Isotonic Contraction of Skinned Muscle Fibers on a Slow Time Base

Effects of Ionic Strength and Calcium

JAGDISH GULATI and RICHARD J. PODOLSKY

From the Laboratory of Physical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205. Dr. Gulati's address is Departments of Medicine, and Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461.

ABSTRACT The force development by calcium-activated skinned frog skeletal muscle fibers and the motion on a slow time base after a quick decrease in load were studied at 0–1°C as a function of the ionic strength and the degree of activation. The ionic strength was varied between 50 and 190 mM by adding appropriate concentrations of KCl to the bathing solution. Under these conditions, the fibers could be maximally activated for several cycles at low ionic strength without developing residual tension. We found that the steady isometric force in fully activated fibers linearly decreased when the KCl concentration was increased from 0 to 140 mM. The steady isotonic motion at a given relative load in fully activated fibers was almost the same at KCl concentrations ≥50 mM. In 0 and 20 mM KCl, the isotonic velocity decreased continuously for more than 300 ms. At a given relative load, the initial velocity of the motion in 0 and 20 mM KCl was about 0.6 and 0.9 times, respectively, that in 140 mM KCl. The initial velocity decreased further when residual tension developed; this observation provides additional evidence that residual tension may reflect the presence of an internal load. The effect of calcium on the motion was examined at 70 mM KCl. In this solution, the motion during the velocity transient at a given relative load appeared to be the same at different levels of activation. The speed of the subsequent motion was almost steady at high calcium levels but decreased continuously in low calcium levels. These results support the idea that at low ionic strength the response of the fiber to calcium is switch-like, but that other factors also affect the contraction mechanism under these conditions.

INTRODUCTION

The effects of ionic strength and calcium on the isotonic contraction transients that occur in skinned muscle fibers during the first 10–50 ms of isotonic motion after the application of load steps in quick-release experiments were recently described (Gulati and Podolsky, 1978). The temperature was kept near 5°C to study the effect of high ionic strength (>190 mM), but when

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ionic strength was varied in the low range (<190 mM) the temperature was decreased to 0°C because the fibers develop irreversible residual tension (measured as the increase in resting tension after activation) in ionic strengths <190 mM when they are activated with calcium at 5–7°C, but do not at 0°C. The presence of residual tension complicates the interpretation of ionic strength and calcium effects on the contraction properties of skinned fibers (Thames et al., 1974) and this was avoided at 0–1°C.

This study describes the effects of low ionic strength and calcium at 0–1°C on the motion beyond the 10–50 ms after the load step. As in previous studies (Thames et al., 1974; Gulati and Podolsky, 1978), ionic strength was controlled by adding appropriate concentrations of KCl to a 50 mM ionic strength solution. For fully activated fibers, this motion was almost the same at KCl concentrations ≥50 mM. In contrast, at lower KCl concentrations, the displacement trace during isotonic motion was curved and the speed decreased continuously as shortening proceeded. The initial velocity of the motion at 20 mM KCl was close to the steady motion at higher ionic strengths, whereas that at 0 mM KCl was significantly less. Repeated activation in 20 mM KCl caused residual tension to appear, and this was associated with a decrease in the initial velocity of the motion.

As regards the effect of calcium, it was found that at 70 mM KCl, the character of the isotonic motion on a slow time base depended on the degree of activation. At high calcium levels, the displacement trace after the velocity transient was nearly linear, but at low calcium levels, the trace was markedly curved. This is different from the influence of calcium on the motion at 140 mM KCl where the displacement trace was linear at both high and low calcium levels and the contraction velocity depended only on relative load (Podolsky and Teichholz, 1970; Gulati and Podolsky, 1978).

Some of this work has already been reported briefly (Gulati and Podolsky, 1974 and 1976).

METHODS

The preparation of skinned fibers from the semitendinosus muscle was as described previously (Gulati and Podolsky, 1978), except that the present data were obtained with preparations from a single species of frog, *Rana pipiens pipiens*. The bathing solution and the experimental procedure were also as described before, with the exception that the time base for the present records was generally 2.5 times slower. The fiber in the relaxing solution was initially brought to near rest length so that the laser (He-Ne gas laser, λ = 6,320 Å beam size 0.8 mm, incident approximately normal to the fiber; model 133; Spectra-Physics Inc., Mountain View, Calif.) diffraction pattern indicated a sarcomere length of 2.2–2.3 μm.

Fiber Selection

In each experiment the fiber was carefully examined with a Wild 5A stereomicroscope (Wild Heerbrugg Instruments Inc., Farmingale, N. Y.) under a magnification of 80 after each of the following steps: (*a*) skinning in cold oil, (*b*) tying of the knot over the notch that forms the end of the attachment rod (flattened, 0.011-inch-diameter stainless steel wire) of the displacement lever, (*c*) tying of the knot over the notch in the identical attachment rod of the force transducer, (*d*) transferring the tied fiber to

relaxing solution and stretching its length to near rest length. The fiber was also examined throughout the first activation and on return to the relaxing solution after each contraction. The fiber was rejected if at any step there were visible nonuniformities, contraction bands, or small nicks along its length. The first activation was generally found to be most critical for discovering these imperfections and a number of fibers pulled out partially or completely through the knots at this stage even though they seemed to be good specimens under the microscope. With these precautions, only about one in five fibers was found to be acceptable for further experimentation. Because sarcomeres were not resolved with the stereomicroscope, some uncertainty regarding the sarcomere uniformity remained. However, when the striation pattern was checked later with immersion optics and laser diffraction (see below), the above criteria were found to be sufficiently stringent to insure sarcomere uniformity.

Striation Patterns During Contraction

In parallel experiments, the first-order laser-diffraction patterns from contracting fibers were compared with the patterns of fibers in the relaxing solution. The laser beam was focused with an Oriel beam expander (pinhole size 50 μm; Oriel Corp. of America, Stamford, Conn.) to ~100 μm in diameter and the diffraction patterns were projected on a screen 5–6 cm above the fiber. The sarcomere length was set at a value between 2.7–3.0 μm because the laser pattern in the activated fibers was most stable under these conditions (see Baskin et al. [1979]). A 1.7-μm diffraction grating was used to calibrate these patterns. In another set of experiments, at 3–4°C, the fibers were visualized with a 40× oil-immersion objective (1.00 numerical aperture, 2.5-mm working distance; Bausch & Lomb, Inc., Rochester, N. Y.) which made contact with the bathing solution. Photographs were made with 6× and 20× oculars and a Polaroid camera (3,000 ASA speed, film 107; Polaroid Corp., Cambridge, Mass.). Sarcomere length was determined by calibrating the micrometer in the 20× ocular with a 10-μm grating. The sarcomere length was set between 2.0 and 2.3 μm, which was the condition used in the mechanical experiments.

Experimental Procedure

In the quick-release experiments, the isotonic motion of the fibers in a test solution was compared with the response in the control solution at the same relative load. For low ionic strength experiments at pCa = 5, the match in relative load was obtained by first recording the force step and displacement during a quick release in the test solution. The set of traces for a given release was completed by recording the zero of force after the fiber was returned to the relaxing solution (140 mM KCl). The three traces were photographed and the relative load was measured (see below). The fiber was then transferred to 140 mM KCl activating solution and additional releases were made until a suitable match to the test release was obtained. With practice this could be done in three to five releases. This procedure made it possible to obtain a number of matched releases in low ionic strength test solutions in the absence of residual tension. A similar protocol was followed for partially activated fibers, but in this case the fibers were recycled in the low KCl solution because the development of residual tension was not a problem under partial activation.

Data Analysis

The length (L_0) of the fiber between ties was measured with a micrometer in the eyepiece (20×) of the steromicroscope. The temperature was generally 0–1°C in low

ionic strength solutions (≤190mM) and 5–7°C in high ionic strength solution (≥190mM). The following notation was used in analysis of the records:

P_0	Isometric force developed by the skinned fiber under a given experimental condition
P_L	Load on the fiber when the contraction is quickly changed from isometric to isotonic
P_L/P_0	P_{rel}
β	Degree of activation, defined as ratio of the isometric force (P_0) at a given pCa to the force at pCa = 5 in solution with the same ionic strength
v_t^i	Velocity of shortening at t ms after the application of the load step. The superscript i refers to the KCl concentration of the activation medium. When $t = 0$, the speed is the initial velocity of shortening measured immediately after the force step.
c	Curvature of the isotonic displacement response of the fiber, defined as the ratio $v_t/v_{(t+250)}$

The relative load for a given record was found by first measuring the mean value of P_0 from the force trace before the quick release and the zero of force trace at the same point. P_L was similarly determined within 50–100 ms after the quick release. The fractional uncertainty of a given P_{rel} could be as great as 0.05–0.1 because (*a*) $P_{rel} < P_0$, and (*b*) the force transducer drifted slowly. Noise in the tension trace before the lever was released introduces additional uncertainty, but this factor generally is negligible compared with the others already mentioned.

