Ionic Strength and the Contraction Kinetics of Skinned Muscle Fibers

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ABSTRACT The influence of KCl concentration on the contraction kinetics of skinned frog muscle fibers at 5–7°C was studied at various calcium levels. The magnitude of the calcium-activated force decreased continuously as the KCl concentration of the bathing solution was increased from 0 to 280 mM. The shortening velocity at a given relative load was unaffected by the level of calcium activation at 140 mM KCl, as has been previously reported by Podolsky and Teichholz (1970. *J. Physiol.* [*Lond.*]. **211:** 19), and was independent of ionic strength when the KCl concentration was increased from 140 to 280 mM. In contrast, the shortening velocity decreased as the KCl concentration was reduced below 140 mM; the decrease in velocity was enhanced when the fibers were only partially activated. In the low KCl range, the resting tension of the fibers increased after the first contraction cycle. The results suggest that in fibers activated at low ionic strength some of the cross bridges that are formed are abnormal in the sense that they retard shortening and persist in relaxing solution.

INTRODUCTION

The influence of ionic strength on the contractile mechanism of muscle cells is of general interest because this parameter affects the biochemistry of the isolated contractile proteins (Szent-Gyorgyi, 1947; Rizzino et al., 1970). However, even before these proteins were well characterized, ionic strength attracted the attention of physiologists because of the striking effects of hypertonic solutions on the mechanical properties of intact muscle cells (Overton, 1902; Ernst, 1926). These solutions, which cause water to move out of the cell, bring about (*a*) an increase in resting tension (Hill, 1968; Lännergren and Noth, 1973), (*b*) a decrease in active tension (Gordon and Godt, 1970), and (*c*) a decrease in contraction velocity (Howarth, 1958), but do not affect the action potential (Hodgkin and Horowicz, 1957). Although the first of these effects does not appear to depend on the activation process, the second and third could arise from an influence of hypertonic solutions on

intracellular calcium release. This possibility was first ruled out, at least as a principal mechanism, when shortening in response to added calcium was found to be considerably slower in skinned fiber preparations in oil made from frog muscles immersed in hypertonic Ringer solution than in those made from muscles immersed in normal Ringer solution (Podolsky and Sugi, 1967). Similar results were reported when calcium was added through a micropipette to intact crayfish fibers in hypertonic solution (April et al. 1968).

Since loss of water both increases the concentration of the intracellular constituents and decreases the lateral spacing between the myofilaments, a number of studies have been made to find out whether one or the other of these changes is responsible for the various mechanical effects. A useful technique in this connection has been to follow the response of the cell to a Ringer solution made hypertonic with KCl, a permeable electrolyte. When the cell is transferred to such a solution from a normal Ringer solution, water first moves out of the cell, which increases the internal ionic strength; then, as KCl enters the cell, water moves back in, which restores the cell volume (and presumably the myofilament spacing) to nearly the original value but keeps the ionic strength at the elevated level. Experiments comparing the mechanical effects in this solution with those in solutions made hypertonic with impermeable solutes suggest that resting tension is associated with filament spacing (Hill, 1968) while active tension and/or contraction velocity is a function of ionic strength (April et al., 1968).

In the present paper we describe the effects of KCl on the contraction kinetics of skinned muscle fibers (Natori, 1954) at 5–7°C. These preparations facilitate studies of the contraction mechanism because they can be directly activated with calcium (Podolsky and Costantin, 1964; Ebashi and Endo, 1968; Hellam and Podolsky, 1969). In addition, quantitative force and velocity data can be obtained at various calcium levels (Podolsky and Teichholz, 1970) and the ionic strength of the bathing solution can be changed without significantly affecting the filament spacing (Matsubara and Elliott, 1972). The experimental results confirm the report that ionic strength controls the magnitude of the calcium-activated force (Gordon and Godt, 1969) and provide evidence that ionic strength has no direct effect on the contraction velocity. The data also indicate, however, that under certain conditions, which depend on ionic strength, some of the cross bridges formed by calcium activation are unusually difficult to break. These *abnormal* cross bridges appear to retard the sliding of the myofilaments produced by normal cross bridges, thereby reducing the contraction velocity below the level expected for a given external load; their influence on the contraction kinetics depends on the level of calcium activation. The possible origin of these abnormal cross bridges is examined. Preliminary accounts of this work have been reported briefly (Teichholz and Podolsky, 1971; Thames and Podolsky, 1973).

METHODS

Fiber Preparation

The method of fiber preparation was as described by Hellam and Podolsky (1969), except that the dissection and skinning were generally carried out in mineral oil at room temperature. The skinned fiber segments varied in length from 5 to 10 mm; the free length after attachment to the transducers ranged from 1.2 to 3.0 mm. Fiber diameter was not measured directly, but previous work (Hellam and Podolsky, 1969) showed that the steady isometric force made by skinned fibers is directly proportional to the cross-sectional area of the fiber. In several experiments the cross-sectional area of the skinned fiber was intentionally reduced by removing an appropriate fraction of the myofibrils.

A few fibers were skinned and mounted in cold liquid silicone fluid (Hellam and Podolsky, 1969; Podolsky and Teichholz, 1970) to determine whether such preparations behaved differently from those prepared as described above. Unless otherwise indicated, muscle fibers were obtained from *Rana pipiens pipiens* collected in the northern part of the United States during winter, spring, and summer.

Bathing Solutions

All bathing solutions contained 5 mM Na_2ATP, 1 mM $MgCl_2$, 10 mM imidazole, and 3 mM total EGTA concentration. The ionic strength was varied by changing the concentration of added KCl; this ranged from no added KCl to 280 mM KCl. The ionic strength of a bathing solution with no added KCl was calculated to be 50 mM. The pCa of the bathing solutions was set at 5, 6.5, 6.6, and 6.7 by addition of the appropriate quantity of $CaCl_2$, which was calculated taking $10^{6.69}$ M^{-1} as the apparent stability constant of the CaEGTA complex at pH 7.0 and 6°C (Sillén and Martell, 1964; Weber et al., 1966; Murphy and Hasselbach, 1968). Relaxing solutions contained no added calcium. The bathing solutions were adjusted to pH 7.0 at 6°C. The experiments were carried out at 5–7°C using the solution changer previously described (Podolsky and Teichholz, 1970).

Force Transducer

At the start of the study this was similar to that used by Podolsky and Teichholz (1970). The signal was amplified with a Tektronix 3A3 dual trace differential amplifier and displayed on a Tektronix 564B storage oscilloscope (Tektronix, Inc., Beaverton, Ore.). The sensitivity was 0.13 mV/mg wt, the overall compliance was 0.03 μm/mg wt, and the natural period was 8 ms. Minimum noise upon amplification was 1 mg wt; stray vibration rarely increased the noise level beyond 4 mg wt.

