Tension in Skinned Frog Muscle Fibers in Solutions of Varying Ionic Strength and Neutral Salt Composition

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ABSTRACT The maximal calcium-activated isometric tension produced by a skinned frog single muscle fiber falls off as the ionic strength of the solution bathing this fiber is elevated declining to zero near 0.5 M as the ionic strength is varied using KCl. When other neutral salts are used, the tension always declines at high ionic strength, but there is some difference between the various neutral salts used. The anions and cations can be ordered in terms of their ability to inhibit the maximal calcium-activated tension. The order of increasing inhibition of tension (decreasing tension) at high ionic strength for anions is propionate$^- \simeq SO_4^{--} < Cl^- < Br^-$. The order of increasing inhibition of calcium-activated tension for cations is $K^+ \simeq Na^+ \simeq TMA^+ < TEA^+ < TPrA^+ < TBuA^+$. The decline of maximal calcium-activated isometric tension with elevated salt concentration (ionic strength) can quantitatively explain the decline of isometric tetanic tension of a frog muscle fiber bathed in a hypertonic solution if one assumes that the internal ionic strength of a muscle fiber in normal Ringer's solution is 0.14–0.17 M. There is an increase in the base-line tension of a skinned muscle fiber bathed in a relaxing solution (no added calcium and 3 mM EGTA) of low ionic strength. This tension, which has no correlate in the intact fiber in hypotonic solutions, appears to be a noncalcium-activated tension and correlates more with a declining ionic strength than with small changes in [MgATP], [Mg], pH buffer, or [EGTA]. It is dependent upon the specific neutral salts used with cations being ordered in increasing inhibition of this noncalcium-activated tension (decreasing tension) as $TPrA^+ < TMA^+ < K^+ \simeq Na^+$. Measurements of potentials inside these skinned muscle fibers bathed in relaxing solutions produced occasional small positive values (<6 mV) which were not significantly different from zero.

INTRODUCTION

Skeletal muscles bathed in hypertonic solutions produce less contractile tension than muscles in normal tonicity solutions. Since Hodgkin and Horo-

wicz (1957) found that in frog muscle fibers normal action potentials could be observed in hypertonic solutions which abolished twitch tension, a number of workers have tried to identify which step beyond activation in the electrochemical coupling process is blocked by the hypertonic solution. Howarth (1958) suggested that the hypertonic solutions, by causing water movement out of the muscle, concentrated the internal salts and directly affected the interaction of the contractile material, causing a decreased contractile tension. Support for this hypothesis was provided by the studies of April et al. (1968) and by us (Gordon and Godt, 1970). April et al. (1968) showed that the tension produced by injection of a fixed amount of calcium into a single crayfish muscle fiber declined steeply as the tonicity of the external solution was increased. We demonstrated previously in frog muscle stimulated either electrically or chemically (caffeine contractures) that the tensions produced by both types of stimulation declined together as the tonicity of the bathing solution increased. Miyamoto and Hubbard (1972) have presented additional data which tend to support this conclusion. On the other hand, Caputo (1966) hypothesized that a major effect of hypertonic solutions was on excitation-contraction coupling in frog muscle fibers. We also found (Gordon and Godt, 1970) that some excitation-contraction uncoupling could result from the hypertonic treatment.

It is important to determine the effects on contractile tension of the raised internal salt concentrations that would be produced by bathing a muscle in a hypertonic solution. This present study was undertaken to measure directly the effects of solution composition on maximum Ca^{++}-activated isometric tension in skinned muscle fibers. In this preparation the composition of the solution bathing the contractile filaments can be controlled and activation produced by elevation of the calcium concentration. It will be shown that the decline in contractile tension of a muscle in a hypertonic solution can be explained quantitatively on the basis of the effect of elevated internal salt concentration, produced by the hypertonic solution, on the maximal calcium-activated tension the muscle can produce. Preliminary reports of these results have appeared (Gordon and Godt, 1969; Gordon et al., 1970). In addition similar results on glycerol-extracted muscle fibers have been reported by Homsher (1969) and by Endo (1967) in a preliminary communication.

METHODS

Preparation and Solutions

The sarcolemma was removed from single frog muscle fibers immersed in oil (Natori, 1954). The methods used in dissection, solution change, and tension measurement were similar to those used by Hellam and Podolsky (1969). The single fiber was isolated from the small bundle of fibers taken from the dorsal or ventral head of the semitendinosus muscle of the frog *Rana pipiens*. All fibers were dissected from the region

opposite the nerve entry to minimize the possibility of obtaining slow fibers. The fiber was skinned in silicone oil (Dow Corning 200 [Dow Corning Corp., Midland, Mich.], 10 cs viscosity). The skinned region of the fiber was mounted between forceps, one end connected to the isometric force transducer, and the other to a rigid mounting. After dissection all fibers were adjusted to their slack length. In several experiments in which the sarcomere length was measured using a phase microscope, this treatment produced sarcomere lengths of 2.0–2.6 μm (in aqueous solutions). The diameter of the fibers in oil was measured with a dissection microscope at a magnification of 100 or 50. No attempt was made to verify that the cross section of the fiber was circular. The fibers were then transferred to an aqueous relaxing solution in which their diameter expanded (by about 25% in diameter if the silicone oil had been saturated with water, somewhat more if the oil was not saturated with water). It is important to transfer the fiber within minutes to the relaxing solution (with ATP) as fibers quickly become stiff when left in the oil. Fibers have a long life time in the relaxing solutions (hours).

The bathing solutions were contained in rectangular wells cut out of a plexiglas block (Rohm and Haas Co., Philadelphia, Pa.). The fibers were transferred from well to well to change the bathing solutions. Measurements were made on the contamination caused by this procedure, and experiments terminated before cross contamination was significant.[1] The bathing solutions in each well were covered with silicone oil and for some experiments the entire block containing the wells was covered with silicone oil so that the fiber could be transferred entirely within liquid. This latter procedure was not usually followed as it did not produce a highly significant increase in fiber performance.

The standard solution had the following composition in mM; $MgCl_2$, 1; Na_2ATP, 5; K_2EGTA, 3; $CaCl_2$, as required; Tris Cl, 10 (pH = 7.0); neutral salt (KCl, etc.), as required. Reagent grade chemicals were used. The Na_2ATP was the Sigma low Ca ATP (Sigma Chemical Company, St. Louis, Mo.). The ionic strength of the solution was computed taking into account all of the contributing species. This problem is not a simple one as anions such as ATP^{-4} bind H^+, Ca^{++}, and Mg^{++} as well as K^+. The problem involves multiple equilibria. In addition, anions such as SO_4^{--} bind K^+ as well as Mg^{++}. Stability constants used in determining concentrations were taken from Sillen and Martell (1964) and (1971). The computation of ionic strength involved the solution of several simultaneous equations. This proved necessary as the various species of ATP and EGTA, etc., contributed significantly to the ionic strength, particularly in the low ionic strength solutions. The ionic strength of the solutions with no added neutral salt was 55 mM. Solutions were generally mixed fresh for each experiment although in some cases solutions were frozen in the final concentration. The pH of each solution was checked before the experiment to be certain that it was 7.00 $\pm$ 0.05.

The value for the free calcium concentration (pCa) was computed using the apparent binding constant of EGTA measured by Ogawa (1968) taking into account the dependence of the apparent stability constant on the concentration of the organic

[1] Transferring directly 50 times back and forth between a solution containing maximum Ca and one with no Ca increased the free calcium concentration of the latter solution only to a pCa of about 8.0. The Ca transferred was less than 1% of the total in the maximum Ca solution.

pH buffer. This gives an apparent binding constant at pH 7.0 and 10 mM Tris buffer of 1.8×10^{6} M^{-1}. This value differs significantly from that given by Schwarzenbach et al. (1957). All of the pCa's in this paper were computed using the Ogawa (1968) value. The contaminating level of Ca in our solutions was about 10 μM. With this apparent stability constant and 3 mM EGTA, the pCa of our relaxing solutions with no added Ca^{++} was about 8.7. All experiments were carried out at room temperature which was normally in the range of 20–22°C. For some of the early studies, the room temperature occasionally reached 29°C.