The curved displacement traces were analyzed by fitting a single exponential to the data. The traces were digitized and the function $A_1 \cdot e^{-A_2 t} + A_3$ was fitted to the data set by a computer program based on the algorithm of Marquardt (1963) for least-square estimation of nonlinear parameters. An example of this technique is shown in Fig. 1. The initial velocity, v_0^i, was taken as $A_1 \cdot A_2$ and the curvature, c, as e^{250A_2}.

The uncertainties associated with mean values are standard errors of the mean.

RESULTS

1. Activation of Skinned Fibers in Low Ionic Strength: The Delayed Appearance of Residual Tension at 0–1°C

The activation of frog skinned muscle fibers at low ionic strength is known to produce effects on both the calcium-sensitive force and the development of residual tension (Gordon et al., 1973; Thames et al., 1974). Residual tension has been defined as the increment in force seen in the relaxing solution of a given ionic strength over the original (before activation) resting tension of skinned fiber in the standard relaxing solution containing 140 mM KCl. The level of residual tension appeared to be sensitive to temperature because at the same value of (low) ionic strength, it was considerably less at 5–7°C (Thames et al., 1974) than at room temperature (Gordon et al., 1973). Thus, in efforts to separate the two effects of low ionic strength, i.e., the effect on calcium-activated tension and the effect on residual tension, we carried out similar experiments with fibers activated at 0–1°C. At this low temperature, the development of residual tension was delayed, as shown in Fig. 2.

Two cycles (the first and the fifth) of activation-relaxation are shown. The fiber in the relaxing solution was brought to near rest length and the initial resting tension of the fiber was ~1 mg. In each cycle from the first to the fifth, the fiber was activated by calcium in 140 mM KCl and then relaxed. This was followed by activation in 20 mM KCl and finally relaxation. (The relaxing solution in each case contained 140 mM KCl.) At the end of each cycle, the fiber was manually shortened by ~5% of its mounted length to find

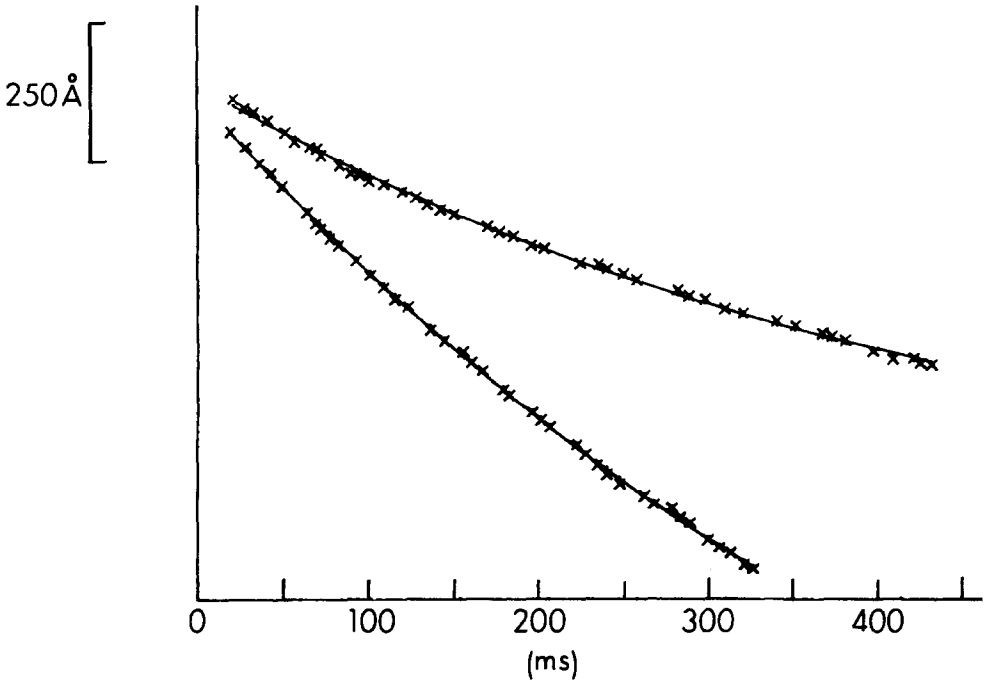

FIGURE 1. Analysis of curved displacement traces. The traces in this example are from the experiment depicted in Fig. 9. The recorded traces were digitized and plotted as crosses; the *lower set* of *crosses* is from the *left panel* of Fig. 9 and the *upper set* is from the *right panel.* The *solid lines* are plots of the best exponential fits to each set of crosses. The zero of time was found by back extrapolating the steady value of the force trace after the step change in load from P_0 to P_L and marking its intersection with the instantaneous force trace during the step (see Fig. 1 of Gulati and Podolsky [1978]).

the zero of force and the resting tension. The fiber was restretched to its original rest length before the next cycle. This procedure of manually shortening the fiber was followed, in part, to compensate for slow drift in the baseline of the force transducer. The resting tension at the end of the first cycle was the same as that before activation (~1 mg). This value was unchanged after the second, third, and fourth cycles. At the end of fifth cycle the tension measured in this way increased to 2–3 mg (~0.1 P_0 in 140 mM KCl). These observations indicate that the onset of residual tension (i.e., the increase in resting tension)

is delayed at a low temperature, even though the effect of ionic strength on calcium-activated tension is seen throughout. Once the residual tension set in, it was irreversible. The onset of residual tension in 0 mM KCl solution occurred sooner, usually after the second cycle. The subsequent studies of the effect of low ionic strength on the isometric and isotonic properties of the skinned fibers were therefore made before the development of residual tension

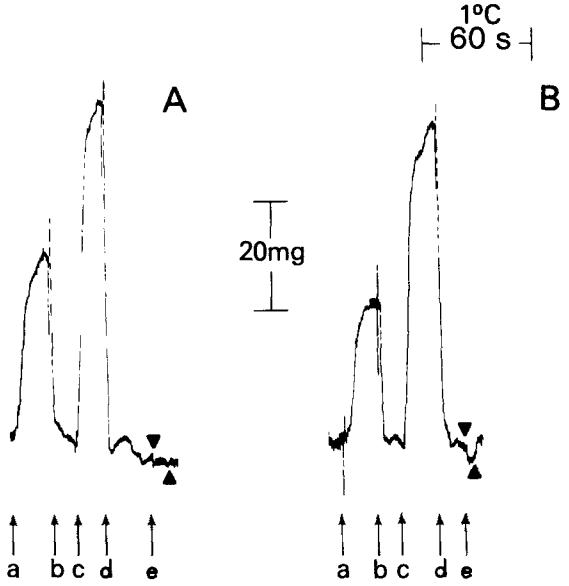

FIGURE 2. The activation of frog skinned fibers in 20 mM KCl solution (pCa = 5.0) at 0–1°C. The fiber was immersed in control relaxing solution containing 140 mM KCl, 5 mM Na_2ATP, 1 mM $MgCl_2$, 5 mM EGTA, and 10 mM imidazole (pH = 7.0 at 0°C). At *a* the fiber was transfered to the control activating solution at pCa = 5.0. The concentration of (EGTA + Ca-EGTA) was fixed at 5 mM. At *b* the fiber was relaxed in the control relaxing solution. At *c*, it was activated in test solution with KCl reduced to 20 mM. At *d*, the fiber was relaxed again as at *b* in the control relaxing solution (KCl = 140 mM). At *e*, the fiber was tested for the presence of residual tension by manually shortening (▼) the fiber by ~5% of its length, L_0, and then restretching (▲) it back to L_0. (*A*) First cycle of activation and relaxation. (*B*) Fifth cycle of activation and relaxation. Notice that the residual tension is present only in *B*. Experiment 24 vi 74; L_0 = 1.5 mm.

except in cases where the effect of residual tension on the isotonic motion was examined specifically.