About half the experiments were made after the force transducer was modified to operate in a differential mode (Meiss, 1971). By using an appropriate light-blocking vane and a pair of photodiodes in opposite arms of the Wheatstone bridge, the sensitivity was increased to 0.63 mV/mg wt and the drift was reduced; the mechanical properties of the transducer were unchanged. The voltage output of the modified transducer was linear for forces up to 1.0 gm wt. In some experiments force was re-

corded continuously on a potentiometric recorder (Leeds and Northrup Speedomax Recorder), which made it possible to detect small changes in force.

Displacement Transducer

This was the same as that previously described by Podolsky and Teichholz (1970). The sensitivity was 2.3 V/mm movement at the tip. The signal, which was linear for displacements up to 2 mm, was displayed on the second channel of the oscilloscope. The equivalent mass of the lever system was 100 mg and the minimum natural period, which is dependent on fiber compliance (Civan and Podolsky, 1966), was about 10 ms.

General Procedure

The method of obtaining force-velocity data from skinned fibers was as previously described (Podolsky and Teichholz, 1970), except that the fiber was set at an initial sarcomere length of 2.4–2.6 μm, as determined by the diffraction pattern from a helium-neon gas laser (Spectra-Physics model, 133 Spectra-Physics, Inc., Mountain View, Calif.; $\lambda = 632$ nm). After mounting in mineral oil, the preparation was transferred to relaxing solution and the sarcomere length was adjusted. Then the fiber was transferred to contracting solution, allowed to develop steady force (P_o), and released to a preset load $P < P_o$ so that it shortened isotonically. After placing the fiber in relaxing solution, the process could be repeated. Experiments were terminated when the steady isometric force fell by more than about 20% and/or the laser pattern of the resting fiber became indistinct. This usually happened after 6–10 contraction cycles, although in one case 20 reversible cycles were recorded (Fig. 3).

The resting tension T was recorded after each contraction cycle. The isometric force P_o was considered to be the difference between the total steady force in contracting solution and T. The isotonic force P was taken as the difference between the total force L presented by the loading spring and T. The ratio of P to P_o was taken as the relative load.

The resting tension at a given sarcomere length was the force measured at that sarcomere length above the zero of force with the fiber in relaxing solution. The zero of force was found by passively shortening the fiber to a length at which the fiber was slightly buckled; slight additional shortening of the fiber resulted in no measurable force change. The resting tension before the first contraction cycle was designated as T_o. The difference between the actual resting tension and T_o was called the residual tension.

RESULTS

High Ca^{2+} Concentration

Isotonic quick release records were obtained from fibers activated in high calcium (pCa 5) at different KCl concentrations. For each experiment, the fiber was first allowed to shorten at a given P/P_o in a reference solution containing 140 mM KCl. The fiber was then activated in a solution with a different KCl concentration and allowed to shorten again. The fiber was

relaxed in a 140 mM KCl solution after each contraction. The speeds of shortening were compared when the relative load was the same for both releases. A typical experiment is shown in Fig. 1. When the KCl concentration of the activating solution was increased from 140 to 210 mM, the isometric force fell 37% in this case, but the speed of shortening for the same

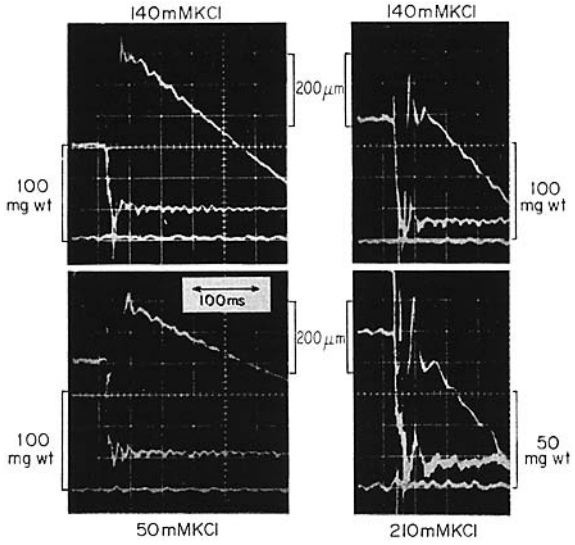

FIGURE 1. Influence of KCl concentration on isometric force and speed of shortening of skinned muscle fibers in high Ca^{2+} (pCa 5). In the oscilloscope records, the upper trace is displacement, the middle trace shows the force, and the lower trace is the resting tension. The records on the left are from a fiber shortening at relative load 0.32 (top) and 0.28 (bottom) in solutions containing 140 and 50 mM KCl, respectively. Those on the right are from another fiber shortening at relative load 0.16 (top) and 0.15 (bottom) in solutions containing 140 and 210 mM KCl. In going from 140 to 50 mM KCl, the isometric force increased from 96 to 132 mg wt while the speed of shortening decreased from 0.75 to 0.48 muscle lengths/s. In going from 140 mM to 210 mM KCl, the isometric force decreased from 125 to 80 mg wt but the speed of shortening was almost the same. Fiber on left: viii 72, segment length 1.9 mm, sarcomere length 2.4 μm, temperature 5–7°C. Fiber on right: 15 viii 72, segment length 2.1 mm, sarcomere length 2.4 μm, temperature 5°C.

P/P_o was unchanged (Fig. 1, right). In contrast, a reduction in KCl concentration from 140 to 50 mM resulted in a 38% increase in isometric force and a reduction in the speed of shortening from 0.75 to less than 0.50 muscle lengths/s (Fig. 1, left).

Data from similar experiments done on 25 fibers are shown in summary form in Fig. 2. When the KCl concentration was raised above 140 mM, isometric force was reduced but the speed of shortening at the same relative load was unaffected. The decrease in isometric force was not due to a shift in the

force-pCa relation because the addition of sufficient $CaCl_2$ to reduce the pCa to 4 did not increase the steady force. In contrast, when the KCl concentration was decreased below 140 mM, the calcium activated force increased, but the speed of shortening decreased continuously.

Fig. 2 also shows the resting tension, T, measured after contraction in solutions with different KCl concentrations. This force, which will be described in detail below, increased as the KCl concentration was reduced.

The experiments represented by open symbols in Fig. 2 were made with the protocol described above. In those represented by closed symbols, the fiber was equilibrated for about 10 s with a relaxing solution containing KCl at the concentration of the activating solution before and after being exposed to the activating solution. Both procedures gave the same results. This would be expected because (*a*) in the first