RESULTS

Tension in Solutions Using KCl

As demonstrated previously by Hellam and Podolsky (1969), skinned fibers produced tension when transferred from a relaxing solution with a pCa greater than 8 to one with a pCa near 5 (see Fig. 1). Fig. 1 demonstrates the

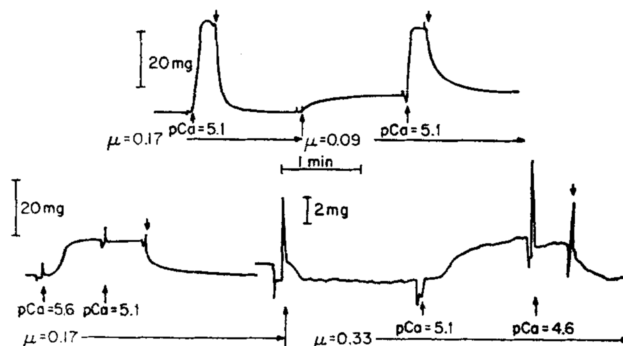

FIGURE 1. Sample records showing tension traces. Initially the fiber is bathed in a relaxing solution with zero added calcium at the indicated ionic strength. Contraction is initiated by transferring the fiber at the time indicated by the upward arrow into a contracting (high [Ca^{++}]) solution at the same ionic strength solution. At the time indicated by the downward pointing arrow the fiber is returned to the original relaxing solution. The same process is then repeated in another set of relaxing and contracting solutions only at different ionic strength. Switching between the sets of solutions at different ionic strengths occurs at the time indicated by the large upward arrow. The upper traces are for fiber 5 (23 July 1969), diameter in oil = 40 μm. The upper traces indicate sample date for transferring from our standard solutions (μ = 0.17) to one of lower ionic strength. The rise in base-line tension on transferring to a relaxing solution of lower ionic strength and the apparent similarity in total tensions at the height of the contraction in the two contracting solutions of different ionic strengths should be noted. The lower traces are for fiber 1 (30 July 1969), a diameter in oil = 40 μm. In the lower trace is indicated first a contraction in a slightly submaximal calcium-activating solution followed by immersion in maximal calcium-activating solution and relaxation. At the indicated large arrow the solution was changed to one with higher ionic strength. Both base-line and maximal tensions decreased in the solutions of higher ionic strength. The fiber had been contracted previously. Its maximum Ca-activated tension in the μ = 0.17 M solution had fallen to 65% of the original control. This record was selected to show the method of verifying that the Ca-activation was maximal.

response of two fibers to the elevated calcium solution; the isometric tension rises slowly to a maximum and then slowly declines to the original level when the calcium concentration is reduced again. The lower part of this figure also shows that as a skinned fiber is transferred from a solution with ionic strength (μ) of 0.17 M to one of μ = 0.33 M (*a*) a reduction of base-line tension occurs in the relaxing solution of higher ionic strength and (*b*) the high calcium concentration produces less tension than it did at the lower ionic strength. Furthermore, transferring a skinned fiber to a solution with a lower pCa produces no additional tension. The upper part of the figure indicates that as another skinned fiber is transferred to a solution of lower ionic strength, 0.17 M–0.09 M: (*a*) the base-line tension increases in the relaxing solution with μ = 0.09; and (*b*) when the solution is changed to one with a pCa of 5.1 with μ = 0.09 the increment in tension is less but the total tension is nearly the same as the tensions produced in the solution with μ = 0.17.

Maximal Ca-activated isometric tensions in these fibers were as high as 3.4 kg/cm^2, expressed per unit cross-sectional area calculated using the diameter measured in oil and assuming a circular cross section. However, since fibers have a smaller diameter in the oil than in the relaxing and contracting solutions, the more appropriate area would be that in relaxing solutions. When this area was used, the average for a series of fibers as 1.1 kg/cm^2 with the maximum being 1.8 kg/cm^2. This is close to the 1.4 kg/cm^2 measured by Hellam and Podolsky (1969) and the 1.5–2.0 kg/cm^2 measured by Endo (1967). In these experiments we did not measure sarcomere length routinely. In one series of experiments where sarcomere length was measured, the average tension/cm^2 area in oil was 3.0 kg/cm^2 correcting each measurement to a sarcomere length of 2.1 μm using the data of Gordon et al. (1966). With the shrinkage in oil, this would correspond to an average tension of about 1.5 kg/cm^2, again in the same range of values given by others. This value of about 1.5 kg/cm^2 is below that found for maximum isometric tetanic tension in intact single fibers (about 2.6 kg/cm^2, see Gordon et al. [1966]). However, considering the fact that a filament lattice distance increases by about 15% as a fresh fiber is skinned (Matsubara and Elliot, 1972), this discrepancy is not as large as it would seem on first consideration.

There was some variability in maximal Ca-activated tensions per square centimeter between fibers and some decline in fiber performance with repeated contractions. In order to compare the data, each fiber served as its own control. Each fiber was cycled between a relaxing solution with no added Ca (pCa $\simeq$ 8.7), high calcium solution (with pCa of either 5.1 or 4.6), and the original relaxing solution, all at the same ionic strength. All tensions are expressed as changes from the base-line tension in the relaxing solution at μ = 0.17 M. The absolute value of this tension was not deter-

mined routinely, but when measured, it was normally not more than 15% of the maximal Ca-activated tensions. The increment in tension seen with the maximizing calcium concentration in a solution with μ = 0.17 M (the standard solution and ionic strength) was considered to be the 100% point to which all other tension increments were compared. Test contractions in solutions of ionic strength different from 0.17 M were always preceded and followed by standard contractions in solutions of ionic strength 0.17 M. A linear correction was used for the decline in tension produced by each contraction in order to interpolate the 100% point (standard contraction tension) associated with the test contraction tension. The experiment with each fiber was terminated when the contractile tensions fell to near 50% of the original tension. Collecting data in this manner, we found that each fiber produced data at several ionic strengths.

Calcium activation must be maximal. Each ionic strength and salt solution used was checked for maximal calcium activation by transferring fibers between solutions of pCa 5.1 and 4.6 and looking for increments in tension (illustrated in Fig. 1).

When the ionic strength was varied by the addition of KCl, the data shown in Fig. 2 were obtained. This figure shows (*a*) the change in base-line tension in a relaxing solution from that in the standard relaxing solution (μ = 0.17 M), (*b*) the increment in tension above the base line produced by a maximal calcium-activating solution at each ionic strength, and (*c*) the total tension (taking the resting tension in the standard relaxing solution as zero), equal to the sum of the base line plus calcium-activated tension at each ionic strength, all plotted as a function of the ionic strength of the solution. All tensions are expressed as percentages of the maximal calcium-activated tension in the standard solution with ionic strength of 0.17 M. Each point is the average of a number of tensions from several individual fibers each normalized to itself as a control. The bars give the standard deviation of the maximal calcium-activated tension points. Each fiber was allowed to equilibrate in a solution of different ionic strength for at least 1 min before being transferred to the contracting solution. Contractions in most of the solutions were reversible except for the high ionic strength solutions which appeared to have a somewhat deleterious effect on the fibers. The fibers immersed in the 0.4–0.5 M solutions tended to give less tension in the standard contracting solution after the treatment. This is entirely consistent with the fact that solutions of this ionic strength are used to extract myosin from myofibrils.