2. Steady Isometric Force as a Function of Ionic Strength of the Activating Solution (pCa = 5): The Effect of Ionic Strength in the Absence of Residual Tension

The effects of ionic strength on isometric force development in the absence of residual tension was determined by comparing the steady force levels reached

in activating solutions of different ionic strengths (pCa = 5; range of KCl: 0–210 mM; range of ionic strength: 50–260 mM) with the force level in 140 mM KCl (control) activating solution. The results are shown in Fig. 3. Ratios of force developed in test solutions containing different amounts of KCl to the force in 140 mM KCl (control) solution were plotted as a function of KCl concentration. The steady isometric force decreased linearly with increasing KCl such that the force in 0 mM KCl was about twice that in 140 mM KCl. Raising the KCl concentration from 140 mM to 210 mM caused a similar

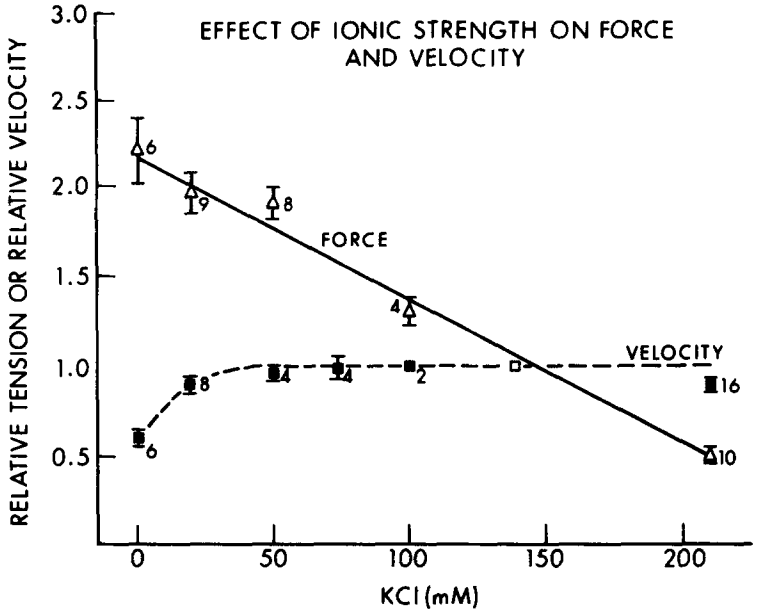

FIGURE 3. Effect of ionic strength on force and velocity. Relative tension (△) and velocity (■) normalized to the values for these parameters in control solution (□). The velocity measurement for each KCl concentration is described in the text. The results were obtained from nine different frogs. The numerical values shown next to the points indicate the number of matched points used for determining the mean value at a given ionic strength. The temperature was 0–1°C below 140 mM KCl and ~5–6°C in 210 mM KCl. *Bars*, SEM.

decrease in the force by a factor of two. The results in low ionic strength (<140 mM KCl) were obtained at 0–1°C and the results in high ionic strength were obtained at ~6°C.

3. Effect of Ionic Strength on Isotonic Motion at pCa = 5

A. HIGH IONIC STRENGTH The study of isotonic motion was begun by examining the effect of raising the ionic strength on the steady isotonic motion of the frog skinned fibers. Experiments were made in activating solutions containing either 140 mM KCl (control) or 210 mM KCl (test) at ~6°C. The

ratios of steady speeds (v^{210}/v^{140}) in the two solutions were determined for various values of matching relative loads on four different fibers and the results are plotted in Fig. 4. The typical isotonic force and displacement records used for determining these velocity ratios were similar to those shown in the earlier paper (see Fig. 4 of Gulati and Podolsky [1978]). The results in Fig. 4 show that for matching relative loads in the range from 0.2 to 0.8 P_0, raising the ionic strength has little effect on the steady speeds. The mean ratio of steady speeds at high KCl to control is found to be 0.90 ± 0.02. This is recorded as a point (filled-in square) for 210 mM KCl on the velocity curve in Fig. 3. This finding extends the earlier results showing almost no effect of high KCl on the speed of shortening at a relative load of 0.3 P_0 (Thames et al., 1974).

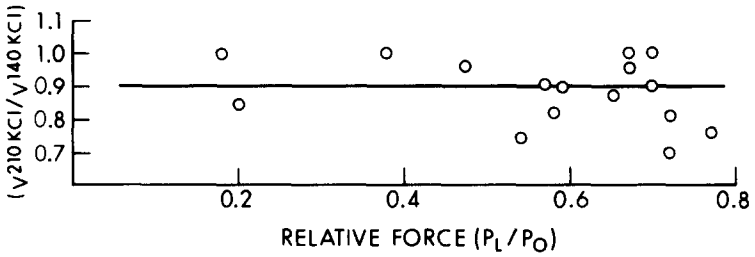

FIGURE 4. The effect of high KCl on the shortening speed. The velocity in 210 mM KCl at a given relative load is normalized to the velocity at the same relative load in 140 mM KCl. The data from four fibers are shown. The line was drawn to show the mean value of all the points. This value (0.90 ± 0.02) is the same as shown in Fig. 3 for KCl = 210 mM. The range of L_0 was 1.2–3.5 mm.

B. LOW IONIC STRENGTH

(*i*) *Isotonic Motion in the Absence of Residual Tension*

(*a*) *50 mM KCl* The effects of lowering the ionic strength on the shortening response of the skinned fibers were studied in 50, 20, and 0 mM KCl solutions. The typical results in 50 mM KCl solution are shown in Fig. 5. As discussed previously, the amplitude of the isotonic velocity transient is very small in this solution (Gulati and Podolsky, 1978). The shortening speed near the end of the transient is unaffected by low ionic strength in the 50 mM KCl solution. This is seen by comparing slopes of the *dashed lines* in the *top traces* of the *left* and *right panels* in Fig. 5, which measure the steady shortening phase of the motion in the test and the control solution.

(*b*) *20 mM KCl* The results are more complex in solutions containing 20 mM KCl. This is seen in Fig. 6 which shows records at relative loads of 0.55 P_0 (*upper panels*) and 0.25 P_0 (*bottom panels*) in both the control (140 mM KCl, *left panels*) and the test (20 mM KCl, *right panels*) solutions. The records in the *upper panels* were made on one fiber and those in the *bottom panels* on another. The velocity transients were completely suppressed in the solution containing

20 mM KCl (Gulati and Podolsky, 1978). Also the displacement trace is seen to be curved at this low ionic strength, so that the shortening speed decreases continuously as the shortening distance increases. Close inspection of the data shows that there is a small curvature even in the control records; this curvature was variable from fiber to fiber and was always less than that in the test solutions. The measurements of the curvature from nine preparations are summarized in Table I. The results show that in 20 mM KCl, the velocity at 250 ms was generally about one-half that at the start of the isotonic motion, and that the effect is somewhat greater at low loads ($P_{rel} \leq 0.4\ P_0$) than at high

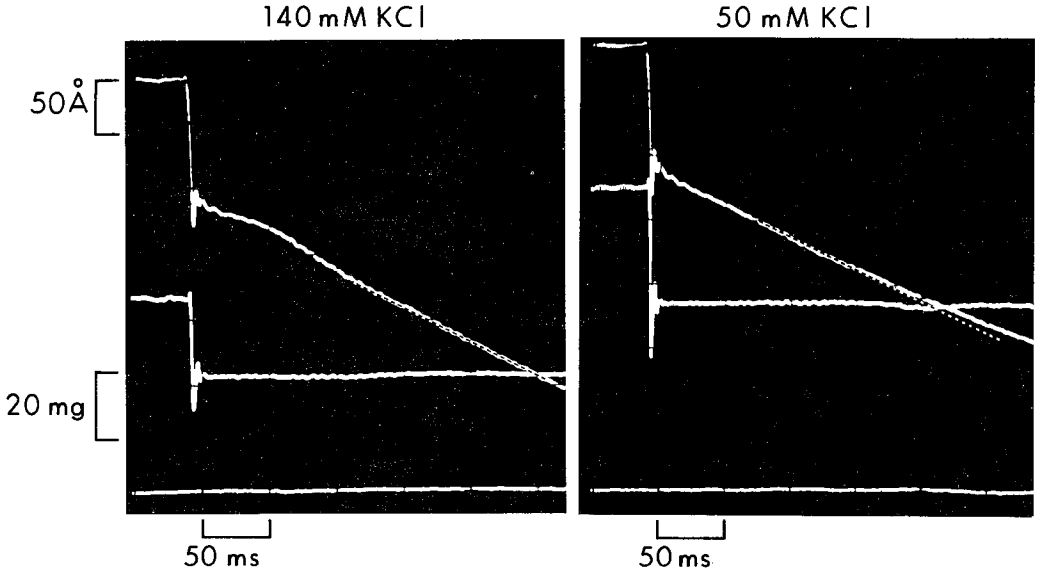

FIGURE 5. Isotonic displacement response of a skinned fiber in 50 ml KCl at 0°C. *Top traces*, displacement; *middle traces*, force; *bottom trace*, zero of force. Fiber length (L_0) = 1.9 mm. Experiment 24 ix 74. Isometric force, P_0: 140 mM KCl, 56 mg; 50 mM KCl, 86 mg; P_{rel} = 0.60. The *dashed lines* are the estimates of the speeds at the end of the velocity transients. Note that the speeds are almost equal. The displacement trace marker (*50 Å*) gives the motion/half sarcomere.