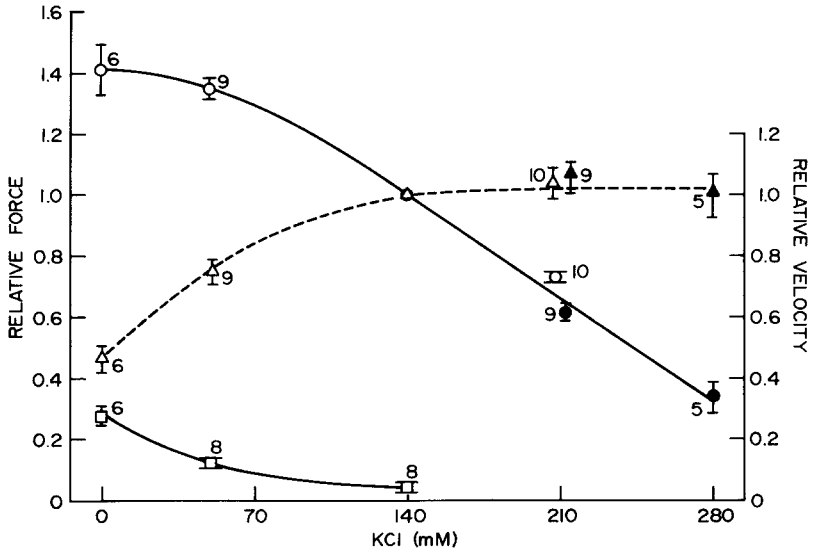

FIGURE 2. Summary of force, velocity, and resting tension measurements from skinned muscle fibers activated in high Ca^{2+} at 5–7°C. The open symbols are from *Rana pipiens pipiens* fibers that were activated by high calcium (pCa 5) at the indicated KCl concentrations and then relaxed (pCa 9) in a solution containing 140 mM KCl. The closed symbols are data obtained by Teichholz and Podolsky (1971) using *Rana pipiens berlandieri* fibers which were equilibrated for 10 s in a relaxing solution at the indicated KCl concentration before and after being activated. Each force (○, ●) and velocity (△, ▲) point is expressed as a fraction of the force and velocity obtained in the same fiber in 140 mM KCl at the same relative load. The resting tension in 140 mM KCl after contraction at the KCl concentration indicated (□) is expressed as a fraction of the calcium-activated force developed in 140 mM KCl. When the KCl concentration was increased above 140 mM, the calcium-activated force decreased while the speed of shortening was unchanged. As the KCl concentration was reduced below 140 mM, the calcium-activated force increased, the speed of shortening decreased, and the resting tension following contraction increased. Error bars give the standard error of the mean.

method, where the separate KCl equilibration step was omitted, KCl equilibrates more rapidly with the myofilament space than does Ca^{2+} (KCl enters the fiber by simple diffusion, which is rapid (Kushmerick and Podolsky, 1969), while the Ca^{2+} concentration in the myofilament space does not reach the contraction threshold until the sarcoplasmic reticulum has been filled (Ford and Podolsky, 1972)), and (*b*) changes in residual tension (see below) are not reversed by changing the KCl concentration.

Low Ca^{2+} Concentration

The experiments of Podolsky and Teichholz (1970) showed that in 140 mM KCl solutions the speed of shortening for the same relative load was the same at high (pCa 5.0) and low (pCa 6.5–6.7) calcium concentrations. We have repeated and confirmed these results in an additional group of 17 fibers (row 2 of Table I). The results from one such fiber are shown in Fig. 3. In this experiment the P_o in low Ca^{2+} was 35% of that in high Ca^{2+} and the extrapolated speed of unloaded shortening, V_{max}, was about 1.8 muscle lengths/s.

Since the speed of shortening in fully activated fibers decreased when the KCl concentration of the bathing solution was reduced, experiments were made to see if the degree of activation affected the shortening velocity in low KCl solutions. Four groups of experiments were done. In each group the KCl concentration in both the relaxing and the contracting solution was held constant. The calcium level in the contracting solution for the first activity cycle was pCa 5, where maximum isometric force was developed. In sub-

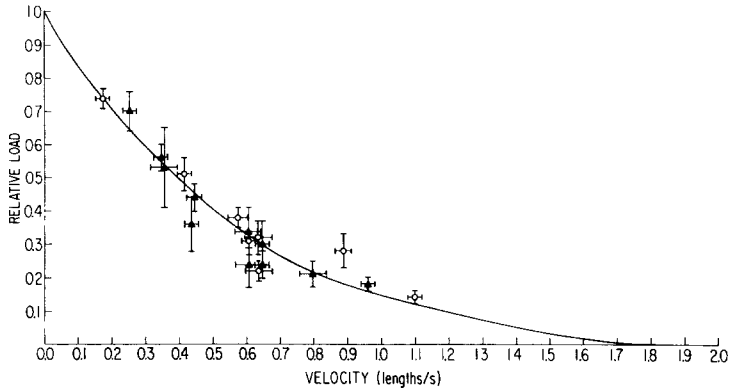

FIGURE 3. Force-velocity data for a skinned fiber segment in 140 mM KCl at high (○) and low (▲) calcium. The graph shows the measured velocities for various loads; error bars give the estimated uncertainties. The high and low calcium releases were alternated, and relative load was varied throughout the experiment. The steady isometric force in low calcium was 35% of that in high calcium. The hyperbola was fitted to the high calcium data; the extrapolated V_{max} is 1.8 muscle lengths/s. The speed of shortening is the same for the same relative load in high and low calcium. Fiber of 22 vi 72, segment length 2.8 mm, sarcomere length 2.4 μm, temperature 5–7°C.

sequent cycles the contracting solution was either pCa 5 or pCa 6.6–6.7, where the steady isometric force was 10 to 50% of maximum. The KCl concentrations of the bathing solutions were 175 mM (6 fibers), 140 mM (11 fibers), 100 mM (10 fibers), and 50 mM (8 fibers).

FIG. 4 shows paired force and displacement records obtained from contractions in the last three solutions. Each pair, obtained from the same fiber,

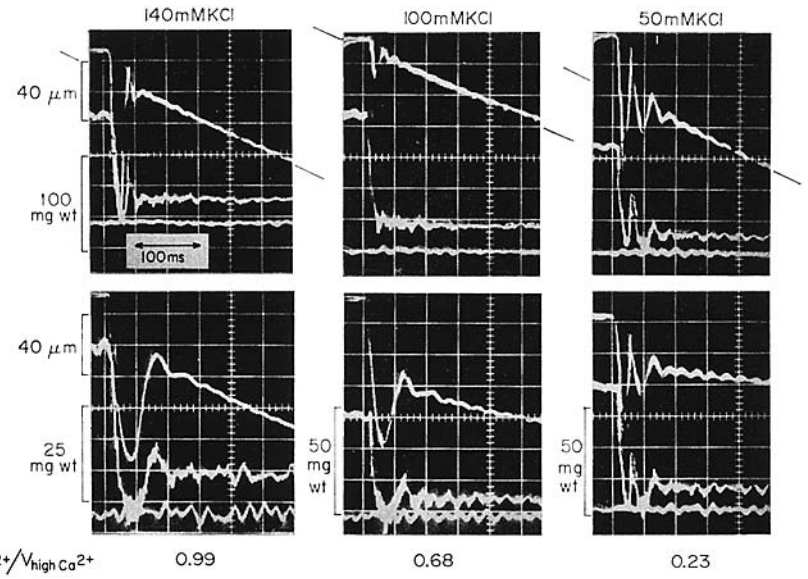

FIGURE 4. Influence of Ca^{2+} concentration on speed of shortening of skinned fibers at different KCl concentrations. Each vertical pair of records was obtained from the same fiber. The upper records were obtained from contraction at pCa 5 and the lower records were obtained in solutions with pCa 6.6–6.7; each pair was matched for relative load. For the three KCl concentrations, reading from left to right, $P_{o\ low\ Ca^{2+}}/P_{o\ high\ Ca^{2+}}$ = 0.38, 0.38, and 0.57 and relative load = 0.22 – 0.24, 0.19, and 0.18. The corresponding preparations were: 30 vi 72, length 2.8 mm, sarcomere length 2.4 μm, 6°C; 3 vii 72, length 2.4 mm, sarcomere length 2.4 μm, 6°C; 5 vii 72, length 2.8, mm, sarcomere length 2.6 μm, 5°C. The way in which the steady velocity was estimated from the displacement trace can be seen by joining the line segments at the sides of each of the upper records.

was matched so that the relative loads were essentially the same. In the first pair, where the KCl concentration was 140 mM and the relative load was about 0.23, the speeds of shortening were the same in high and low calcium. In the 100 mM KCl records, where the relative load was 0.19, the speed of shortening in low calcium was 0.7 times the speed in high calcium. The velocity difference in 50 mM KCl was even greater.