As can be seen from Fig. 2, the maximal calcium-activated tension falls off dramatically as the ionic strength of the solution is increased, declining to near zero in a solution of ionic strength near 0.5 M. At lower ionic strength the calcium-activated tension decreases. In contrast, the tension produced

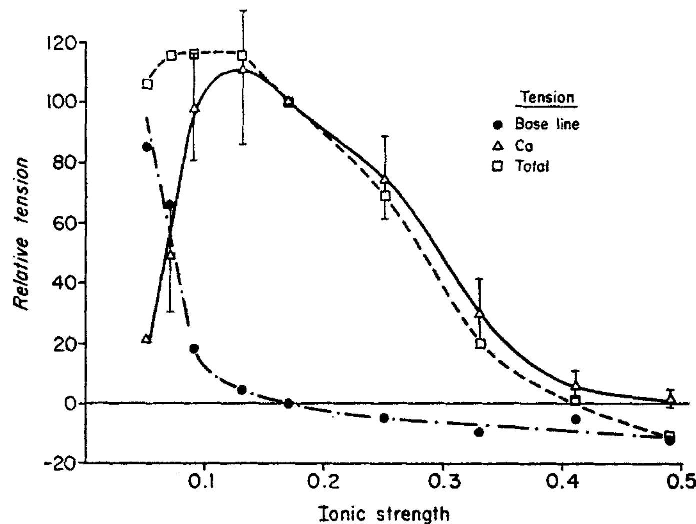

FIGURE 2. Relative tension normalized to the maximal calcium-activated tension of the fiber in a standard solution with ionic strength of 0.17 M plotted against the ionic strength of the solution which was calculated by including the contribution of all ionic species. Three tensions are plotted: ●, the base-line tension change or the change from the tension in the standard relaxing solution (solution with zero added calcium, 3 mM EGTA and μ = 0.17 M); △, the increment in tension produced by transferring the fiber to a maximal calcium-activating solution; □, the total tension, or the sum of the normalized calcium-activated plus base-line tension change. KCl concentration was changed to vary ionic strength in this experiment. The bars indicate the standard deviations.

in the relaxing solutions with pCa greater than 8 increases markedly at low ionic strengths and decreases somewhat at high ionic strengths. This produces a total tension vs. ionic strength curve, using KCl to vary μ, which has a plateau at low ionic strengths and decreases as the ionic strength is increased. In this discussion we will deal primarily with the calcium-activated tension and deal later in the paper with the increased base-line tension.

The decrease in calcium-activated tension seen at increased ionic strength is very reminiscent of both the decline of tension of muscle in a hypertonic solution and also the decline in ATPase activity of myofibrils or actomyosin seen by a number of workers at increased ionic strengths (cf. Katz, 1966; Hasselbach, 1952; Weber and Herz, 1963; Portzehl et al., 1969). However, this decline of ATPase activity occurs at a lower ionic strength than does the decline in tension in the skinned muscle preparation. A decline in Ca^{++}-activated tension in a glycerol-extracted muscle with elevated ionic strength has been shown by Homsher (1969) to occur over a similar range of ionic strengths as those shown here for skinned fibers.

Comparison of Skinned Fiber Data to Whole Muscle Data

The major question is whether this decline of tension with elevated salt concentration in a maximal calcium-activated contraction can explain quantitatively the decline of tension of the muscle in hypertonic solutions. In order to compare the two, a number of assumptions have to be made. Given the decline of tetanic tension with elevated tonicity of the muscle in hypertonic solutions (see Fig. 1 in Gordon and Godt, 1970), one can compare this quantitatively to the decline of tension with increased ionic strength assuming: (*a*) the ionic strength inside the muscle fiber in a normal tonicity solution is known; (*b*) ionic strength will increase in proportion to the increase in tonicity of the external solution; (*c*) the tension produced by tetanic stimulation is the same as the maximal calcium-activated tension at an ionic strength near 0.17 M; (*d*) the increase in concentration of the other constituents inside muscle produced by the hypertonic solutions acts only through increases in total ionic strength (i.e. the change in [Mg], [organic phosphates], will not change the maximal Ca-activated tension per se, but only through changing the ionic strength); (*e*) KCl is a representative neutral salt, i.e., there are no specific ion effects; and (*f*) the composition of the solution around each myofilament is identical with the solution bathing the skinned fiber.

When all these assumptions are made and the data from Gordon and Godt (1970) on the decline of tetanic tension of the muscle in hypertonic solutions are plotted with our present data on the decline of maximal calcium-activated tension in skinned fiber as a function of ionic strength, the curves in Fig. 3 result. Here is plotted the data from the maximal calcium-activated tension of a skinned muscle fiber as the triangles and the lines representing tetanic tension of intact muscle from Fig. 1 in Gordon and Godt (1970). If an internal ionic strength of 0.14 M is assumed with a normal tonicity bathing solution for the intact muscle, the fit is excellent between the two sets of data. This indicates that the majority of the decline of tension of the muscle in a hypertonic solution can be explained quantitatively on the basis of the effect of elevated internal salt concentration (or ionic strength) on the ability of the muscle proteins to generate tension under maximal calcium activation.

The six assumptions above need to be justified. On the first there is no precise measurement of the internal ionic strength in normal tonicity solution. There are measurements on the total constituents of muscle, but the numbers needed are the free concentrations in the sarcoplasm which are different because of binding and compartmentalization. Using data from Conway (1957) plus estimations of binding, ionic strengths in the range of 0.1–0.2 M are found. This calculation neglects the charge groups on the proteins which

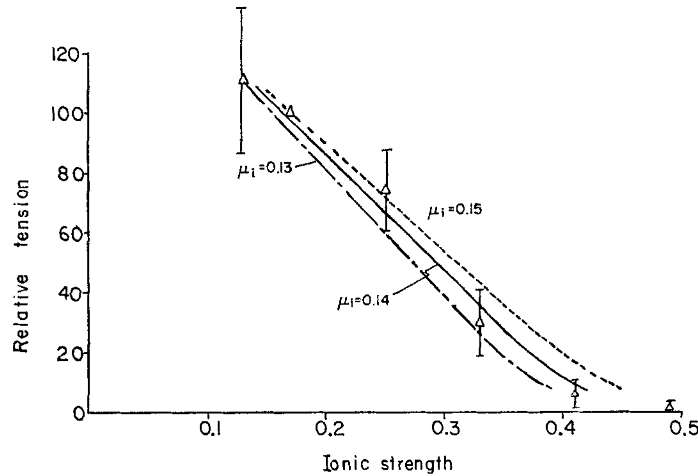

FIGURE 3. Plotted here are the relative tensions of a skinned fiber maximally activated with calcium and the tetanic tension of intact muscle against the ionic strength of the solution in molar quantities. The triangles are from the maximal calcium-activated tensions in skinned fibers. The lines are drawn from the data of Gordon and Godt (1970, see their Fig. 1) for the decline of tension of a tetanic tension of a muscle in hypertonic solutions. The initial internal ionic strengths for the intact muscle used in computing these curves are labeled. The method of computation is indicated in the text.

contribute to charge balance but not in a direct way to ionic strength. A further uncertainty is caused by the fact that these charged proteins (polyelectrolytes) may affect the activity of counter-ions depending on the ionization of the polyelectrolytes (Katchalsky, 1964). The value that we use to fit the data (see Fig. 3), 0.14 M, is certainly within the acceptable range.

The second assumption that the ionic strength increases in proportion to the tonicity is well justified from the data of Blinks (1965) on changes in muscle volume with osmotic strength of the bathing solution. Deviations might occur due to the concentration dependence of binding, but the majority of the ionic strength is still contributed by ions like K^+, HCO_3^-, etc., which probably do not bind to a significant extent (Kushmerick and Podolsky, 1969).

The third assumption, that the tetanic tension is equivalent to the maximal calcium-activated tension, is justified to within 10%. Hodgkin and Horowicz (1960) showed that a maximal potassium contracture could produce 10% more tension than tetanic stimulation in single fibers from the English frog, *Rana temporaria*. Thus the assumption is an underestimation of the maximal tension which can be produced. The close agreement between the maximal caffeine contracture tension and tetanic tension measured at the same tonicity (Gordon and Godt, 1970) further supports this assumption.

On the fourth assumption, several controls were done to show that changes in concentration of the solution constituents other than the one contributing most to ionic strength, KCl, did not produce changes in tension. The maximal Ca^{++}-activated tension is fairly insensitive to increases in [Mg^{++}] and [Mg ATP], both free and added. This curve was no different when the added Mg^{++} was increased from 1 mM to 5 mM with the accompanying change in free ATP^{-4} from near 1.2 to 0.2 mM. Godt (1971) observed very little change in maximal calcium-activated tension at an ionic strength of about 0.15 M as [MgATP] varied from 20 μM to 2 mM. Kerrick and Donaldson (1972) showed that no significant change occurred in maximal calcium-activated tension at $\mu = 0.14$ and 0.20 as the free [Mg^{++}] varied from 0.3 mM to 2 mM. Varying the pH buffer concentration from 3 mM to 13 mM also did not produce any significant shifts in this tension vs. ionic strength curve.

On the fifth assumption that there are no specific ion effects and that KCl is acting only through ionic strength, we will present data below to indicate that some variation in the decline of tension depends upon the particular neutral salt used.