loads ($P_{rel} \geq 0.4\ P_0$). There was a sixfold range of force in these preparations (which corresponds to the more than twofold range of diameter (Hellam and Podolsky, 1969)). The magnitude of the force does not seem to be a factor in the curvature, which suggests that the fiber diameter (and, therefore, substrate limitation) has no influence on the curvature. However, because all but two of these experiments were within only a twofold range of force, more data points at the extremes of the range would be needed to establish this point conclusively.

To see the effect of ionic strength on contraction velocity, the results in Fig. 6 were analyzed and the initial speeds (v_0^{20}) in 20 mM KCl at each relative load were compared with the steady speeds (v_{steady}^{140}) at corresponding relative

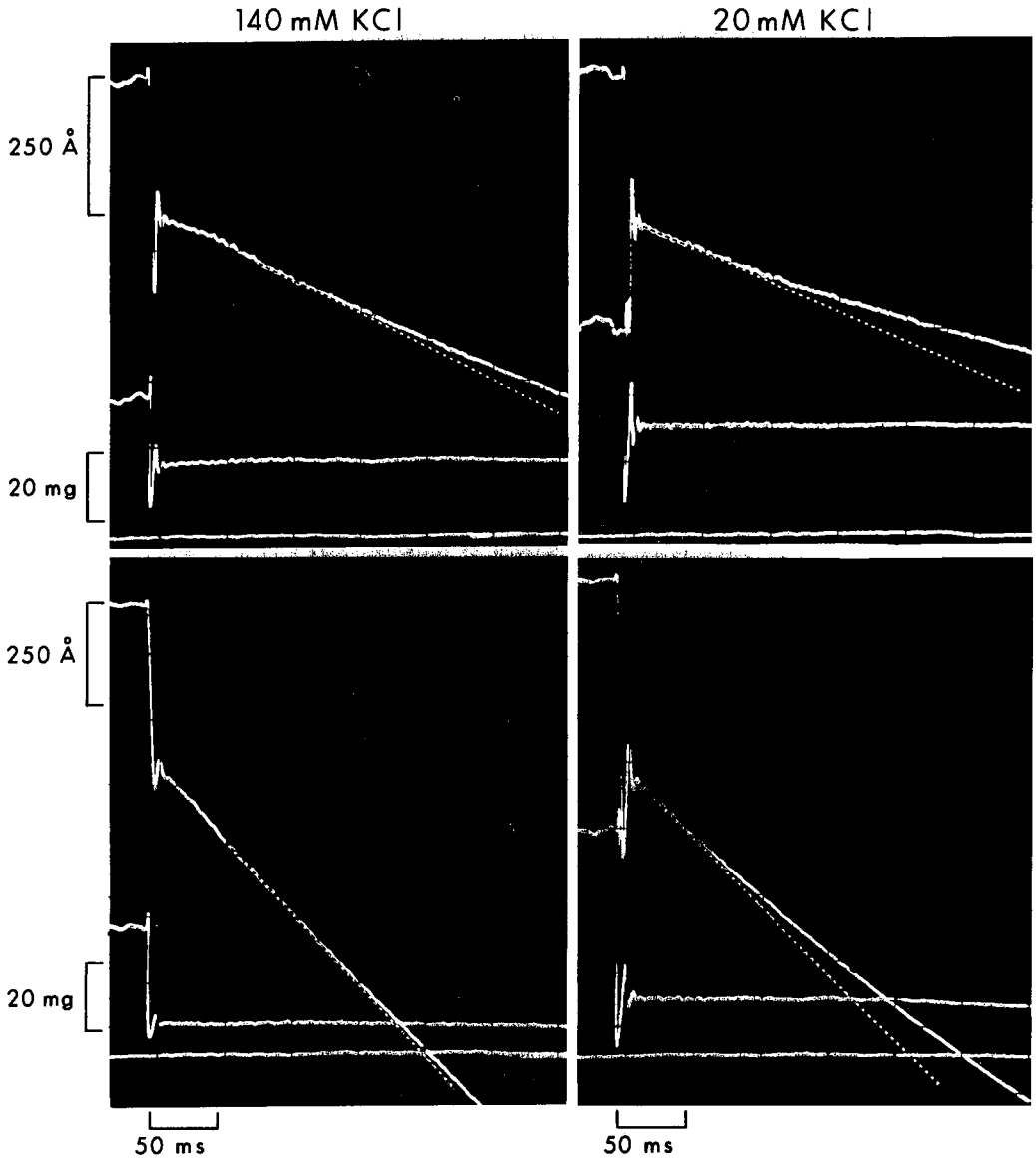

FIGURE 6. Isotonic displacement response in 20 mM KCl at 0–1°C. In each panel: *top trace*, displacement; *middle trace*, force; *bottom trace*, zero of force. *Top panels*: experiment 8 i 74a; L_0 = 0.8 mm. P_0 in control, 41 mg; P_0 in 20 mM KCl, 60 mg; P_{rel} = 0.55. *Bottom panels*: experiment 8 i 74b; L_0 = 1.5 mm; P_0 in control, 36 mg; P_0 in 20 mM KCl, 66 mg; P_{rel} = 0.24. The *dashed line* in the left panel is an extrapolation of the speed at the end of the velocity transient and indicates the steady speed. In the *right panels*, the *dashed lines* represent the initial speeds of shortening calculated from the computer fit of the curved trace. The curvature calculated for the fitted displacement curve is 1.7 in the *upper right panel* and 1.4 in the *lower right panel*.

loads in the control solution. These results and others for five fibers are summarized in Fig. 7 where the ratio $v_0^{20}/v_{steady}^{140}$ is plotted as a function of relative load from 0.1 to 0.6 P_0. The mean value for all these points was found to be 0.9 ± 0.1, which is shown by the *solid line* in Fig. 7. Statistical analysis of

TABLE I

CURVATURE OF THE ISOTONIC MOTION IN 20 mM KCl

Experiment	Force (P_0) in solutions of pCa = 5; 140 mM KCl	P_{rel}	Curvature in 20 mM KCl
	mg		
24 ix 73	40	0.06	2.0
18 xi 73	20	0.12	1.6
22 xi 73	39	0.48	1.5
23 xi 73	45	0.26	1.9
24 xi 73	39	0.35	2.3
25 xi 73	57	0.09	1.8
8 i 74a	43	0.56	1.5
8 i 74b	40	0.25	1.9
22 ii 74	30	0.52	1.3
26 ix 74	130	0.70	1.3
Range of P_o	20–130		
Range of P_{rel}		0.06–0.70	
Mean ± SEM			1.7±0.1

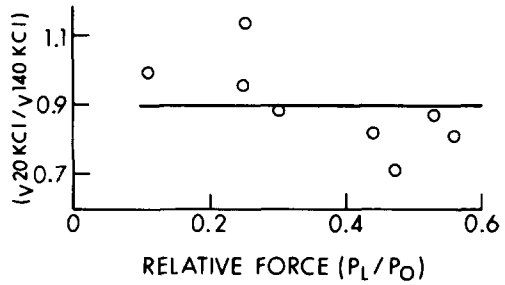

FIGURE 7. The effect of 20 mM KCl on contraction velocity at various loads. The initial velocity in 20 mM KCl at a given relative load is normalized to the steady speed in 140 mM KCl control solution at the same relative load. The data from five fibers are shown. The *solid line* represents the mean of all values. This value (0.9 ± 0.1) is also plotted in Fig. 3. The least-squares fit (not shown) to the points is y = 1.12 − 0.60 x; the standard errors of the intercept and slope are 0.09 and 0.23, respectively.

the data (see legend to Fig. 7) shows a tendency for the velocity ratio to decrease with increasing relative load, and that at low relative loads, the velocity in the two solutions is the same within the experimental error of ~10%.