Table I shows in summary form the paired data from experiments in high and low calcium for the three KCl concentrations shown in Fig. 4 and

TABLE I

SUMMARY OF PAIRED COMPARISONS FOR VELOCITY MEASUREMENTS OBTAINED AT THE SAME RELATIVE LOAD IN HIGH AND LOW Ca^{2+} IN SOLUTIONS WITH VARIOUS KCl CONCENTRATIONS

For each KCl concentration the fiber shortened in high and low Ca^{2+} at the same relative load (±0.05) and the relative velocity difference $d = (V_{\text{low } Ca^{2+}} - V_{\text{high } Ca^{2+}})/V_{\text{high } Ca^{2+}}$ was computed. The force ratio is the ratio of the steady force at the low and the high Ca^{2+} concentration. $\bar{d}$ is the mean difference for N pairs of observations. Probabilities were calculated according to the t distribution of $\bar{d}$ and the standard error of $\bar{d}$. $p(d = 0)$ is the probability that the data were sampled from a population with $V_{\text{high } Ca^{2+}} = V_{\text{low } Ca^{2+}}$. The data exclude relative velocity differences larger than Δ at the significance level indicated. All calculations were carried one extra decimal point before rounding.

KCl	Force ratio	N	$\bar{d}$	$(SE)_{\bar{d}}$	$p(d = 0)$	$\Delta(P < 0.05)$
mM						
175	0.30–0.62	11	−0.02	0.04	>0.6	−0.14 to 0.10
140	(i) 0.20–0.30	6	−0.04	0.02	>0.1	−0.13 to 0.05
	(ii) 0.31–0.40	6	0.02	0.025	>0.3	−0.07 to 0.11
	(iii) 0.41–0.58	5	0.05	0.025	>0.1	−0.02 to 0.04
	(i) + (ii) + (iii)	17	0.01	0.02	>0.6	−0.02 to 0.04
100	0.27–0.56	14	−0.28	0.05	<0.001	
50	0.36–0.83	15	−0.43	0.05	<0.001	

also results from experiments made in solutions containing 175 mM KCl. Within the experimental uncertainty, there were no differences in the speeds of shortening for the same P/P_o in high and low calcium with KCl concentrations of 140 and 175 mM. (At higher KCl concentrations the values of P_o at high calcium were reduced to levels that made comparisons at high and low calcium technically difficult. In addition, exposure of skinned fibers to KCl concentrations greater than 175 mM resulted in a gradual loss of the ability of the fiber to develop force in high calcium solutions.) When the KCl concentration of the bathing solutions was lowered to 100 mM and below, a significant difference between the speeds of shortening in high and low calcium for the same P/P_o became increasingly evident.

Fig. 5 shows the effect of decreasing the KCl concentration on the speed of shortening of skinned fiber segments in *low* Ca^{2+}. The speed of shortening is reduced by considerably more than 50% in going from 140 and 175 mM KCl to 50 mM KCl. In contrast, the average speed of shortening in *high* calcium is only reduced by 25% for the same drop in KCl concentration (Fig. 2).

Resting Tension

Since the decrease in speed of shortening when the KCl concentration was lowered below 140 mM is associated with an increase in resting tension (Fig. 2), the nature of the resting tension was studied.

EFFECT OF CONTRACTION The resting tension of a relaxed skinned

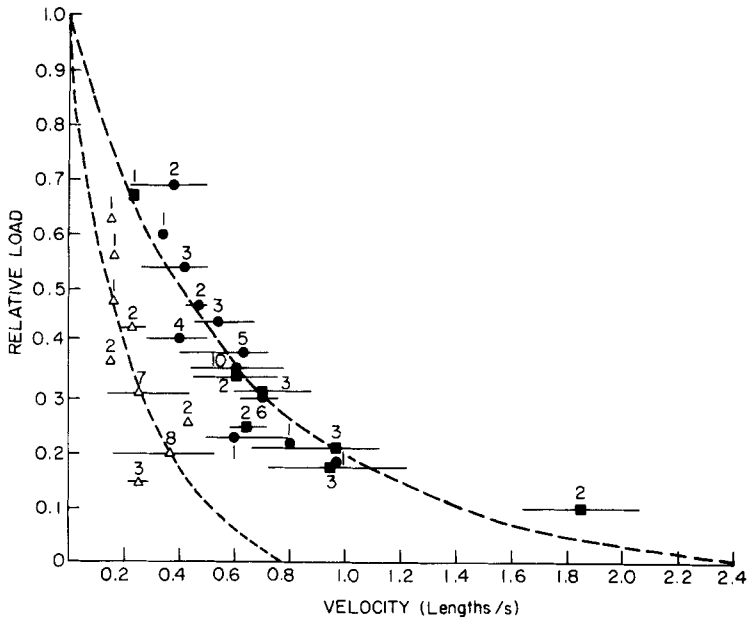

FIGURE 5. Summary of the effect of KCl concentration on the speed of shortening of skinned fiber segments in low Ca^{2+} at 5–7°C. The data were taken from 17 fibers contracting in 140 mM KCl (●), 11 fibers contracting in 175 mM KCl (■), and 15 fibers contracting in 50 mM KCl (△). The pCa of the bathing solution was 6.6–6.7 and the steady isometric force was 20–83% of that in high Ca^{2+} (pCa 5). The abscissa for each class interval is the average value of the velocity for the indicated number of observations; the ordinate is the midpoint of the class interval; the horizontal bars give the velocity range. The curves are hyperbolae fitted to the combined 140 and 175 mM data (upper line) and to the 50 mM data (lower line). V_{max} at high KCl concentrations is close to 2.4 muscle lengths/s; V_{max} in low KCl is 0.8 muscle lengths/s.

fiber was not directly affected by changes in the ionic strength of the relaxing solution. The resting tension at a sarcomere length of 2.4 μm was measured (in random order) in relaxing solutions with KCl concentrations of 50, 140, and 210 mM. The resting tension in the three solutions averaged 4.9, 4.8, and 4.9 mg wt (four fibers), respectively, and was, thus, independent of the KCl concentration over the range studied.

