 24, 28), the findings presented herein do not appear to be particularly compatible with a shortening model based upon changes in the strength of the electrostatic repulsive forces between the filaments.

The other plausible alternative appears to involve models which propose a direct chemo-mechanical interaction between the thin filament and the cross bridge of the thick filament. The cross bridges must be flexible or extensible because the contact between the filaments must be maintained over a rather wide range of distances. Pepe (54, 55) has proposed a mechanism for the maintenance of bridge contact. Experiments using antibody-staining techniques in frog muscle lead him to conclude that as the lattice expands, part of the light meromyosin component of the myosin molecule bends away from the myosin filament to maintain contact with the thin filament active site. This concept has received some support from X-ray diffraction evidence (47) and has been modified and expanded by H. E. Huxley (46), Hill (38), and A. F. Huxley and Simmons (42). The results presented herein are compatible with shortening models which propose chemo-mechanical interaction of flexible cross bridges between thick and thin filaments.

While our previous papers in this series (5, 6) support a hypothesis for the liquid-crystalline nature of the resting myofilament lattice and this paper has discussed the relationship between myofilament spacing and tension, little attempt has been made herein to correlate these phenomena. Subsequent papers (3, 4) will deal with some physical-chemical aspects of the myofilament lattice and the role of electrostatic forces in normal muscle function.

We wish to thank Mr. Robert Demarest for assistance with the graphics and Doctors Gerald F. Elliott, Harry Grundfest, and John P. Reuben for their valuable discussions. This work was supported in part by National Institutes of Health grants (NB-03728, NB-05328, NS-05910, and GM-00256). Dr. April is also grateful to the Grass Foundation supporting a portion of this work at the Marine Biology Laboratory, Woods Hole, Mass.

*Received for publication 31 May 1972.*

#### BIBLIOGRAPHY

1. APRIL, E. W. 1969. The effects of sarcomere length and tonicity on the myofilament lattice. Ph.D. Thesis. Columbia University, New York.
2. APRIL, E. W. 1971. The effect of myofilament spacing upon tension. *J. Gen. Physiol.* 57:242.



3. APRIL, E. W. 1973. The myofilament lattice. In *Muscle Research*. A. Stracher, editor. Plenum Publishing Corporation, New York.
4. APRIL, E. W. 1973. The non-equilibrium nature of the myofilament lattice of living muscle. *Biophys. Soc. Annu. Meet. Abstr.* **13**:179a.
5. APRIL, E. W., P. W. BRANDT, and G. F. ELLIOTT. 1971. The myofilament lattice: studies on isolated fibers. I. The constancy of the unit-cell volume with variation in sarcomere length in a lattice in which the thin-to-thick myofilament ratio is 6:1. *J. Cell Biol.* **51**:72.
6. APRIL, E. W., P. W. BRANDT, and G. F. ELLIOTT. 1972. The myofilament lattice: studies on isolated fibers. II. The effects of osmotic strength, ionic concentration, and pH upon the unit-cell volume. *J. Cell Biol.* **53**:53.
7. APRIL, E. W., P. W. BRANDT, J. P. REUBEN, and H. GRUNDFEST. 1968. Muscle contraction: the effect of ionic strength. *Nature (Lond.)*. **220**:182.
8. APRIL, E. W., and J. P. REUBEN. 1968. Length-tension relation and intracellular ionic strength in crayfish muscle fibers. *Fed. Proc.* **27**:375.
9. APRIL, E. W., J. P. REUBEN, and P. W. BRANDT. 1968. The effects of tonicity on the length-tension relation in crayfish muscle fibers. *Anat. Rec.* **160**:308.
10. AXELSSON, J., and S. THESLEFF. 1958. Activation of the contractile mechanism in striated muscle. *Acta Physiol. Scand.* **44**:55.
11. BIANCHI, C. P. 1962. Kinetics of radiocaffeine uptake and release in frog sartorius. *J. Pharmacol. Exp. Ther.* **138**:41.
12. BIANCHI, C. P. 1968. *Cell Calcium*. Appleton-Century-Crofts, New York.
13. BOYLE, P. J., and E. J. CONWAY. 1941. Potassium accumulation in muscle and associated changes. *J. Physiol. (Lond.)*. **100**:1.
14. BRANDT, P. W., and H. GRUNDFEST. 1968. Sarcomere and myofilament changes accompanying local contractile activation in crayfish muscle fibers. *Fed. Proc.* **27**:375.
15. BRANDT, P. W., E. LOPEZ, J. P. REUBEN, and H. GRUNDFEST. 1967. The relationship between myofilament packing density and sarcomere length in frog striated muscle. *J. Cell Biol.* **33**:255.
16. BRANDT, P. W., J. P. REUBEN, E. LOPEZ, and H. GRUNDFEST. 1964. An electron microscopic study of the effect of osmotically induced volume changes on myofilament spacing and structure. *Anat. Rec.* **148**:263.
17. CALDWELL, P. C., and G. WALSTER. 1963. Studies on the micro-injection of various substances into crab muscle fibres. *J. Physiol. (Lond.)*. **169**:353.
18. CAPUTO, C. 1966. Caffeine- and potassium-induced contractures of frog striated muscle fibers in hypertonic solutions. *J. Gen. Physiol.* **50**:129.
19. CHIARANDINI, D. J., P. W. BRANDT, and J. P. REUBEN. 1967. Drug-evoked contractions and caffeine-induced action potentials in isolated crayfish muscle fibers. *J. Gen. Physiol.* **50**:2501.
20. CHIARANDINI, D. J., J. P. REUBEN, P. W. BRANDT, and H. GRUNDFEST. 1970. Effects of caffeine on crayfish muscle fibers. I. Activation of contraction and induction of Ca spike electrogenesis. *J. Gen. Physiol.* **55**:640.
21. CHIARANDINI, D. J., J. P. REUBEN, L. GIRARDIER, G. M. KATZ, and H. GRUNDFEST. 1970. Effects of caffeine on crayfish muscle fibers. II. Refractoriness and factors influencing recovery (repriming) of contractile responses. *J. Gen. Physiol.* **55**:665.
22. EDMAN, K. A. P., and K. E. ANDERSON. 1968. The variation in active tension with sarcomere length in vertebrate skeletal muscle and its relation to fibre width. *Experientia (Basel)*. **24**:134.
23. EISENBERG, E., and C. MOOS. 1970. Actin activation of heavy meromyosin adenosine triphosphatase. *J. Biol. Chem.* **245**:2451.
24. ELLIOTT, G. F. 1968. Force-balances and stability in hexagonally-packed polyelectrolyte systems. *J. Theor. Biol.* **21**:71.
25. ELLIOTT, G. F., J. LOWY, and C. R. WORTHINGTON. 1963. An X-ray and light-diffraction study of the filament lattice of striated muscle in the living state and in rigor. *J. Mol. Biol.* **6**:295.