(*c*) *0 mM KCl* The results in 0 mM KCl, where ionic strength due to other constituents in the solution is estimated to be ~50 mM, are shown in Fig. 8. The amplitude of the velocity transient is suppressed and the displacement trace is curved. The initial speed in 0 mM KCl was 0.5 times the steady speed in 140 mM KCl control solution when the relative loads were matched (*dashed line* in Fig. 7). The ratio of these speeds (v_0^0/v_{steady}^{140}) at nearly matching relative loads on four fibers ranged from 0.4 to 0.8. The average value for the curvature in the isotonic motion of these fibers was 1.5 ± 0.1, which is close to the value for 20 mM KCl. The influence of KCl on both the speed and the curvature of the motion in the absence of residual tension was completely reversible.

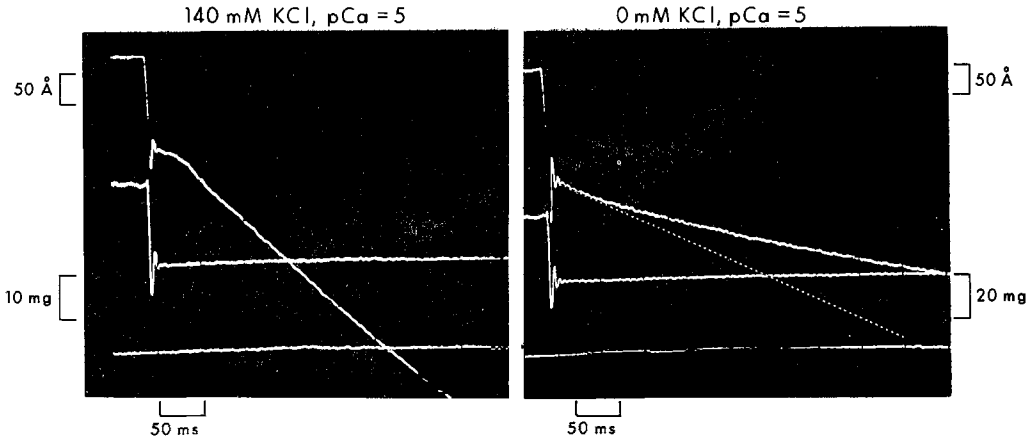

FIGURE 8. Isotonic displacement response in 0 mM KCl at 0–1°C. *Top trace*, displacement; *middle trace*, force; *bottom trace*, zero of force. Experiment 17 v 75; L_0 = 1.8 mm; P_0 in control, 37 mg; P_0 in 0 mM KCl, 63 mg; P_{rel} = 0.52. The *dashed line* in 0 mM KCl represents the initial speed calculated from the computer fit of the data. Curvature in 0 mM KCl is 1.5. Note that the initial velocity in 0 mM KCl is 0.5 times that of the steady motion in 140 mM KCl.

The effects of ionic strength on the steady speeds of shortening in high KCl and the initial speeds of shortening in various low KCl solutions are summarized in Fig. 3.

(*ii*) *Isotonic Motion in the Presence of Residual Tension* To study the way in which the shortening response of the fibers was affected by the presence of residual tension, the isotonic motion at nearly matched relative loads was compared before and after residual tension appeared in 20 mM KCl, as shown in Fig. 9. A residual tension of ~10 mg was present when the response in the *right panel* was recorded. The *left panel* record was obtained before the residual tension appeared. The isotonic velocity transient is suppressed and the displacement trace is curved in both cases. The initial speed obtained from the fitted exponential (Methods) decreased twofold in the presence of residual tension. The curvature was nearly the same in both cases.

4. Effect of Calcium Concentration in Low (70 mM) KCl Solution on Isotonic Motion in the Absence of Residual Tension

Previous work at 5–7°C showed that the relative force-velocity relation of frog muscle fibers is unaffected by the level of ionized calcium in bathing solutions containing 140 mM KCl, but that at lower salt concentrations the calcium effect is more complex (Julian, 1971; Thames et al., 1974). Because it appeared that the complexity was associated with the presence of residual tension, it seemed worthwhile to reexamine the calcium effect in low salt at 0–1°C, where the development of residual tension is delayed.

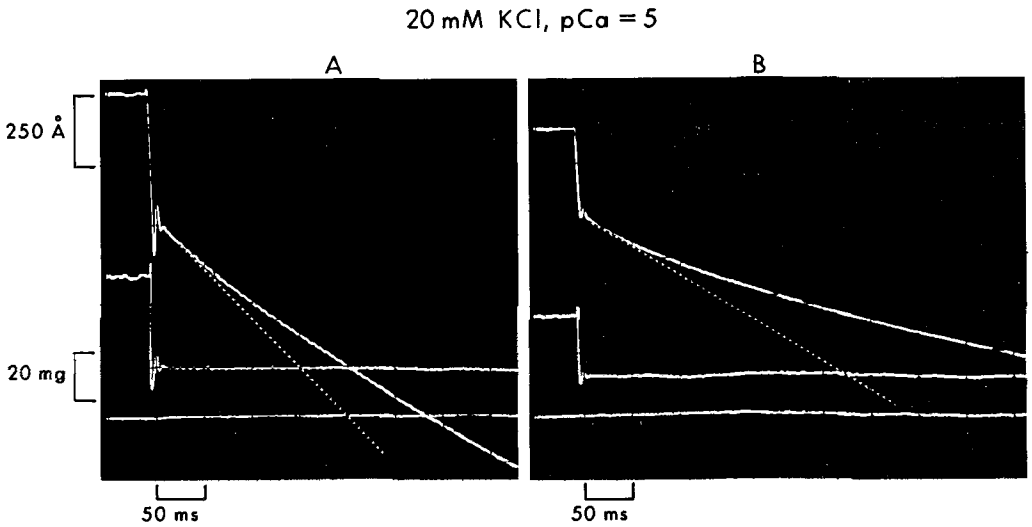

FIGURE 9. Effect of residual tension on isotonic motion in 20 mm KCl. Experiment 8 i 74b; L_0 = 1.7 mm. T = 1°C. *Top traces*, displacement; *middle traces*, force; *bottom traces*, zero of force. *Left panel*: P_0 = 58 mg, no residual tension present, P_{rel} = 0.34; *right panel*: P_0 = 42 mg, residual tension = 10 mg, P_{rel} = 0.38. There were 10 activations in 20 mM KCl before the release in the right panel was recorded. The initial speeds calculated from the computer fits are indicated by the *dashed lines* in both panels. Note that the initial speeds in the presence of residual tension is ~0.6 times that in control. The curvature values are 1.5 and 1.6 in the *left* and *right panels*, respectively.

Typical results in 70 mM KCl are shown in Fig. 10 which contains records of the isotonic motion of a fiber in three different solutions. The relative load was the same in each case. The control record in 140 mM KCl is shown in the *left panel*. In the *middle panel*, KCl was 70 mM, pCa was 6.9. The steady force was close to that in the *left panel* and the degree of activation (β) was estimated to be ~0.9. The shortening velocity at the end of the fast isotonic transient (Podolsky et al., 1974) was close to that in the control solution.