In the low KCl solutions, development of force by calcium activation was required before the resting tension increased. Force records of the first isometric contraction cycle of two different fibers in 140 and 50 mM KCl are shown in Fig. 6. Although the resting tension after contraction T in 140 mM KCl was the same as that present before the first contraction T_o (Fig. 6, right), activation in 50 mM KCl resulted in a large increase in the resting tension (Fig. 6, left). This increase was greatest after the first contraction; very much smaller increases were seen in subsequent contractions.

Fig. 7 shows T and T_o as a function of KCl concentration. The resting

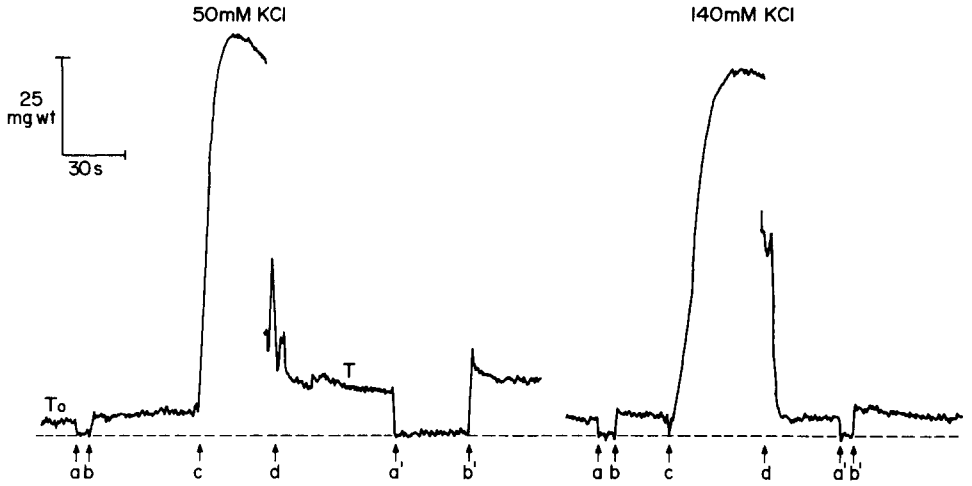

FIGURE 6. Contraction cycle of skinned fiber at 5°C. Left, preparation in solutions containing 50 mM KCl; right, another preparation in solutions containing 140 mM KCl. In both preparations, the sarcomere length was set at 2.4 μm after the fiber was skinned and transferred to relaxing solution; the resting tension T_o was determined by shortening the fiber until it just buckled (*a*). The sarcomere length was reset at 2.4 μm (*b*), the fiber was activated at pCa 5 (*c*) for about 30 s and then relaxed at pCa 9 (*d*). The zero of force was checked (*a'*) and the sarcomere length was reset at 2.4 μm (*b'*). Although T and T_o are nearly equal in solutions containing 140 mM KCl, T is greater than T_o after contraction in solutions containing 50 mM KCl. Fiber on the left, 23 vi 72, length 2.0 mm, sarcomere length 2.4 μm; fiber on the right, 12 v 72, length 2.4 mm, sarcomere length 2.4 μm.

tension following contraction increased progressively as the KCl concentration was reduced from 140 to 50 mM KCl. The increase in resting tension at low KCl concentrations was not affected when the concentration of EGTA in the relaxing solution was increased from 3 to 12 mM, and it also occurred after the activated fiber had been allowed to shorten isotonically.

IRREVERSIBILITY The residual tension ($T-T_o$) that appeared after contraction and relaxation in 50 and 100 mM KCl was not reduced by soaking the fiber in 140 mM KCl relaxing solution for periods of up to 30 min. In one of the nine fibers that were examined, however, an exceptionally large residual tension developed, which in this case fell to about half value during a 5-min immersion in 140 mM KCl relaxing solution. The experiments show that ionic strength changes alone have practically no effect on resting tension in skinned fibers but that residual tension develops following contraction in solutions with low KCl concentration and that this force cannot be reversed by increasing the ionic strength of the relaxing solution.

FIBER DIAMETER The resting tension could have developed because diffusion of ATP into the contracting fiber was so slow that the ATP concen-

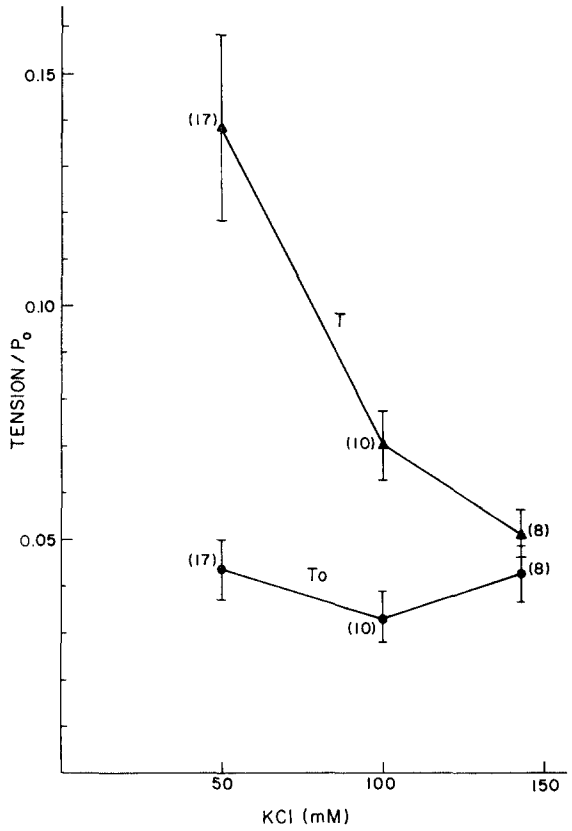

FIGURE 7. Effect of KCl concentration and activation at pCa 5 on the resting tension of skinned fiber segments at 5°C. T_o (●) is the average resting tension measured before the first contraction and T (▲) is the average resting tension after the first contraction in solutions with KCl concentrations of 50, 100, and 140 mM. The data for each segment are normalized according to P_o. The number in brackets is the number of preparations and the vertical lines give the standard error of the mean.

tration near the fiber axis fell to the level at which rigor bonds form. We examined this possibility in 50 mM KCl by comparing the ratio T/T_o in preparations having a wide range of cross-sectional area. As can be seen in Fig. 8, for nearly an order of magnitude increase in P_o (which corresponds to about a three-fold range of diameter) there was no change in T/T_o. Therefore, slow diffusion of ATP does not appear to account for the observed development of resting tension in the low KCl solutions.