26. ELLIOTT, G. F., J. LOWY, and B. M. MILLMAN. 1965. X-ray diffraction from living striated muscle during contraction. *Nature (Lond.)*. **206**:1357.
27. ELLIOTT, G. F., J. LOWY, and B. M. MILLMAN. 1967. Low-angle X-ray diffraction studies of living striated muscle during contraction. *J. Mol. Biol.* **25**:31.
28. ELLIOTT, G. F., and E. M. ROME. 1969. Liquid-crystalline aspects of muscle fibers. In *Molecular Crystals and Liquid Crystals*. Gordon and Breach Science Publishers, London. **8**:215.
29. ELLIOTT, G. F., E. M. ROME, and M. SPENCER. 1970. A type of contraction hypothesis applicable to all muscles. *Nature (Lond.)*. **226**:417.
30. FENN, W. O. 1936. The role of tissue spaces in the osmotic equilibrium of frog muscles in hypotonic and hypertonic solutions. *J. Cell. Comp. Physiol.* **9**:93.
31. GORDON, A. M., and R. E. GODT. 1970. Some effects of hypertonic solutions on contraction and excitation-contraction coupling in frog skeletal muscles. *J. Gen. Physiol.* **55**:254.
32. GORDON, A. M., A. F. HUXLEY, and F. J. JULIAN. The length-tension diagram of single vertebrate striated muscle fibres. *J. Physiol. (Lond.)*. **171**:28. 30P.
33. GORDON, A. M., A. F. HUXLEY, and F. J. JULIAN. 1966. Tension development in highly stretched vertebrate muscle fibres. *J. Physiol. (Lond.)*. **184**:143.
34. GORDON, A. M., A. F. HUXLEY, and F. J. JULIAN. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol. (Lond.)*. **184**:170.
35. HANSON, J., and H. E. HUXLEY. 1955. The structural basis of contraction in striated muscle. *Symp. Soc. Exp. Biol.* **92**:228.
36. HASSELBACH, W. 1953. Giftwirkungen auf den Arbeitszyclus des Fasermodells. *Z. Naturforsch. Teil B.* **8**:212.
37. HERZ, R., and A. WEBER. 1965. Caffeine inhibition of Ca uptake by muscle reticulum. *Fed. Proc.* **24**:208.
38. HILL, T. L. 1970. Sliding filament model of muscular contraction. V. Isometric force and interfilament spacing. *J. Theor. Biol.* **29**:395.
39. HODGKIN, A. L., and P. HOROWICZ. 1957. The differential action of hypertonic solutions on the twitch and action potential of the muscle fibre. *J. Physiol. (Lond.)*. **136**:17.
40. HOWARTH, J. V. 1958. The behaviour of frog muscle in hypertonic solutions. *J. Physiol. (Lond.)*. **144**:167.
41. HUXLEY, A. F., and R. NIEDERGERKE. 1954. Structural changes in muscle during contraction. Interference microscopy of living muscle fibers. *Nature (Lond.)*. **173**:971.
42. HUXLEY, A. F., and R. M. SIMMONS. 1972. Proposed mechanism of force generation in striated muscle. *Nature (Lond.)*. **233**:533.
43. HUXLEY, H. E. 1951. *Discuss. Faraday Soc.* **11**:148.
44. HUXLEY, H. E. 1953. Electron microscope studies of the organisation of the filaments in striated muscle. *Biochim. Biophys. Acta.* **2**:387.
45. Huxley, H. 1961. The contractile structure of cardiac and skeletal muscle. *Circulation.* **24**:328.
46. Huxley, H. E. 1969. The mechanism of muscular contraction. *Science (Wash. D. C.)* **164**:1356.
47. Huxley, H. E., and W. Brown. 1967. The low-angle X-ray diagram of vertebrate striated muscle and its behavior during contraction and rigor. *J. Mol. Biol.* **30**:383.
48. HUXLEY, H., and J. HANSON. 1954. Changes in the cross-striation of muscle during contraction and stretch and their structural interpretation. *Nature (Lond.)*. **173**:973.
49. HUXLEY, H. E., S. PAGE, and D. R. WILKIE. 1963. An electron microscopic study of muscle in hypertonic solutions (appendix to The osmotic properties of muscle fibers in hypertonic solutions by Dydyńska and Wilkie). *J. Physiol. (Lond.)*. **169**:312.
50. KOMINZ, D. R. 1971. Role of swelling in muscle contraction. *J. Theor. Biol.* **31**:255.
51. KOREY, S. 1950. Some factors influencing the contractility of a non-conducting fiber preparation. *Biochim. Biophys. Acta.* **4**:58.
52. OVERTON, E. 1902. Beiträge zur allgemeinen Muskel und Nervenphysiologie. II. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **92**:346.