At a lower calcium level, where the steady isometric force was decreased about threefold (β = 0.3), the shortening trace in 70 mM KCl contains a fast transient and is then curved (Fig. 10, *right panel*). The motion during the

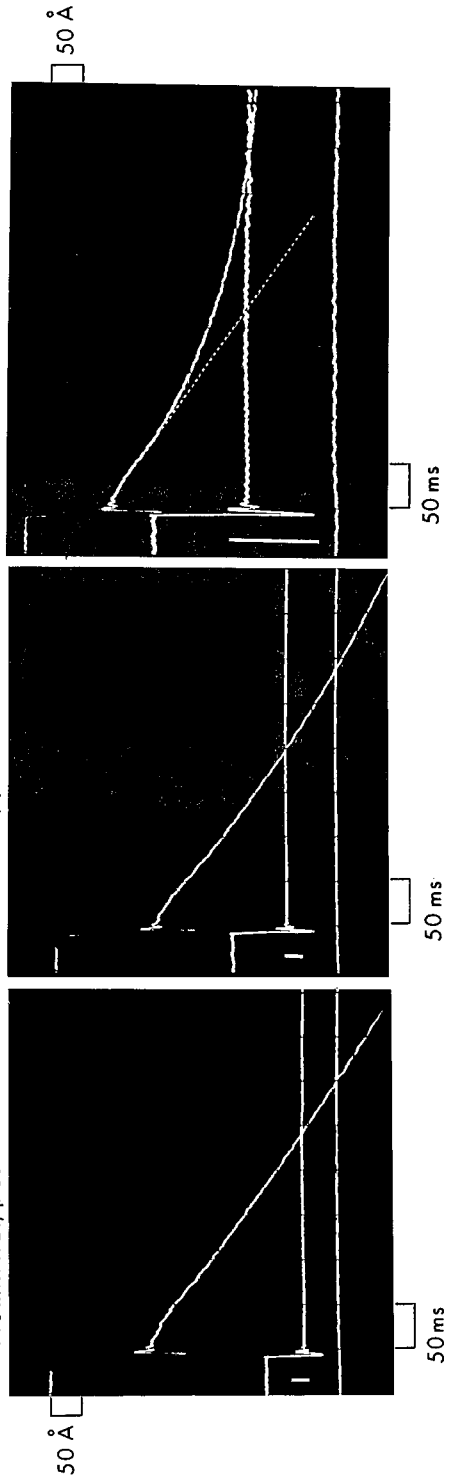

FIGURE 10. Isotonic motion at different free calcium concentrations in 70 mM KCl. Experiment 12 vi 75b; L_0 = 1.5 mm, temperature 0°C; P_{rel} = 0.46. *Top traces*, displacement; *middle traces*, force; *bottom traces*, zero force. *Left panel*: control solution, 140 mM KCl, pCa = 5, P_0 = 80 mg, β = 1.0; *middle panel*: 70 mM KCl, pCa = 6.9 P_0 = 112 mg, β = 0.9; *right panel*: 70 mM KCl, pCa = 7.1, P_0 = 40 mg, β = 0.3. The *dashed line* in the *right panel* indicates the speed calculated from the exponential fit of the displacement curve after the velocity transient whose duration was estimated to be ~80 ms. Note that the motion during the transient is nearly the same in the three panels, but that the *trace* after the transient is curved in the *right panel*.

transient was nearly the same in high and low calcium and the velocity at the end of the transient in both cases was close to that in 140 mM KCl. Thus, lowering the calcium level appears to have little effect on the first 70–80 ms of the isotonic motion at a given relative load. Similar effects were found at relative loads varying from 0.2 to 0.7 P_0 (Fig. 11).[1]

In low calcium, the curvature in the displacement trace after the velocity transient increased when the relative load was decreased. It is important to note that this curvature in low calcium occurred in the absence of measurable residual tension and was fully reversible. Control releases made in 140 mM KCl (pCa = 5) were essentially the same before and after the test releases.

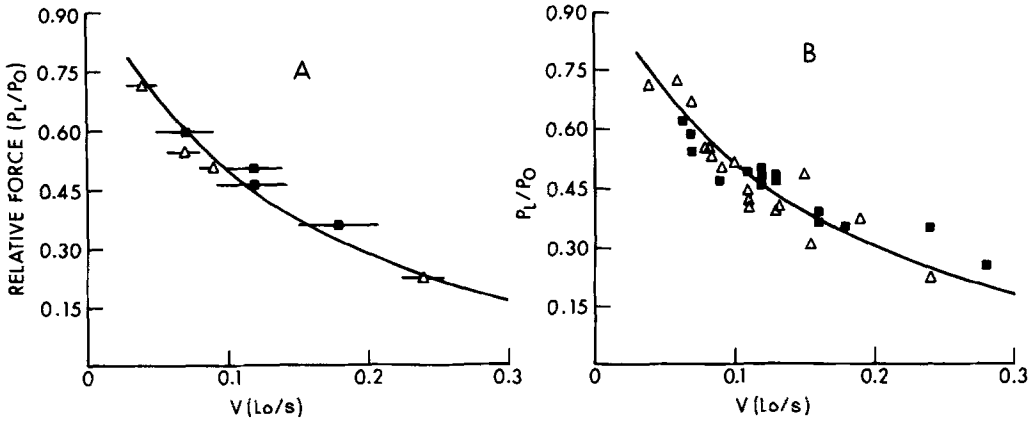

FIGURE 11. Effect of calcium on relative force-velocity relation. The speeds were measured at the end of the transient. In the case of partially activated fibers where the traces were curved, the speed at the end of the transient was calculated from the fitted function. (■) measurements made in 140 mM KCl, pCa = 5; (Δ) measurements in 70 mM KCl, pCa = 7.1. (*A*) All measurements were made on one skinned fiber. *Horizontal bars* indicate the uncertainty of each measurement which in the case of curved records includes the uncertainty in the estimate of the transient duration. (*B*) Cumulative data from four fibers from different frogs.

The experiments shown in Fig. 10 make it unlikely that the curvature in this case is due to a limitation in availability of the substrate or to the accumulation of products within the fiber, because the curvature was more

[1] The presence of curvature makes it impossible to construct a unique "force-velocity" relation and requires additional assumptions to use this method to characterize the kinetic response of a fiber. For the data in Fig. 11 we assumed that the appropriate velocity was that at the end of the isotonic transient, i.e., at the start of the exponential part of the displacement trace. The time at which this velocity was taken depends on relative load, as the duration of the isotonic transient depends on this parameter. This assumption is not needed at high calcium levels, because in this case the contraction at the end of the isotonic transient is practically steady. In any case, the similarity of the "force-velocity" relations, defined in this way, at high and low calcium levels shows that degree of activation has almost no effect on the early part of the isotonic motion over a wide range of relative loads.

pronounced in the low level of free calcium (Fig. 10, *right panel*), when the isometric force was reduced. Because there is a good correlation between the isometric force at different calcium levels and the rate of ATP hydrolysis (Levy et al., 1966), the ATP and ADP levels in the core of the fibers should have been closer to those in the bathing solution in the case of activation with low levels free calcium than with high levels free calcium.

Sarcomere Uniformity During Contraction

To see whether the curvature at low free calcium is produced by sarcomore length dispersion, microscopic observations of relaxed and activated fibers were made with an overall magnification of 250, which made it possible to record striation spacing over a length of 0.4 mm. Fig. 12 shows these results for the central region of a fiber in 70 mM KCl. The striation pattern in the activated fiber (Fig. 12*B*, pCa = 7.1) is similar to that in the relaxed fiber (Fig. 12*A*), although the activated fiber appears to contain some regions where the striations have become more oblique relative to the longitudinal axis (arrow). The average sarcomere length is 2.30 μm (range 2.20–2.35 μm) in Fig. 12*A* and 2.25 μm (range 2.09–2.35 μm) in Fig. 12*B*. The two regions of the fiber near the ties (pictures not shown) also gave an average sarcomere length of 2.30 μm (range 2.08–2.35 μm). The fiber was left in the contracting solution for 20 min without significant change in the sarcomere length distribution. Similar results were obtained on another fiber at an average sarcomere length of 2.0 μm.

The sarcomere distribution in the isometrically contracting fibers in pCa = 5 solutions containing 140 mM KCl or 70 mM KCl was less stable. Similar observations have been made by Moss (1979). Uniformity was apparent after force became steady, but the photographs (with 5- and 15-s exposure times) were blurred, apparently because blocks of sarcomeres shifted slightly but continuously throughout the fiber volume. These conditions were not investigated further because shortening here was almost linear after the velocity transient and the magnitude of the steady contraction velocity was close to the physiological value for intact fibers (Civan and Podolsky, 1966).

The first-order laser diffraction patterns in activated fibers in general appeared to be less intense than those in the relaxing solution (see also Hill [1953]; Kawai and Kuntz [1973]; Fujime [1975]; Paolini et al. [1976]; Halpern [1977]; Zite-Ferenczy and Rudel [1978]; Rudel and Zite-Ferenczy [1980]). Clear patterns in activated fibers, with little shift in the position of the first-order diffraction line, were more frequently obtained with partial than with full activation. In selected preparations, these patterns were sharp and stable for at least 20 s during the force plateau under fully activated (pCa = 5; KCl, 140 and 70 mM) as well as in partially activated conditions (pCa = 7.1; 70 mM KCl). Although these experiments were made with fibers at sarcomere lengths between 2.7 and 3.0 μm, the results are essentially the same as those obtained by direct microscopic observations and they provide evidence that the striation pattern in activated fibers, particularly under partially activated conditions, is reasonably uniform.