The final assumption is that the composition of the external bathing solution is identical with the composition of the solution bathing the myofilaments. The diffusion measurements of Kushmerick and Podolsky (1969) indicate that ions can migrate freely through the sarcoplasm of the skinned fiber. On the other hand, if there were large potentials and high resistances inside the fiber, there could be quite substantial differences in concentration between inside and outside due to the Gibbs-Donnan equilibrium. Collins and Edwards (1971) have recently described potentials measured in glycerol-extracted muscle fibers. To see if these potentials exist in the skinned fibers and if they are of the magnitude that Collins and Edwards describe, measurements were done on potentials inside these skinned fibers using microelectrodes of varying tip diameters and resistances (0.5–60 MΩ). The potentials were so small that it was difficult to differentiate between real potential differences in the fiber and potentials due to bending of the electrode tips, plugging of the electrodes, or changes in electrode tip potential. To try to differentiate electrodes with tip potentials under 2 mV were used. Values were used only if the electrode resistance did not increase substantially (5%) and if the electrode could be visualized in the fiber. Most fibers produced potentials accompanied by electrode resistance changes, when the electrode tip was obviously bent.

For the few penetrations that satisfied the above criteria, if there was a potential inside these skinned fibers, it was slightly positive averaging 2.0 $\pm$ 1.9 mV (mean $\pm$ SD of 23 measurements) in the 0.17 M ionic strength solution and 5.2 $\pm$ 1.8 (mean $\pm$ SD of six measurements) 0.07 M ionic

strength solution. These values are opposite in sign to those of Collins and Edwards (1971). The reason for the difference is not clear. The uncertainty in the experimental measurements is great enough so that the potential is not significantly different from zero. Thus no corrections need to be made for differences in ionic composition between the sarcoplasm of the skinned muscle fiber and the external bathing solution.

On the other hand, this says little about the actual concentrations of ionic species near a charged myofilament. This problem cannot be solved at this time. With these considerations in mind, ionic strength as calculated here may not be the proper variable. McLaughlin et al. (1971) have shown that monovalent and divalent ions have a different ability to screen surface charge and thus affect the surface potential on charged membranes than would be anticipated from the relative ionic strengths as computed here. ($\mu = \Sigma C_i Z_i^2$ where C_i is the concentration of the ith species with valence Z_i) from the Debye-Huckel theory which assumes that the electrical potential is less than RT/F (25 mV). This may not be the case for the surface potential near charged myofilaments.

To test that these electrodes could measure a negative potential inside a body with fixed negative charges, beads of carboxymethyl cellulose were penetrated while in the solutions used with the skinned muscle fiber. Negative potentials were measured which varied with ionic strength in a way that would be predicted for fixed negative charges, but with the concentration of fixed negative charges only about $\frac{1}{10}$ of that given for the material used. This is not surprising considering the shunting effect of the fluid in the beads and the fact that the electrode is somewhat distant from the actual charge groups. In any case, with our technique, negative potentials inside bodies with fixed negative charges could be measured.

Again, on this point of equilibration between external and internal solutions, no ATP-regenerating system was added in these experiments. It was assumed that ATP would diffuse in from the external solution to replenish that which was hydrolyzed. If ATP hydrolysis is diffusion limited, this might affect the maximal tension. To test this, a regenerating system was added (10 mM creatine phosphate plus 0.1 mg/ml creatine phosphokinase). This produced no effect on the maximal Ca^{++}-activated tension vs. ionic strength curve other than that which would be predicted from the additional ionic strength added with the creatine phosphate and neutralizing ions. Thus if a regenerating system is needed under some conditions, it is not needed to produce the maximal tension at room temperature.

Calcium-Activated Tension in Solutions with Different Neutral Salts

In the previous discussion, only KCl was used as a neutral salt to vary the ionic strength. A question exists, however, on the mechanism of its action.

Is the KCl acting only to raise ionic strength or are there specific ion effects or general structure-disrupting effects of the neutral salt? To help us answer this question, we repeated the experiments looking at the calcium-activated tension and the base-line tension as the ionic strength was varied with a variety of neutral salts. In all experiments we tested for maximal calcium activation and compared the contraction with the particular neutral salt to a test contraction in KCl for each fiber.

The data in Fig. 4 show the effect of varying the anion with a fixed cation, K^+. For ionic strengths at and above 0.17 M, six or more fibers were used to provide the data for each plotted point. As can be seen, propionate and sul-

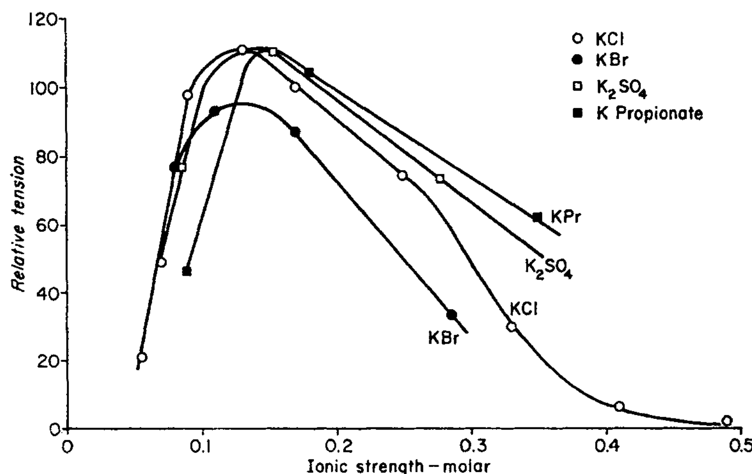

FIGURE 4. Shown here is the relative tension as a percentage of the maximal calcium-activated tension in a standard solution of 0.17 M ionic strength made up with KCl plotted against ionic strength. The KCl curve here is the one obtained from the data for Fig. 2. The other curves are drawn from data obtained in solutions with different anions and are labeled as such. The lines are fitted by eye to the points.

fate are least effective with the anions falling in the sequence of propionate $\simeq SO_4^{--} > Cl^- > Br^-$ in order of increasing dimunition of calcium-activated tension. The sulfate data was corrected for binding of K^+ to SO_4^{--} in the computation of ionic strength. With this correction, there is not a significant difference between the propionate and sulfate data at high ionic strengths ($P \simeq 0.5$). The other differences $SO_4^{--} > Cl^- > Br^-$ are statistically significant ($P < 0.1$) for $\mu \geq 0.17$ M. For example at $\mu = 0.17$ M, the maximal calcium-activated tension in KBr was 87.0 ± 13.8% (mean ± SD for 11 fibers) of the tension in KCl with no KBr contraction producing more tension than the previous contraction in KCl.

Fig. 5 demonstrates what happens when the cation used in the neutral salt was varied. In addition to the contraction with K^+ and Na^+, contrac-

tions were done in solutions with tetralkylammonium salts. These tetralkylammonium salts were used in the form available in highest purity, the Br^- salts. Contractions at a variety of ionic strengths were done only for tetramethylammonium bromide (TMABr) and tetrapropylammonium bromide (TPrABr). Contractions were also done at one ionic strength (0.17 M) for tetraethylammonium bromide (TEABr) and tetrabutylammonium bromide (TBuABr). Contractions were done in NaCl and compared to contractions in KCl. For all solutions data from five or more fibers were averaged for each point in Fig. 5 with $\mu \geq 0.15$ M. As can be seen in Fig. 5, there is

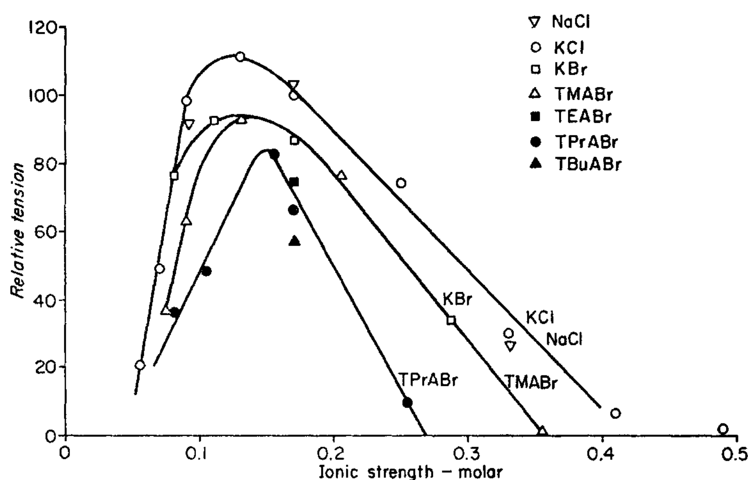

FIGURE 5. The ordinate shows the relative tension as a percentage of the maximal calcium-activated tension in a standard solution of ionic strength of 0.17 M made with KCl. In all cases the maximal calcium-activated tension in a particular solution is indicated as a function of the ionic strength. A number of different neutral salts are used to make up the solutions for the various curves. The solution composition data are indicated in the Methods section. The key in the upper right-hand corner indicates the various points. In this case the salt used in making up the solutions of various ionic strengths contains a different cation for each curve. In addition, because of the fact that Br^- is used in many of the curves, the difference between the standard KCl curves and the KBr curves is indicated.