53. OVERTON, E. 1904. Beiträge zur allgemeinen Muskel und Nervenphysiologie. III. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* 105:176.
54. PEPE, F. A. 1966. Some aspects of the structural organization of the myofibril as revealed by anti-body staining methods. *J. Cell Biol.* 28:505.
55. PEPE, F. 1971. Structural components of the striated muscle fibril. In *Subunits in Biological Systems*. S. N. Timasheff and G. D. Fasman, editors. Marcel Dekker, Inc., New York.
56. PERRY, S. V. 1956. Relation between chemical and contractile function and structure of skeletal muscle cell. *Physiol. Rev.* 36:1.
57. PODOLSKY, R. J., and H. SUGI. 1967. The influence of external hypertonic solutions on the contractile mechanism of skeletal muscle fibers. *J. Gen. Physiol.* 50:2496.
58. RAMSEY, R. W., and S. F. STREET. 1940. The isometric length-tension diagram of isolated muscle fibers of the frog. *J. Cell Comp. Physiol.* 15:11.
59. REUBEN, J. P., P. W. BRANDT, M. BERMAN, and H. GRUNDFEST. 1971. Regulation of tension in the skinned crayfish muscle fiber. I. Contraction and relaxation in the absence of  $Ca^{++}$  ( $pCa > 9$ ). *J. Gen. Physiol.* 57:385.
60. REUBEN, J. P., P. W. BRANDT, and H. GRUNDFEST. 1967. Tension evoked in skinned crayfish muscle fibers by anions, pH, and drugs. *J. Gen. Physiol.* 50:2501. (Abstr.)
61. REUBEN, J. P., L. GIRARDIER, and H. GRUNDFEST. 1964. Water transfer and cell structure in isolated crayfish muscle fibers. *J. Gen. Physiol.* 47:1141.
62. REUBEN, J. P., E. LOPEZ, P. W. BRANDT, and H. GRUNDFEST. 1963. Muscle: volume changes in isolated single muscle fibers. *Science (Wash. D. C.)*. 142:246.
63. ROME, E. 1968. X-ray diffraction studies of the filament lattice of striated muscle in various bathing media. *J. Mol. Biol.* 37:331.
64. SANDOW, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* 17:265.
65. SHEAR, D. B. 1969. The electrical double layer, long range forces and muscle contraction. *Physiol. Chem. Phys.* 1:495.
66. SHEAR, D. B. 1970. Electrostatic forces in muscle contraction. *J. Theor. Biol.* 28:531.
67. SPENCER, M., and C. R. WORTHINGTON. 1960. A hypothesis of contraction in striated muscle. *Nature (Lond.)*. 187:388.
68. WEBER, A. 1968. The mechanism of the action of caffeine on sarcoplasmic reticulum. *J. Gen. Physiol.* 52:760.
69. WEBER, A., and R. HERZ. 1961. Requirement for calcium in the syneresis of myofibrils. *Biochem. Biophys. Res. Commun.* 6:364.
70. WORTHINGTON, C. R. 1961. X-ray diffraction studies on the large-scale molecular structure of insect muscle. *J. Mol. Biol.* 3:619.
71. WORTHINGTON, C. R. 1964. Impulsive (electrical) forces in muscle. In *Biochemistry of Muscle Contraction*. Chapter 47. J. Gergely, editor. Little, Brown and Company, Boston. 511.