We conclude from these observations that the curvature seen in the displacement trace in partially activated fibers in 70 mM KCl probably reflects events that take place on the sarcomere level. While the striation pattern is less stable in fully activated fibers, the linearity and magnitude of the motion seen in such fibers strongly suggests that the overall shortening in these cases also reflects events that take place on the sarcomere level. The reason for this may be that the selection of successful preparations under the low-power

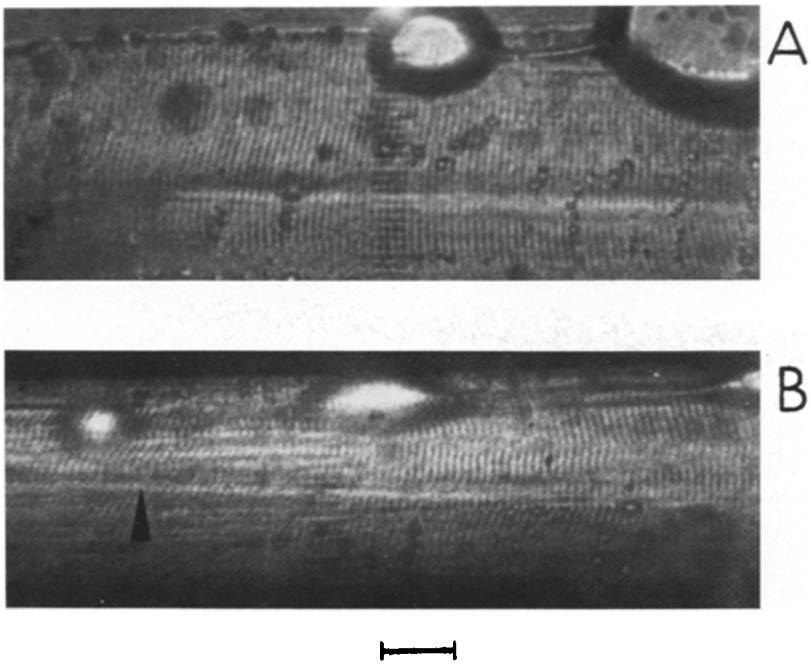

FIGURE 12. Light-microscope photography of a skinned fiber segment in relaxing solution (*A*), and activating solution 70 mM KCl, pCa = 7.1 (*B*). The force in *B* was 40% of the force made by the same fiber in pCa = 5, 70 KCl solution ($\beta = 0.4$). Experiment 28 vi 80. Average sarcomere length in (*A*) 2.30 μm and in (*B*) 2.25 μm. Polarized light was used in *B*. The apparent diameter of the fiber was different in the two photographs because the plane of focus was different. *Bar*, 20 μm. $T = 3°C$.

microscope produces fibers that are uniform on the time scale necessary for the present measurements.

DISCUSSION

The contraction kinetics of the skinned fibers are very sensitive to the temperature and ionic strength of the bathing solution. This study was made on a slow time base with no residual tension. Under these conditions (0–1°C), the steady motion of fully activated fibers was independent of ionic strength

over a wider range of KCl concentrations than was found at 5–7°C. In addition, we found that at low ionic strength (70 mM KCl), the isotonic motion during the velocity transient was unaffected by calcium, which was the case also in higher ionic strength (Gulati and Podolsky, 1978). However, the motion in low ionic strength after the transient depends on the degree of activation. These results support the idea that, at low ionic strength, calcium acts like a simple "on-off" switch as in 140 mM KCl, but also that there are additional factors in low ionic strength that affect the contraction mechanism and influence the motion that follows the velocity transient.

Effect of Ionic Strength

FORCE DEVELOPMENT At 0–1°C, calcium-activated force development decreases linearly with ionic strength in bathing solutions containing KCl concentrations ranging from 0 to 140 mM (Fig. 3). This differs from results found when all the measurements were made at 5–7°C or at 20–22°C and residual tension was present in the low ionic strength solution (Fig. 13). Under the former condition (5–7°C), ionic strength had a smaller effect on calcium-activated force in solutions containing <140 mM KCl than in solutions containing more than this value (Thames et al., 1974; and Fig. 13*A*, *dashed line*). At 20–22°C, the calcium-activated force decreases when ionic strength is less than ~120 mM (Gordon et al., 1973; and Fig. 13*A*, *dotted line*). It is interesting that the relation describing total tension, i.e., the sum of calcium-activated tension and residual tension, as a function of ionic strength, is nearly linear at 5–7°C (Fig. 13*C*, *dashed line*).[2] Also at 20–22°C, where the residual tension is much greater, the decrease in *total* tension in low ionic strength (Gordon et al., 1973; and Fig. 13*C*, *dotted line*) is much less than is the case for calcium-activated tension. These results suggest that the residual and the calcium-activated tension may have a common origin. That is, residual tension may be produced when cross-bridges form without the activating effect of added calcium. The greater the number of such bridges, the smaller may be the number of additional bridges that can form in the presence of calcium.

ISOTONIC MOTION Ionic strength affects the isotonic motion of fully activated skinned fibers in two ways. First, as shown earlier (Gulati and Podolsky, 1978), in solutions containing ≥50 mM KCl, the effect of ionic strength is mainly on the velocity transient, which indicates that some aspect of the cross-bridge cycle is affected by ionic stength.

A second effect of ionic strength becomes prominent in solutions containing 0 and 20 mM KCl, where the transient response is suppressed. In these low ionic strengths, the displacement trace is curved (Figs. 6 and 8, Table II). However, despite the presence of curvature, the initial contraction velocity in 20 mM KCl at a given relative load is close to the steady speed in the control solution. Thus, in solutions where the KCl concentration ranged from 20 to 210 mM, an appropriate measure of "contraction velocity" (initial velocity in

[2] The decrease in tension found by Thames et al. (1974) at 210 mM KCl relative to that at 140 mM KCl was ~20% less than that found in our experiment. This difference is at the uncertainty limits of the two studies.

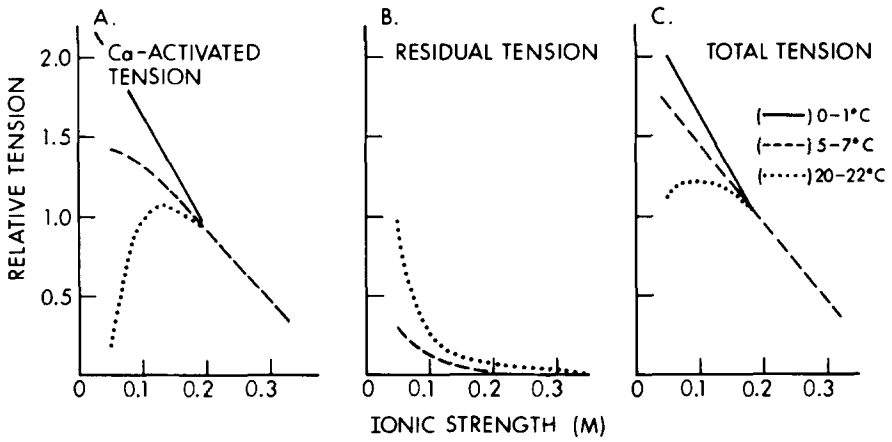

FIGURE 13. Effect of ionic strength on the tension-generating properties of skinned fibers. The *solid line* is from the data in this study. The data for the *dashed lines* at 5–7°C are from Thames et al. (1974) and the data for the *dotted lines* at room temperature are from Gordon, et al (1973). The *dotted line* in *A* and *C* is practically coincident with the dashed line at ionic strength >190 mM. The data for total tension were normalized to the measurements of total tension in 190 mM ionic strength.

TABLE II

SUMMARY OF THE EFFECTS OF LOW IONIC STRENGTH AT 0–1°C ON RESIDUAL TENSION, SPEED OF SHORTENING, AND CURVATURE

KCl concentration	Residual tension*	Speed of shortening‡	Curvature*	Remarks
mM				
		Full activation (pCa = 5)		
70	0	1.0	0	—
50	0	1.0	+	The curvature is small and reversible
20				
(i)	0	0.9	+	The residual tension develops after several activations; it is irreversible and is associated with reduction in the initial velocity; the curvature is the same before and after the appearance of residual tension
(ii)	+	0.5	+	
0	0	0.6	+	The speed of shortening is reversibly reduced, but there is no measurable residual tension until after one to two activations
		Partial activation (pCa = 7.1)		
70	0	1.0	++	The curvature increases at low activation levels and is reversible

* 0, indicates no change relative to 140 mM KCl; +, an increase in the parameter; ++, greater effect.
‡ The ratio of the appropriate measure of contraction speed in the test solution (see Results) and the steady speed in 140 mM KCl at the same relative load.

the case of solutions <50 mM KCl and steady velocity after the transient for >50 mM KCl) has the same value at the same relative load. Lowering the temperature from 5–7°C to 0–1°C appears to extend the flat part of the KCl-velocity curve to lower values of KCl.