no significant difference between tensions in NaCl and KCl. Since the other salts were Br^- salts, data were needed on the effect of changing the anion from Cl^- to Br^-. The mean values of the maximal calcium-activated tensions in KBr are significantly lower than those in KCl as described above. The KBr curve will be the one to which the others are compared. There is no significant difference between the maximal calcium-activated tension in KBr and TMABr. On the other hand, at all ionic strengths the tensions in TPrABr were lower than in KBr ($P < 0.02$ at $\mu = 0.17$ M, <0.05 at $\mu = 0.25$ M). Also, as the chain length of the alkyl is increased, the maximal calcium-activated ten-

sion at a particular ionic strength (μ = 0.17 M) appears to decrease in order from KBr (the standard) TMABr to TEABr to TPrABr to TBuABr. This conclusion is less clear-cut than that for KBr and KCl since although the tensions in KBr and TMABr at 0.17 M are all significantly higher than those for the rest of the sequence and the maximum tensions fall in exactly this sequence, there is enough scatter in the data to keep the significance of the inner sequence differences to $P > 0.1$. On the other hand, stepping the fiber from a maximal calcium-activating solution with one tetralkyl to another verifies that tension increases as one goes from $TMBuA^+$ to $TPrA^+$ to TEA^+ to TMA^+ and declines in the reverse order. This stepping procedure clearly shows the order but not the absolute ratios of tensions. The long-chain length tetralkyls, particularly TBuABr, did irreversible damage to the fibers. After a contraction in TBuABr, the subsequent control or standard contraction tension was significantly lower. This happened to a much lesser extent in the TPrABr.

Figs. 4 and 5 show that, for all the neutral salts used, the maximal calcium-activated tension declines as the salt concentration is increased. On the other hand, there are significant differences between tensions in solutions with a given concentration of various highly ionized neutral salts, so that ionic strength is certainly not the sole or unique variable.

Tension in Solutions of Salt Concentration in the Absence of Calcium

As shown in Figs. 1 and 2, when a skinned muscle fiber was transferred from our standard solution of 0.17 M ionic strength with 3 mM EGTA and no added calcium (pCa of greater than 8) to a similar solution but with lower ionic strength, the increase in the base-line tension was substantial. This tension could approach 100% of the maximal calcium-activated tension of the fiber in the 0.17 M solution. This appears to be a tension which is not controlled by the calcium concentration in the fiber, but may be caused by the same mechanism as the calcium-activated tension. The total tension (the sum, base-line plus calcium-activated) is approximately constant so that as the base-line tension increases, the Ca-activated tension decreases. Also, if the skinned muscle is released (slackened) manually, this increased base-line tension will redevelop at the new muscle length, as would Ca-activated tension. On the other hand, this tension does not occur in normal fibers. Okada and Gordon (1972) did not observe an increased resting tension in frog muscles immersed in solutions with tonicities down to 0.3 times normal [μ = 0.05 assuming μ = 0.17 in Ringer's and the muscle fiber is a perfect osmometer (Blinks, 1965)]. The reason for this discrepancy is not clear.

We did several experiments to investigate the cause of this elevation in base-line tension in the skinned fibers. It was difficult to change solution constituents to any great extent since ionic strength had to be held fairly constant because of the very strong dependence of this tension on salt con-

centration (or ionic strength) per se. We suspected initially that the muscle fiber may have become more sensitive to calcium at this low ionic strength so that the contaminating levels of calcium present, (near 10 μM total) even with the 3 mM EGTA, might be sufficient to activate the system. On the other hand, increasing the concentration of EGTA to about 7 mM, decreasing KCl to keep the ionic strength constant, did not significantly decrease this tension. The addition of EGTA alone in almost all experiments decreased tension only by the amount that would have been expected by the increase in ionic strength added by the EGTA and neutralizing potassium. Thus this tension did not appear to be the result of activation by trace amounts of calcium.

We next questioned whether the ATP supply was being limited so that the interior of the fiber might be experiencing very low ATP levels and that the base-line tension might be more of a rigor tension. To test this hypothesis we added an ATP-regenerating system to the solutions. To have much effect, the compound from which the ATP is regenerated needs to be in a concentration somewhat higher than the ATP in the external solution. As the $[ATP]_{added}$ was 5 mM, this poses a problem because of the additional ionic strength added to the solutions by large quantities of the charged species. In a series of experiments, we replaced the KCl in a solution containing no added calcium with an ionic strength equivalent of 4.2 mM creatine phosphate and 0.5 mg/ml creatine phosphokinase. The solution with the ATP regenerating system did not produce relaxation in the fiber but instead produced a slight increase in tension above that seen without the creatine phosphate.

We investigated changing $[Mg]_{free}$ and $[MgATP]_{free}$ on this noncalcium-activated tension. Again because of the constraints placed by the low ionic strength, it was difficult to vary concentration of these ions over wide ranges. In changing $[Mg]_{free}$ from 10 μM to 120 μM [MgATP] from 0.5 mM to 2.0 mM, the majority of the variation seen in tension could be explained entirely on the basis of the changes in ionic strength of the solution. Thus over this narrow range of changes investigated, tension still correlated more directly with ionic strength than with any of the changes in $[Mg]_{free}$ or $[MgATP]_{free}$.

This increased tension at low salt concentration is probably not due to tension in an osmotically active component in the skinned muscle fiber (like the sarcoplasmic reticulum) since replacing salt with osmotically equivalent amounts of sucrose results in the same increased tension that occurs with low salt concentrations alone.

Additional experiments were conducted to determine if the increase in base-line tension which occurred when a fiber was transferred from the standard relaxing solution to a lower ionic strength relaxing solution was de-

pendent upon a prior contraction in the presence of a high concentration of Ca^{++}, a condition which possibly promotes loading of the sarcoplasmic reticulum with Ca^{++}. In this series of experiments done at a room temperature of 20°C, the maximum increase in base-line tension observed upon changing the ionic strength of bathing solution from 0.17 M to 0.07 M was 20% of the maximum Ca^{++}-activated tension at 0.17 M ionic strength. This was somewhat less than the average observed previously but within the range of values seen. This increase in base-line tension in the absence of Ca^{++} in the bathing solution occurred in several fibers before these fibers had been contracted by maximal Ca^{++}-activation in a solution of 0.17 M ionic strength. Single or repeated Ca^{++}-activated contractions did not appear to increase the magnitude of the base-line increase in tension elicited by decreasing the ionic strength of the relaxing solution. Soaking the fibers in a 0.17 M relaxing solution containing 18 mM caffeine for 1–2 min, which should cause the release of Ca^{++} from the sarcoplasmic reticulum, never abolished and did not appear to decrease the magnitude of the base-line shift. A few fibers did not show an increased base-line tension when the ionic strength of the relaxing solution was decreased but contracting them in a standard ionic strength solution with pCa = 5, sometimes repeatedly, did not change their behavior. Only one fiber failed to demonstrate the base-line shift before a Ca^{++}-activated contraction and then responded with an increase in base-line tension after a Ca^{++}-activated contraction. It is concluded that the increase in base-line tension associated with a lowering of the ionic strength of the relaxing solution is not significantly dependent upon a prior Ca^{++}-activated tension or loading of the sarcoplasmic reticulum with Ca^{++}.