With reference to the relation between P_o and cross-sectional area in 50 mM KCl, when a skinned fiber segment was split lengthwise into two nearly equal halves, the P_o in high calcium for each half was approximately half of that produced by a control segment from the same fiber. Thus, the proportionality between cross-sectional area

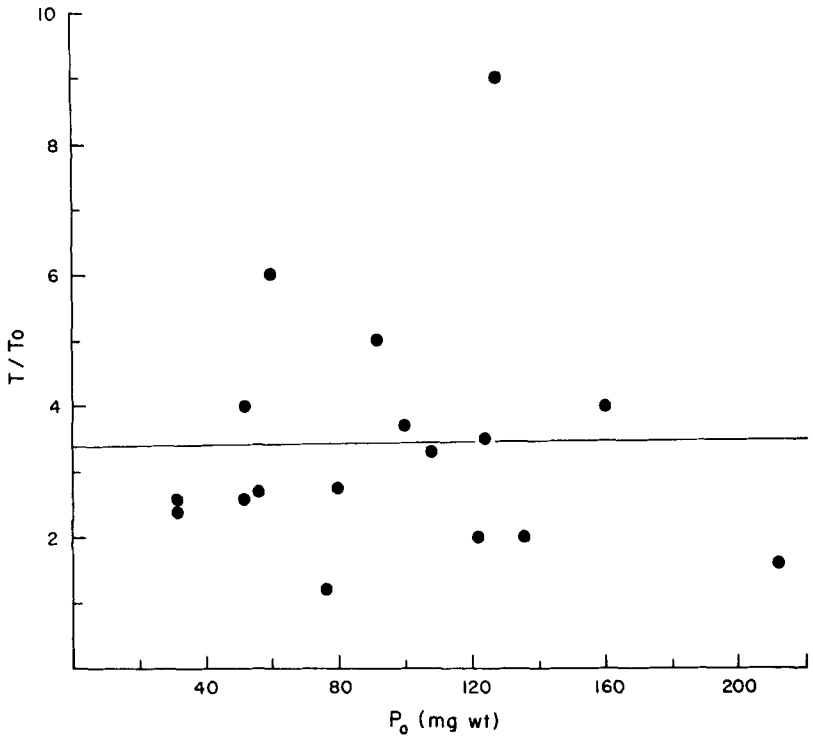

FIGURE 8. Effect of cross-sectional area on the resting tension of skinned muscle fibers after activation in 50 mM KCl. Abscissa, steady isometric force at pCa 5; ordinate, ratio of the resting tension after activation, and the resting tension before activation. The curve is the least squares fit of the data to a straight line. The data show that the increase in resting tension associated with activation is independent of fiber diameter over a three-fold range.

and P_o appears to be valid for fibers which contract in 50 mM KCl solutions as well as in 140 mM KCl solutions (Hellam and Podolsky, 1969).

ATP CONCENTRATION To further investigate the possible role of ATP concentration in the generation of residual tension, we studied the effect on resting tension of lowering the ATP concentration of the bathing solution from 5 to 2 mM; 30 mM potassium propionate was added to the solution to compensate for the reduction in ionic strength. Fibers were placed initially in 140 mM KCl relaxing solution, transferred to 140 mM KCl high calcium contracting solution, and returned to 140 mM KCl relaxing solution. The resting tension after contraction was unchanged. The same fiber was transferred to relaxing solution containing 50 mM KCl and, again, no increase in resting tension was observed over a 5-min period. Finally, the resting tension was measured following contraction in high calcium 50 mM KCl solu-

tion and was found to have increased from an average value of 5.2 mg wt (eight fibers, SEM, 0.3 mg wt) to 9.8 mg wt (SEM, 0.7 mg wt), a change similar to that observed in solutions containing 5 mM ATP (Fig. 7).

SARCOMERE LENGTH Since the increase in T in low KCl occurred only after contraction, it seemed possible that the development of residual tension depended on some interaction between the thick and thin filaments. This was tested by examining the influence of filament overlap on the development of residual tension. T_o was measured at an initial sarcomere length of 2.4 μm and the fiber was then passively stretched to a sarcomere length of 4.0 μm, placed in a contracting solution (50 mM KCl) for at least 1 min, returned to relaxing solution (50 mM KCl), and passively shortened to 2.4 μm where T was measured; the sarcomere lengths were calculated from the laser diffraction pattern. In three fibers, the residual tension at this stage was zero (T/T_o = 0.9–1.0). Each fiber was then returned to contracting solution with an initial sarcomere length of 2.4 μm and about a three-fold increase in T was noted after relaxation. These results show that the development of residual tension requires filament overlap as well as activation by calcium.

The dependence of the magnitude of the residual tension on sarcomere length was studied in fibers passively stretched in relaxing solution from a sarcomere length of 2.0 μm to 2.4, 2.9, and 3.5 μm, before and after activation (pCa 5.0) at a sarcomere length of 2.0 μm. Fibers in 140 mM KCl showed the same passive length-tension relation before and after contraction (seven preparations), while those in 50 mM KCl produced a greater resting tension at each sarcomere length after the fiber had been carried through a contraction cycle (seven preparations) (Fig. 9). Apparently the change in the fiber brought about by contraction in low KCl at 2.0 μm produces additional tension when the sarcomere length is increased.

ABILITY TO SHORTEN Residual tension was induced by contraction in a low KCl, high calcium solution. The fibers were then subjected to quick release from T to some tension less than T and force and displacement were recorded. The zero of force was taken as the base line. Fig. 10 shows a record of such a release. Following an initial quick displacement, there was isotonic shortening at a rate of 0.04 muscle length/s, which is less than $\frac{1}{10}$ the contraction speed of a calcium activated fiber at this relative load. In six preparations that had developed residual tension in 50 mM KCl, the fibers shortened with speeds ranging from 0.01 to 0.10 lengths/s at relative loads between 0.05 and 0.5. In contrast, fibers stretched to a sarcomere length of 3.0 μm before or after contraction in 140 mM KCl had a large resting tension but, when released to a fraction of this tension, produced only a quick displacement without further shortening in the following second (velocity $<$ 0.01 lengths/s). The increased tension produced by passively stretching the fiber

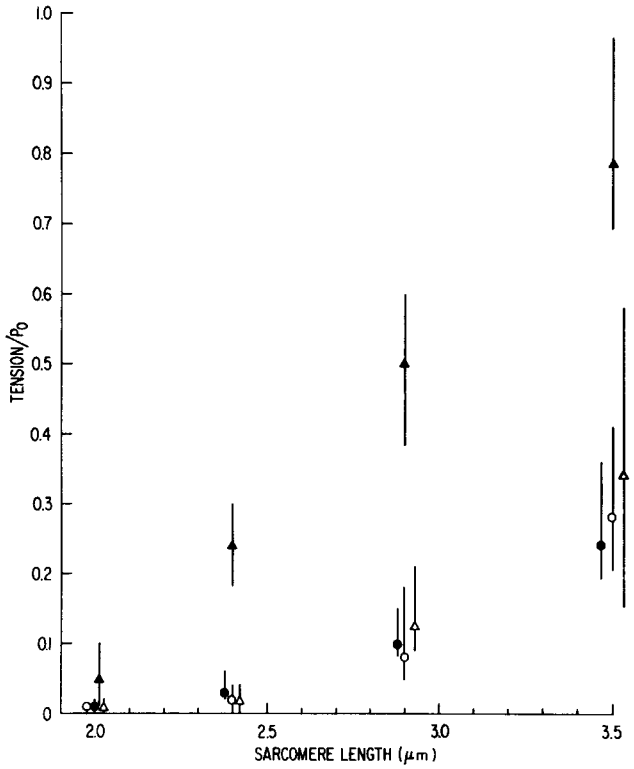

FIGURE 9. Effect of KCl concentration and contraction at sarcomere length 2.0 μm on the resting length-tension relation in skinned fiber segments at 5°C. Abscissa, sarcomere length; ordinate, the average value of the resting tension, normalized for P_o, before (open symbols) and after contraction (closed symbols) in solutions with pCa 5 and KCl concentrations of 50 (triangles, six fibers) and 140 mM (circles, seven fibers). The vertical bars give the range for each group.

and the residual tension observed following contraction in bathing solutions with low KCl concentrations seem to be basically different with regard to the ability to produce motion.