When the KCl concentration was reduced to 0 mM, the initial velocity of the motion was ~0.6 times that at higher ionic strength. This effect was reversible. Thus a sufficiently low ionic strength causes a decrease in the contraction velocity.

Residual Tension

Although at 0–1°C residual tension was absent after a few contraction cycles at a concentration of 20 mM KCl, it eventually became measurable. When this happened, the initial velocity of fully activated isotonic motion decreased twofold and the curvature remained the same (Fig. 9; Table II). Thus residual tension appears to reflect the presence of an internal load within the sarcomere, as suggested by Thames et al. (1974).

The influence of residual tension probably accounts for the difference in the response seen by Thames et al. (1974), and by us in 50 mM KCl solutions. Thames et al. found the contraction velocity in this solution to be ~0.7 times in 140 mM KCl, whereas we find almost the same speed in the two solutions (Fig. 3). However, the former experiments were made at 5–7°C, where residual tension was present and the latter were done at 0–1°C, where residual tension was not measurable. The large decrease in speed associated with the presence of residual tension implies that its effect on the force-velocity relation is greater than that expected from an internal load of equal magnitude. The presence of residual tension therefore appears to be an indicator of a state of the myofilaments that is mechanically equivalent to a significantly greater internal load.

The influence of temperature on both residual tension and calcium-activated tension (Fig. 13) supports the idea that residual tension is produced within the sarcomere. Additional evidence that this is the case comes from recent experiments of Julian and Moss (1981). They confirmed the finding of Thames et al. (1974) that residual tension is irreversibly produced after contraction in low ionic stength solutions at 5–7°C and noted, on the basis of microscopic examination, that striation uniformity is maintained, which appears to exclude sarcomere length dispersion as a factor in residual tension under these conditions.

Effect of Calcium at 70 mM KCl

The present experiments show that certain aspects of the motion in 70 mM KCl depend on calcium concentration (Fig. 10). The early part of the motion, which contains the isotonic velocity transient, is the same at both high and low levels of activation. In contrast, the displacement trace after the velocity transient depends on the calcium concentration. The trace is nearly linear at high levels of activation and is strongly curved at low calcium levels. Photomicroscopic observations (Fig. 12) and laser diffraction measurements indicate

that the striation pattern is stable under the latter condition, which suggests that the curvature seen there reflects properties of the sarcomeres. Thus, the similarity in the early part of motion at different activation levels supports the idea that at low KCl concentrations calcium acts as a switch that controls cross-bridge number, but the effect of calcium on later parts of the motion indicates that additional factors are also involved under these conditions.

The lack of effect of Ca in 70 mM KCl (120 mM ionic strength) at 0°C on the force-velocity relation (Fig. 11) is similar to the response seen in 140 mM KCl (190 mM ionic stength) at 5°C (Table III) and in intact muscle fibers (Edman, 1979). However, calcium appears to have an effect on the force-velocity relation of skinned fibers at 50 and 100 mM KCl (100 and 150 mM

TABLE III

SUMMARY OF THE EFFECT OF CALCIUM ON RELATIVE FORCE-VELOCITY RELATION IN FROG SKINNED FIBERS AT DIFFERENT IONIC STRENGTHS AND TEMPERATURES

	Ionic strength (mM)			
Temperature	90–100	120	140–150	180–190
°C				
0–1		0*		
4–7	+ ‡		+ ‡§ ‖	0 ¶‡**
10	+ ‖		+ ‖	+ ‖

0, the velocity at a given relative load is the same in partially activated and in fully activated fibers; +, the velocity at a given relative load is less in partially than in fully activated fibers.

* This paper.

‡ Thames et al. (1974).

§ Julian (1971).

‖ Julian and Moss (1981).

¶ Podolsky and Teichholz (1970).

** Gulati and Podolsky (1978).

ionic stengths) at 4–7°C and in these solutions as well as 180 mM ionic stength at 10°C (see Table III). The Ca effect at 4–7°C in lower ionic strength solutions has been explained in terms of an internal load within the sarcomere, as indicated by the presence of residual tension under these conditions (Thames et al., 1974); an internal load would be expected to retard the motion of partially activated more than that of fully activated fibers. The Ca effect at 10°C may also be due to a similar mechanism, although this has been questioned (Julian and Moss, 1981). Further quantitative experiments along these lines are needed to clarify this point.

The influence of degree of activation (β) on the linearity of the motion in low ionic stength has not been remarked upon in previous studies. However, the effect can be seen in records of Thames et al. (1974). In their Fig. 4,

curvature is clearly present in the 100 mM KCl, low-calcium trace, where the degree of activation is 0.36. Curvature is almost absent in the corresponding 50 mM KCl trace, where the degree of activation is 0.57. This is consistent with the present observation that curvature at low ionic stength is more apparent when the degree of activation is low (Fig. 10).

MECHANISMS OF THE CURVATURE The question arises as to whether the mechanism of the curvature seen in the displacement traces recorded from fully activated skinned fibers in 0 and 20 mM KCl solutions is the same as that found in traces from partially activated skinned fibers in 70 mM KCl. In the fully activated fibers, the entire trace could be fitted by an exponential function. This could not be done for the partially activated fibers in 70 mM KCl, where the exponential part of the displacement trace began near the end of the velocity transient rather than at the beginning of the motion. This suggests that the underlying mechanisms of the curvature may be different in the two conditions.

A possible mechanism for the curvature in the displacement trace is that the fibers in low ionic stength produce two types of cross-bridges which differ in their kinetic properties. One type, the predominant species, has normal properties, whereas the second type is supposed to turn over relatively slowly. In this case the "slow" bridges can be thought of as perturbing the motion produced by the normal bridges. That myosin within a single muscle cell can be heterogeneous (Gauthier and Lowey, 1979; Lutz et al., 1979) supports the idea that different types of bridges can be formed within each sarcomere. The observation that *N*-ethylmaleimide (NEM) treatment of myosin in solution causes a certain fraction of the treated myosin to remain complexed to actin in relaxing solution (Pemrick and Weber, 1976) raises the possibility that certain conditions in the fiber system (e.g., low ionic strength) may also produce heterogeneity in properties of cross-bridges. The finding that differences in the actin-activated ATPase activities of the two isoenzymes of myosin S-1 subfragments with A1 and A2 alkali light chains are modulated by ionic strength (Wagner et al., 1979; Reisler, 1980), provides another possible mechanism for heterogeneity among cross-bridges. The hypothesis that there are two types of bridges can explain the observation that the curvature is greater when the degree of activation is decreased if, at low ionic stength, the number of slow bridges is independent of the degree of activation so that they make up a greater fraction of the total bridges.

Another explanation for the curvature of the displacement trace is a deactivation mechanism in which the force the fiber can develop decreases as a consequence of active shortening. In addition, the affinity of calcium to binding sites on the regulatory proteins may depend on the number and/or distribution of cross-bridges, which could change during shortening (Huxley, 1957). The latter effect would be expected to produce curvature only in partially activated fibers since in this condition the extent of calcium binding to the regulatory proteins is sensitive to the calcium affinity. Because the curvature in low ionic strength solutions is present over distances that are large (300–400 Å) relative to the presumed reach of the cross-bridge (100 Å),

the effects of shortening would appear to operate over several cross-bridge cycles. It should be pointed out that active shortening has a depressant effect on the contractile force in intact cells (Edman, 1980), which may be related to the effect described here.

It is interesting to note that curvature is also seen in the motion of intact amphibian slow muscle fibers (Aidley, 1965; Lannergren, 1978), cardiac muscle (Forman et al., 1972; Brenner and Jacob, 1980), and smooth muscle preparations (Hellstrand and Johansson, 1979; Mulvany, 1979). Because the present observations provide evidence that curvature reflects properties of the contraction mechanism of fast frog muscle fibers, they support the idea that the curvature seen in these other cells is also an intrinsic property of their contraction mechanism.

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