On the other hand, this noncalcium-activated tension is sensitive to the neutral salt used. In particular, it seems to be highly dependent on the particular cation used in the solutions. Fig. 6 illustrates the effect of changing anion and cation on this noncalcium-activated tension. As can be seen, there is no significant difference between KCl and the KBr. On the other hand, there is a significant shift as the cation is changed from K^+ to TMA^+ to $TPrA^+$. K^+ is best at inhibiting this tension followed by TMA^+ and $TPrA^+$. Thus although this noncalcium-activated tension does depend strongly on salt concentration (ionic strength) there are some specific ion effects. This increased interaction at low ionic strengths has been seen by a number of people before, including Katz (1966) and Portzehl et al. (1969), who measured increased myofibrillar ATPase activity at low ionic strengths in solutions with EGTA present and no added calcium.

An interesting phenomenon was observed in these low ionic strength solutions. If a muscle was taken first from the standard relaxing solution (zero added calcium) to the low ionic strength relaxing solution (in which the base-line tension increased), and then to a maximal calcium-activating bathing

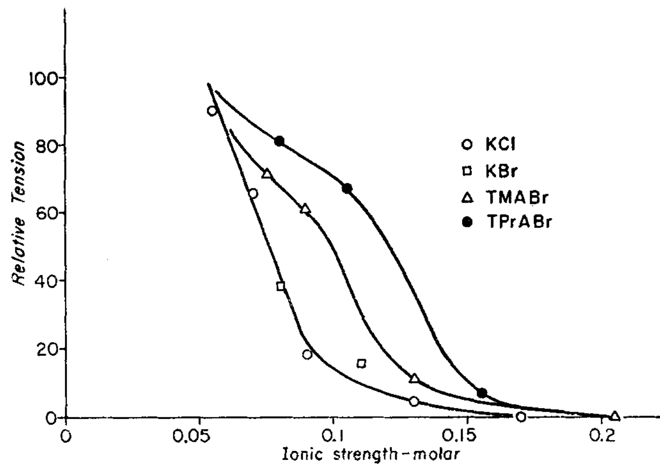

FIGURE 6. Plotted here is the increase in base-line tension seen in a solution with low ionic strength plotted as a percentage of the maximal calcium-activated tension for the standard solution of 0.17 M ionic strength made up of KCl. Curves are plotted for four different neutral salts indicating the major shifts seen with changes in cations from K^+ to TMA^+ to $TPrA^+$. The curves were fitted to the points by eye.

solution at this low ionic strength (in which tension increased further), quite often upon returning the muscle to the low ionic strength-relaxing solution, relaxation back to the original level in that solution did not occur. Tension would often hang or "catch" at an elevated value. Putting some mechanical stress on the fiber by taking it through one of the air-fluid interfaces frequently produced rapid relaxation back to the expected value. On the other hand, small manual releases of the fiber were followed by some redevelopment of tension. Once the system had been turned on with calcium, removing the calcium in a low ionic strength solution did not seem to lead to complete relaxation. This phenomenon was not investigated further.

DISCUSSION

The major conclusion in this paper is that the decline in tension of a muscle bathed in a hypertonic solution can be explained quantitatively by the effects of the elevated salt concentration inside the muscle on the contractile protein interaction. A number of assumptions were made to compare the data on the maximal calcium-activated tension of a skinned muscle fiber to the tetanic tension of a whole muscle fiber bathed in a solution of greater than normal tonicity, but these assumptions are fairly well-justified. These data obtained from using various concentrations of KCl produced an estimate of internal ionic strength of a muscle fiber in normal Ringer's solution of about 0.14 M. If the solution on the inside of the muscle were considered to be more similar to K_2SO_4, then the internal ionic strength would be about

0.17 M. Each of these values is reasonable and does not invalidate the conclusion.

Additional information about the internal ionic strength can be obtained by using the data on the effect of hypotonic solutions on tetanic tension in frog muscle fibers (Okada and Gordon, 1972). Tetanic tension is potentiated in hypotonic solutions by 10–20% down to a tonicity of half the normal value. (See Fig. 1 of Okada and Gordon, 1972). This potentiation is certainly reminiscent of the increase in total tension for KCl solutions with $\mu < 0.17$ M. These data on intact fibers can be used to estimate internal ionic strength in much the same way as the hypertonic solution data used above. However, we do not know if the potentiation which occurs in hypotonic solutions is related to the fact that the tetanic tension in normal solutions may not be maximal tension since it can be less than the K-contracture tension (Hodgkin and Horowicz, 1960). In this case potentiation might occur because hypotonic solutions lead to a more effective electrically stimulated Ca^{++} release. If a change occurred in E-C coupling efficiency, the hypotonic solutions' data could be fit with an internal ionic strength near 0.14 M. If we assume that the tetanic tension is the maximal tension at all tonicities (or at least the same fraction of maximal tension), an internal ionic strength of near 0.17 M gives a better fit to the data. These are the same numbers discussed above and are consistent with the hypothesis regarding the decline of tension of muscles in hypertonic solutions. On the other hand, the increase in noncalcium-activated tension in the skinned fiber is not matched by an increase in resting fiber of the intact fibers in hypotonic solutions.

All of this is not to say that under some circumstances there may be some true excitation-contraction uncoupling of a muscle in a hypertonic solution. We (Gordon and Godt, 1970) were able to demonstrate a small uncoupling at very high tonicities. Ashley and Ridgway (1970) demonstrated a decrease in the calcium transient in the barnacle muscle fiber in hypertonic solutions. The presence of activation heat in muscles in hypertonic solutions (Hill, 1958; Gibbs et al., 1966) has been taken as evidence that Ca^{++} release is little affected in muscles in hypertonic solutions, but Smith (1972) has shown that activation heat does decline for tonicities above 2.5 times normal. Even if the Ca^{++} release is unaffected, more Ca^{++} could be required for activation. Godt (1971) demonstrated that changes in the MgATP concentration will shift the relationship between tension and calcium concentration so that a higher free calcium concentration is required to reach a given percentage of maximal activation with the higher MgATP concentration. This was also demonstrated by Weber (1970) for ATPase activity. It is also known that as the ionic strength is increased, the calcium sensitivity of the actomyosin ATPase activity shifts so that more calcium is required to attain a given percentage of maximal activity (Weber and Herz, [1963], from μ =

0.09 to 0.15, and Portzehl et al. [1969], from μ = 0.05 to 0.10, but not above 0.10). The amount of calcium bound to the myofibrils to produce a given level of activation may be the same in these cases, but the amount released must provide both the bound and free calcium. Thus the increase of MgATP concentration or the elevated ionic strength in the muscle might raise the free calcium requirement of the muscle in hypertonic solutions to a point where even if the amount of calcium released on stimulation were the same as that produced by muscle in normal Ringer's solution, it might produce much less activation. Although there are several mechanisms by which hypertonic solutions could uncouple excitation and contraction, the major decline of tension seems to be accounted for by the effect of salt concentration on the contractile proteins, on the tension produced by maximal Ca^{++} activation.

A question may arise whether the skinned muscle fiber is an adequate model of a fiber with the sarcolemma intact. What is the effect of removal of the sarcolemma? Matsubara and Elliott (1972) have shown that the filament lattice of a frog muscle fiber expands when it is skinned in a solution which is similar to our standard relaxing solution. This expansion depends on sarcomere length and, over the range of lengths they investigated, the expansion is smallest (10–15%) at the sarcomere lengths of the fibers used in these experiments (2–2.5 μm). In addition, they showed that at the sarcomere lengths used in our study, decreasing the ionic strength by half or replacing the chloride with propionate did not significantly affect the lattice spacing but that replacing the chloride with sulfate caused less than a 5% shrinkage. In all cases, the changes in filament lattice seen in the skinned muscle fiber were small over the range of sarcomere lengths in this study. In addition, the work of Gordon et al. (1966) and of Edman and Andersson (1968) indicates that the tension generated per bridge may be relatively insensitive to filament lattice spacing. Thus the skinned fiber is probably a good model for the intact cell.