Magnitude of Velocity

The maximum speed of shortening obtained by extrapolation of the measured force-velocity data was 1.8–2.4 muscle lengths/s. In order to compare this result with previously reported data (Podolsky and Teichholz, 1970), the apparently minor differences in protocol for the two studies were examined.

The earlier data were obtained from fibers skinned in cold silicone fluid and in the present study fibers were skinned in mineral oil at room temperature. However, in the present solutions force-velocity measurements obtained from fibers skinned by both procedures were comparable.

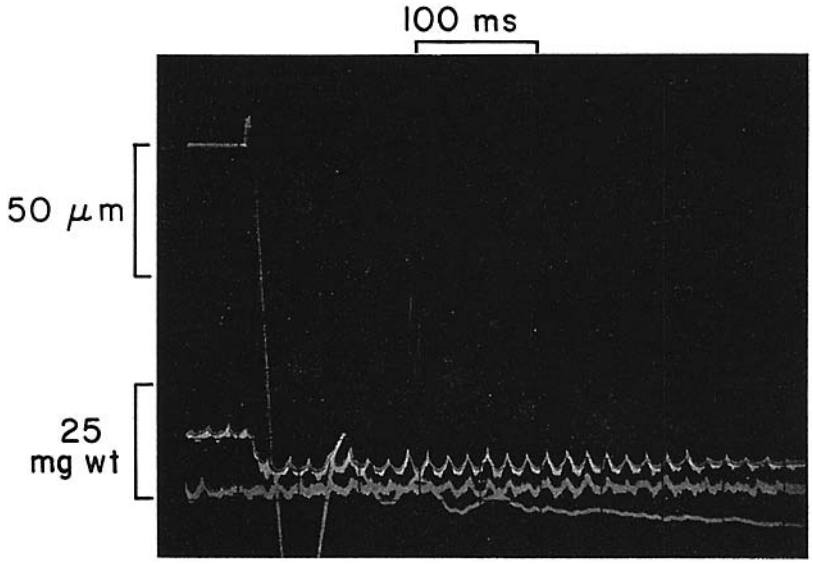

FIGURE 10. Quick release from resting tension after the development of residual tension. The upper trace is displacement, the middle trace shows the force, and the lower trace is the zero of force. The resting tension following contraction in 70 mM KCl pCa 5 solution was 12 mg wt. The fiber was released to a tension of 4 mg wt, where it shortened isotonically at 0.04 muscle lengths/s. Experiment of v 70, fiber from *Rana pipiens berlandieri* skinned in cold silicone oil, initial sarcomere length 2 2 μm, fiber length 1.5 mm, temperature 5°C.

The initial sarcomere length for the fibers used in the present experiments was 2.4–2.6 μm while those used by Podolsky and Teichholz (1970) were 2.0–2.2 μm. In three fibers the speed of shortening (in millimeters per second) for the same relative load was determined at different sarcomere lengths and was found to be independent of initial sarcomere length over the range 2.0–2.9 μm.

DISCUSSION

High KCl Concentration

The results summarized in Fig. 2 show that the calcium-activated force of skinned muscle fibers at 5–7°C decreases continuously as the KCl concentration in the bathing solution is increased; a similar response has previously been reported at room temperature (Gordon and Godt, 1969). The contraction velocity, however, increases and reaches a plateau at about 140 mM KCl. Thus in the concentration range between 140 and 280 mM, which will be called *high KCl*, the contraction velocity is unaffected by ionic strength even though the force is considerably reduced.

The simplest explanation of the effect of high KCl on the force and speed

of shortening of skinned fibers is that the number of sites at which cross bridges can be formed is reduced, but that the kinetic properties of the remaining sites are unchanged. The same kind of mechanism has been put forward to account for the mechanical changes seen when the degree of activation by calcium is reduced in solutions containing 140 mM KCl (Podolsky and Teichholz, 1970). Since calcium activates by binding to troponin, a protein associated with the actin-containing filament (Ebashi et al., 1968; Fuchs and Briggs, 1968), and since, at ionic strength 90 mM, one troponin molecule controls the activity of about seven actin molecules (Bremel and Weber, 1972), a possible explanation of the high KCl effect is that the conduction of information from calcium-saturated troponin to actin is, in some way, inhibited when the ionic strength of the activating solution is increased, so that in high KCl fewer than seven actin molecules are switched on when calcium binds to troponin. If this suggestion were correct, one would expect the activation of myosin ATPase by the actin-tropomyosin-troponin-calcium complex to be reduced at high ionic strength in vitro. Another possibility along the same line is that the availability of myosin sites is reduced when the ionic strength is increased.

When KCl is added to the actin-heavy meromyosin ATPase system, the ATPase rate in the presence of Mg^{2+} is reduced, apparently because the binding of actin to heavy meromyosin is reduced (Rizzino et al., 1970). This observation raises the possibility that the force of contraction in the physiological system is lowered by high KCl because the binding of myosin "heads" to sites on the actin-containing filament is decreased. Since this explanation also implies that f, the rate function for cross-bridge formation, is decreased by high KCl, the fact that the shortening velocity is unchanged by high KCl requires that the magnitude of f be large relative to the other parameters of the system, as argued by Podolsky et al. (1969), and not small, as suggested by Huxley (1957).

Low KCl Concentration

When the KCl concentration was reduced below 140 mM, which we refer to as the *low KCl* range, the contraction velocity was progressively decreased. This result was unexpected because intact fibers in hypotonic solution (which reduces the internal ionic strength) shorten at close to the normal speed (Howarth, 1958). This suggests that the decrease in velocity seen in skinned fibers in low KCl is probably associated with the increased resting tension that develops under these conditions. A similar association between increased resting tension and decreased velocity is found in intact fibers in hypertonic solution (Howarth, 1958; Hill, 1968; Lännergren and Noth, 1973); it is unlikely that the relatively slow velocity in this case can be attributed to the increase in internal ionic strength per se since skinned fibers in high KCl contract at the normal rate

By taking into account the responses of both intact and skinned fibers, it seems reasonable to conclude that contraction velocity is probably independent of ionic strength over a wide range provided the resting tension does not increase above the normal level. Increased resting tension appears to reflect the presence of an internal load within the fiber, which reduces the contraction speed observed with a given external load. This point will be taken up again below.