As discussed previously, this decline in contractile protein interaction with increasing salt concentration is not a new finding. Hasselbach (1952) described this, and it is described by many other workers including Katz (1966) whose Fig. 2 looks very similar to our curve on calcium-activated and noncalcium-activated tensions except that his changes occur at lower ionic strengths. Fig. 11 in Kominz (1970) has data on turbidity of myofibrillar suspensions which when plotted against ionic strength show a similar sort of dependence as in both this paper and Katz's (1966). The effects of various neutral salts on muscle have also been investigated before. Warren et al., (1966) investigated the effects of various neutral salts on the ATPase activity of myosin and found that there was a distinct ordering for both anions and cations in decreasing this activity at high salt concentration. Although they worked at somewhat higher salt concentrations for those

ions where duplication occurs, there is agreement between our results and theirs except in the cation sequence where they find a difference between TMA^+, K^+, Na^+ unlike our study. Jacobs and Guthe (1970) also describe a series relating the effects of various anions on contraction produced in glycerol-extracted muscle fibers by the elevation of pH. They, as do we, find that Br^- inhibits contraction more than Cl^-. Sorenson et al. (1973) also found an ordering of anions in terms of their effectiveness in decreasing the maximum Ca-activated tension in skinned crayfish muscle fibers which agrees with our results where there is duplication, i.e., Cl^- is more effective in reducing tension than propionate. In contrast to our data Katz (1968) found that the ATPase activity of reconstituted actomyosin was stimulated when sodium was substituted for potassium. This was not seen in our study but a difference of 5% would not have been visible. Thus there is evidence for both the effect of increased salt concentration (or ionic strength) as well as specific ion effects on contractile proteins.

Which of these two is responsible for the decline of tension and what is the mechanism of decline? Our data show that ionic strength is not the sole culprit. However, it is not clear from our data whether the tension decline is due to a smaller number of interacting sites, to less force generated per interaction, or to a combination of the two. Moos (1973) describes work by himself and his co-workers that, with isolated proteins (actin and heavy meromyosin (HMM) or subfragment −1) elevated ionic strength decreases the actin-myosin interaction (as reflected in the K_{app}) but does not affect the maximum ATPase rate at infinite actin (V_{max}) up to $\mu = 0.1$ M or the Michaelis constant for the acto-HMM ATPase. This could be produced if the elevated ionic strength produces increased screening and decreased electrostatic interaction of charged sites on actin and myosin. Kominz (1970) has proposed that, alternatively, high ionic strength promotes the dissociation of actin and myosin by increasing ATP binding to a clearing site on myosin. He indicates that his data could be fit by assuming that a small part of the myosin molecule with molecular weight from 10,000 to 20,000 was involved in this.

On the other hand, a wealth of literature has accumulated relating to the hypothesis of von Hippel and Schleich (1969) that neutral salts order in their effects on protein structure (either salting in or salting out, stabilizing or destabilizing the native form) on the basis of their effects on water structure. They and their co-workers have produced a series of anions and cations which is impressive in its similarity in affecting the physical properties of a number of proteins. The data of Warren et al., (1966) are included in this. Table I indicates the ordering of the anions and cations used in this study in terms of their effects in decreasing the maximal calcium-activated tension produced at high salt concentrations. Comparing this series to that of von

TABLE I

IONS AFFECTING TENSION*

Inhibition maximal Ca^{++}-activated tension

decreasing tension →

increasing inhibition →

$C_2H_5COO^- \simeq SO_4^{--} < Cl^- < Br^-$

$K^+ \simeq Na^+ \simeq (CH_3)_4N^+ < (C_2H_5)_4N^+ < (C_3H_7)_4N^+ < (C_4H_9)_4N^+$

Inhibition of non-Ca^{++}-activated tension

decreasing tension →

increasing inhibition →

$(C_3H_7)_4N^+ < (CH_3)_4N^+ < K^+ \simeq Na^+$

$Cl^- \simeq Br^-$

* Indicated here are the sequences of the ions as they affected the maximal calcium-activated tension in the upper part of the table, and in the lower part of the table as they affected the noncalcium-activated tension (the increased base-line tension). In the upper part from left to right they move from those ions which inhibited tension at high ionic strength the least to those that inhibited tension the most. In the lower part moving from left to right is the sequence of those ions that allowed the largest base-line tension in the low ionic strength solutions to those on the right that inhibited base-line tension the most in low ionic strength solutions.

Hippel, we see an exact comparison with little or no rearranging. (Fig. 1 in von Hippel and Schleich, 1969.) Generation of this series does not give any indications as to the precise mechanism of the effect of these neutral salts, but it does connect this study with the more general studies of the effects of ions on macromolecules. Further data on how much of the tension decline is due to ionic strength elevation and how much to the structural disrupting effects of the neutral salts might be obtained by investigating the influence on the calcium-activated tension of different alcohols which may act as structure disrupters. Alcohols have been shown to depress contractile force in whole, isolated cardiac muscle in proportion to the length of their carbon chains (independent of osmotic activity) (Nakano and Moore, 1972), so that one might suspect that they would have an effect on skinned fiber contractile tension.

It is interesting to note that different muscles have different ionic strength sensitivities of their ATPase activity. Buller et al. (1969) have shown that the ATPase activity of myosin extracted from a fast muscle varies more with ionic strength than does myosin extracted from a slower mammalian muscle. It would be an interesting test of the hypothesis to see if those muscles whose myosin is less sensitive to ionic strength would show less dependence of tetanic tension on tonicity. In frog muscles, the tonic muscle fibers appear to be less sensitive to elevated tonicity and less sensitive to elevated ionic strength when skinned (Godt and Kirby, personal communication). It is also of interest that the light molecular weight subunits of myosin, which may be the

major difference between the myosins from the faster and slower muscles, have molecular weights in the 20,000 range. This is comparable to Kominz's estimation of the protein subunit controlling the actin and myosin interaction and affected by ionic strength.

There are other factors that affect the nucleotide triphosphatase activity besides salt concentration (ionic strength). These include the nucleotide used (Muir et al., 1971), pH (Kaldor et al., 1968) and the method of preparation of the proteins, and the treatment of SH group modifiers (Muir, et al., 1971; Dancker and Hasselbach, 1971). These need to be taken into account in any proposed mechanism of action of high salt concentrations.

In addition, we have been considering only the effect of ions on the maximal Ca-activated tension. Ions can act by shifting the Ca^{++}-sensitivity. Sorenson et al. (1973) found that the anions differed in their ability to shift the Ca^{++}-sensitivity with SCN^- solutions requiring more Ca^{++} for a given percentage of activation than propionate solutions. Thus different anions and cations can do more than simply change the tension in the absence of calcium and tension under maximal calcium activation, the two values measured in this study.

The data on the increased base-line tension in the low ionic strength solutions with pCa greater than 8 indicate that in the low salt strengths, the troponin-tropomyosin complex loses its ability to prevent interaction of actin and myosin in these skinned fibers. This same effect is seen with isolated proteins (see Fig. 2 in Katz, 1966). Tropomyosin and/or troponin might be removed by the low ionic strength solution. Another way to look at this is that, in the terminology of Bremel and Weber (1972), enough "rigor complexes" may be formed to "turn on" many actin molecules at this ionic strength and MgATP level. The lower part of Table I shows the order of the ions in terms of their ability to decrease the tension at low ionic strengths. K^+ and Na^+ seem to be the best inhibitors with TMABr and TPrABr following. Br^- and Cl^- have equal effects. This is the inverse of what we found for the Ca-activated contractions. Considering von Hippel's sequences, this implies that the molecular processes are different for the neutral salt effects on the Ca-activated and nonCa-activated tensions.

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Note Added in Proof: Since the acceptance of this manuscript for publication, a paper has appeared (April, E. W., and P. W. Brandt. 1973. The myofilament lattice: studies on isolated fibers. III. The effect of myofilament spacing upon tension, *J. Gen. Physiol.* **61**:490) providing additional evidence using crayfish muscle that hypertonic

solutions can decrease isometric tension if they produce an elevated internal ionic strength which affects the tension-generating capacity of the muscle through affecting the contractile proteins activated by calcium. April and Brandt "further demonstrated that interfilament spacing changes encountered during shortening and with variation in osmotic strength have no effect upon the tension-generating capacity of muscle."

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REFERENCES

April, E., P. W. Brandt, J. P. Reuben, and H. Grundfest. 1968. Muscle contraction: the effect of ionic strength. *Nature (Lond.).* **220**:182.

Ashley, C. C., and E. B. Ridgway. 1970. On the relationship between membrane potential, calcium transient, and tension in single barnacle muscle fibres. *J. Physiol. (Lond.).* **209**:105.

Blinks, J. R. 1965. Influence of osmotic strength on cross-section and volume of isolated single muscle fibres. *J. Physiol. (Lond.).* **177**:42.

Bremel, R. D., and A. Weber. 1972. Cooperation within actin filament in vertebrate skeletal muscle. *Nat. New Biol.* **238**:97.

Buller, A. J., W. F. H. M. Mommaerts, and K. Seraydarian. 1969. Enzymic properties of myosin in fast and slow twitch muscles of the cat following cross-innervation. *J. Physiol. (Lond.).* **205**:581.

Caputo, C. 1966. Caffeine- and potassium-induced contractures of frog striated muscle fibers in hypertonic solutions. *J. Gen. Physiol.* **50**:129.

Collins, E. W., Jr., and C. Edwards. 1971. Role of Donnan equilibrium in the resting potentials in glycerol-extracted muscle. *Am. J. Physiol.* **221**:1130.

Conway, E. J. 1957. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* **37**:84.

Dancker, P., and W. Hasselbach. 1971. Dependence of actomyosin ATPase activity on ionic strength and its modification by thiol group substitution. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **16**:272.

Edman, K. A. P., and K.-E. Andersson. 1968. The variation in active tension with sarcomere length in vertebrate skeletal muscle and its relation to fibre width. *Experientia (Basel).* **24**:134.

Endo, M. 1967. Regulation of contraction-relaxation cycle of muscle (in Japanese) *Proc. XVII Gen. Ass. Japan Med. Congr.* **1**:193.

Gibbs, C. L., N. V. Ricchiuti, and W. F. H. M. Mommaerts. 1966. Activation heat in frog sartorius muscle. *J. Gen. Physiol.* **49**:517.

Godt, R. E. 1971. Ca^{++}-activated tension of skinned muscle fibers: Dependence on MgATP concentration. Ph.D. thesis. University of Washington, Seattle.

Gordon, A. M., and R. E. Godt. 1969. Effects of variations of ionic strength on contractile tension of skinned muscle fibers activated by calcium. *Physiologist.* **12**:238.

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.

Gordon, A. M., R. E. Godt, and J. W. Woodbury. 1970. Ionic strength as a determinant of calcium-activated tension in skinned muscle fibers in various salt solutions. *Fed. Proc.* **29**:656.

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.

Hasselbach, W. 1952. Die Umwandlung von Aktomyosin-ATPase in L-Myosin-ATPase durch Aktivatoren und die resultierenden Aktivierungseffekte. *Z. Naturforsch.* **7b**:163.

Hellam, D. C., and R. J. Podolsky. 1969. Force measurements in skinned muscle fibres. *J. Physiol. (Lond.).* **200**:807.

Hill, A. V. 1958. The priority of the heat production in a muscle twitch. *Proc. R. Soc. Lond. B. Biol. Sci.* **148**:397.

Hodgkin, A. L., and P. Horowicz. 1957. The differential action of hypertonic solutions on the twitch and action potential of muscle fibre. *J. Physiol. (Lond.).* **136**:17P.

HODGKIN, A. L., and P. HOROWICZ. 1960. Potassium contractures in single muscle fibres. *J. Physiol.* (*Lond.*). **153**:386.

HOMSHER, E. E. 1969. On the mechanism of the inhibition of muscular contraction by hypertonic solutions. Ph.D. thesis. University of Pittsburgh, Pittsburgh.

HOWARTH, J. V. 1958. The behaviour of frog muscle in hypertonic solutions. *J. Physiol.* (*Lond.*). **144**:167.

JACOBS, H. K., and K. F. GUTHE. 1970. Anions and the contraction of glycerol-extracted muscle fibers. *Arch. Biochem. Biophys.* **136**:36.

KALDOR, G., P. K. CHOWRASHI, and Q.-S. HSU. 1968. Studies on the interaction of actomyosin with polyions. *Arch. Biochem. Biophys.* **128**:261.

KATCHALSKY, A. 1964. Polyelectrolytes and their biological interactions. *Biophys. J.* (Suppl.) **4**:9.

KATZ, A. M. 1966. Purification and properties of a tropomyosin-containing protein fraction that sensitizes reconstituted actomyosin to calcium-binding agents. *J. Biol. Chem.* **241**:1522.

KATZ, A. M. 1968. Effects of alkali metal ions on the Mg^{2+}-activated ATPase activity of reconstituted actomyosin. *Biochim. Biophys. Acta.* **162**:79.

KERRICK, W. G. L., and S. K. B. DONALDSON. 1972. The effects of [Mg^{2+}] on submaximal calcium activated tension in skinned frog skeletal muscle fibers. *Biochim. Biophys. Acta.* **275**:117.

KOMINZ, D. R. 1970. Studies of adenosine triphosphatase activity and turbidity in myofibril and actomyosin suspensions. *Biochem.* **9**:1792.

KUSHMERICK, M. J., and R. J. PODOLSKY. 1969. Ionic mobility in muscle cells. *Science* (*Wash., D.C.*). **166**:1297.

MATSUBARA, I., and G. F. ELLIOTT. 1972. X-ray diffraction studies on skinned single fibres of frog skeletal muscle. *J. Mol. Biol.* **72**:657.

MCLAUGHLIN, S. G. A., G. SZABO, and G. EISENMAN. 1971. Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.* **58**:667.

MIYAMOTO, M., and J. I. HUBBARD. 1972. On the inhibition of muscle contraction caused by exposure to hypertonic solutions. *J. Gen. Physiol.* **59**:689.

MOOS, C. 1973. Actin activation of heavy meromyosin and subfragment −1 ATPases; steady state studies. *Cold Spring Harbor Symp. Quant. Biol.* **37**:137.

MUIR, J. R., A. WEBER, and R. E. OLSON. 1971. Cardiac myofibrillar ATPase: A comparison with that of fast skeletal actomyosin in its native and in an altered conformation. *Biochim. Biophys. Acta.* **234**:199.

NAKANO, J., and S. E. MOORE. 1972. Effect of different alcohols in the contractile force of the isolated guinea-pig myocardium. *Eur. J. Pharmacol.* **20**:266.

NATORI, R. 1954. The property and contraction process of isolated myofibrils. *JiKeiKai Med. J.* **1**:119.

OGAWA, Y. 1968. The apparent binding constant of glycoletherdiaminetetraacetic acid for calcium at neutral pH. *J. Biochem.* (*Tokyo*). **64**:255.

OKADA, R. D., and A. M. GORDON. 1972. Excitation, contraction, and excitation-contraction coupling of frog muscles in hypotonic solutions. *Life Sci., Pt* 1. **11**:449.

PORTZEHL, H., P. ZAORALEK, and J. GAUDIN. 1969. The activation by Ca^{2+} of the ATPase of extracted muscle fibrils with variation of ionic strength, pH and concentration of MgATP. *Biochem. Biophys. Acta.* **189**:440.

SCHWARZENBACH, G., H. SENN, and G. ANDERREGG. 1957. Komplexone XXIX. Ein grosser Cheleteffekt besonderer Art. *Helv. Chim. Acta.* **40**:1886.

SILLEN, L. G., and A. E. MARTELL. 1964. Stability constants of metal-ion complexes, *Chem. Soc. Spec. Publ.* 17.

SILLEN, L. G., and A. E. MARTELL. 1971. Stability constants of metal-ion complexes, *Chem. Soc. Spec. Publ.* 25, Supplement 1.

SMITH, I. C. H. 1972. Energetics of activation in frog and toad muscle. *J. Physiol.* (*Lond.*). **220**:583.

SORENSON, M., M. J. DAWSON, J. P. REUBEN, and P. W. BRANDT. 1973. Modes of action of anions which attenuate tensions in skinned muscle fibers of the crayfish. *Biophys. Soc. Annu. Meet. Abs.* 182 *a*.

von Hippel, P. H., and T. Schleich. 1969. Ion effects on the solution structure of biological macromolecules. *Acc. Chem. Res.* **2**:257.

Warren, J. C., L. Stowring, and M. F. Morales. 1966. The effect of structure-disrupting ions on the activity of myosin and other enzymes. *J. Biol. Chem.* **241**:309.

Weber, A. 1970. The dependence of relaxation on the saturation of myosin with adenosine triphosphate. *In* Physiology and Biochemistry of Muscle as a Food. E. J. Briskey, R. G. Cassens, and B. B. Marsh, editors. University of Wisconsin Press, Madison. 383.

Weber, A., and R. Herz. 1963. The binding of calcium to actomyosin systems in relation to their biological activity. *J. Biol. Chem.* **238**:599.