Residual Tension

Under our experimental conditions, a fiber that had been activated by calcium in solutions containing less than 140 mM KCl did not relax to the initial resting tension T_o but relaxed to a new resting tension $T > T_o$. The difference $T - T_o$ is called the *residual tension*[1]. This change in resting tension was not reversed in a relaxing solution containing 140 mM KCl. Since residual tension did not develop when the filaments were out of overlap and the calcium concentration was raised to pCa 5, the effect is not due to partial inactivation of the troponin-tropomyosin system at low ionic strength. It does not appear to be due to a deficiency of ATP within the fiber volume since its magnitude was unaffected when the fiber diameter was varied over about a three-fold range or when the ATP concentration in the bathing solution was reduced from 5 to 2 mM.

The mechanism responsible for the residual tension is not clear. However, since this force caused fibers in relaxing solution to shorten isotonically (Fig. 10) at a rate greater than that seen when the force on an extensively stretched fiber was lowered by a quick release, it is probably generated by cross bridges that form and can turn over in the absence of calcium. The additional observations that (*a*) the motion produced by the residual tension in relaxing solution is considerably slower than that produced by calcium-activated fibers in 140 mM KCl, and (*b*) residual tension is associated with the presence of an internal load during calcium-activated shortening, suggest that motion retarding interfilamentary links exist, both in the presence and absence of calcium, when residual tension is recorded. Thus two kinds of cross bridges seem to be formed when residual tension is measured in relaxing solution: cross bridges that are normal in the sense that they produce motion and those that are abnormal since they only retard motion.

[1] Some of the previous studies of perfused skinned fibers from this laboratory (Hellam and Podolsky, 1969; Ford and Podolsky, 1972) were made at room temperature (19–22°C) rather than at the low temperature (5–7°C) used here. The primary reason for working at a low temperature is to reduce the shortening velocity, which was not measured in the room temperature studies. However, another advantage of the low temperature is that fibers in solutions containing 140 mM KCl can be carried through a large number of reversible contraction cycles, which is difficult to do at room temperature. The question of whether the residual tension that develops at room temperature is related to that studied here has not been investigated yet.

Since the number of normal cross bridges increases when the fiber is activated by calcium, the effect of the abnormal cross bridges on the motion would be expected to be more conspicuous in partially activated, as opposed to fully activated, fibers. This appears to be the case, as the data in Fig. 4 show that the shortening velocity at a given relative load in solutions of low ionic strength (the condition required for the development of residual tension) decreases when the degree of activation is reduced.

These ideas are consistent with some recently published biochemical observations of Bremel and Weber (1972). These authors demonstrated that the actin-tropomyosin-troponin system activates myosin ATPase in the absence of calcium after S1, a subfragment of the myosin molecule, has interacted with actin-tropomyosin-troponin. In the present context, this result implies that a condition that leads to the formation of abnormal links between the myosin-containing and actin-containing filaments would also cause some of the sites on the actin-containing filament to become activated, and thereby allow normal cross bridges to develop tension in the absence of calcium. Furthermore, if the external load were reduced, the normal cross bridges would turn over and produce motion; the velocity would be relatively slow because of the retarding effect of the abnormal cross bridges.

The residual tension increased when a fiber that had been activated in low KCl at a sarcomere length of 2.0 μm was stretched to longer sarcomere lengths (Fig. 9). The reason for this is not known, since the number of sites available for forming abnormal cross bridges would be expected to decrease when filament overlap is decreased. However, the response could simply be an effect of strain on abnormal cross bridges that remain after the fiber is stretched. Additional studies are needed to fully explain this effect.

D. K. Hill (1968) recently described experiments with resting muscle in which he related a "short-range elastic component" with an active "filamentary resting tension." He suggested that the short-range elastic component was due to cross bridges present in the resting muscle, which is the same kind of concept as our abnormal cross bridge. The analogy can be carried a step further if we associate his filamentary resting tension with our residual tension. In intact fibers both the short-range elastic component and the filamentary resting tension increase in hypertonic solution, apparently because the spacing between the filaments is decreased. The fact that the filament spacing in skinned fibers is relatively insensitive to ionic strength (Matsubara and Elliott, 1972) could explain, at least in part, why the analogous effects are not seen in skinned fibers in high KCl.

Relation to Other Studies of Contraction Kinetics

MAGNITUDE OF THE CONTRACTION VELOCITY The speed of shortening in the present study was 1.5-2 times greater than that reported by Podolsky and Teichholz (1970). The experiments of Podolsky and Teichholz were made

in the summer with muscles from large "tropical" frogs that had been collected in Mexico near the Rio Grande valley; these animals belonged to the subspecies *Rana pipiens berlandieri*. The present study made use of muscles from *Rana pipiens pipiens* collected in the northern part of the United States, usually New England. In both studies the frogs were stored in the laboratory for periods up to several weeks at 5°C. Minor differences in technique (the type of oil used in the skinning step, the temperature of this oil, the initial sarcomere length before the quick release) did not affect the shortening speed. We believe, therefore, that the quantitative difference between the present results and those of Podolsky and Teichholz is due to the difference in experimental material. When the data were appropriately normalized, fibers from the two subspecies of frog responded to changes in ionic strength in the same way (compare open and closed symbols in Fig. 2; also note that the left-hand panels in Fig. 4 are essentially the same as Fig. 3 in Podolsky and Teichholz, 1970).

GLYCERINATED FIBERS The influence of degree of activation on contraction velocity has been examined in extensively glycerinated rabbit psoas fibers (Wise et al., 1971) and in lightly glycerinated frog muscle fibers (Julian, 1971). In both studies the authors found that the velocity at a given relative load appeared to be less at partial activation than at full activation.

The KCl concentration was 60 mM in the experiments with psoas fibers; the ionic strength of the other constituents of the bathing solution was about 55 mM (as opposed to a value of 50 mM for our solutions). If these were the only parameters involved in characterizing the contraction kinetics, one would expect the shortening speed under these conditions to be both relatively low (Fig. 2) and dependent on the degree of activation (Fig. 4). Wise et al. (1971) reported a V_{max} (about 0.4 length/s) which was probably lower than normal for the muscle used and the temperature at which the experiments were performed. Associated with the slow speed of shortening in high calcium was a strong dependence of shortening speed on the degree of activation.

The experiments with lightly glycerinated frog fibers were carried out in solutions similar to those used here, with the KCl concentration set at 100 mM. Fig. 4 shows that the contraction velocity of skinned fibers at partial activation is less than that at full activation in this solution but not in 140 mM KCl. Fig. 7 indicates that resting tension in the 100 mM KCl solution is only slightly increased above that in 140 mM KCl, but apparently the abnormal cross bridges associated with this effect are numerous enough to affect the motion when the number of calcium-activated bridges is relatively small.

Further experiments will be required to see whether the force-velocity relations for these preparations will be independent of the calcium ion level at

ionic strengths where this has been shown to be the case for skinned frog fibers.

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