Contraction Transients of Skinned Muscle Fibers:
Effects of Calcium and Ionic Strength

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ABSTRACT Calcium and ionic strength are both known to modify the force developed by skinned frog muscle fibers. To determine how these parameters affect the cross-bridge contraction mechanism, the isotonic velocity transients following step changes in load were studied in solutions in which calcium concentration and ionic strength were varied. Analysis of the motion showed that calcium has no effect on either the null time or the amplitude of the transients. In contrast, the transient amplitude was increased in high ionic strength and was suppressed in low ionic strength. These results are consistent with the idea that calcium affects force in skeletal muscle by modulating the number of force generators in a simple switchlike "on-off" manner and that the steady force at a given calcium level is proportional to cross-bridge number. On the other hand, the effect of ionic strength on force is associated with changes in the kinetic properties of the cross-bridge mechanism.

INTRODUCTION

The force developed by frog skinned muscle fibers is modulated by both the calcium ion concentration (Hellam and Podolsky, 1969) and the ionic strength (Gordon et al., 1973) of the bathing solution. The mechanisms of these effects have been examined by studying the steady-state force-velocity relations because these relations contain information about the cross-bridge kinetics (Huxley, 1957). In the case of calcium, the motion at pCa 5, which gives full isometric force, was compared with that at lower calcium levels. The relative force-velocity relation was found to be the same at different calcium levels, provided the ionic strength was 190 mM or more (Podolsky and Teichholz, 1970; Thames et al., 1974). This observation is consistent with the idea that in these fibers calcium regulates force by controlling the number of actin sites at which actomyosin cross-bridges can be formed (Ebashi and Endo, 1968; Weber and Murray, 1973).

As regards the effect of ionic strength, the motion is complex at ionic strengths below 190 mM under conditions used in previous studies (Thames et al., 1974). At ionic strengths above this value, which reduce the full isometric force, the relative force-velocity relation appears to be the same at different force levels, which is similar to the calcium effect. However, in biochemical
studies ionic strength affects the actomyosin ATPase kinetics in the absence of the calcium-sensitive regulatory proteins (Rizzino et al., 1970). Thus, the lack of influence of ionic strength on velocity in skinned fibers could be explained if (a) ionic strength has an on-off, switchlike effect on the activity of one of the components of the contraction mechanism, and/or (b) ionic strength changes certain kinetic properties of the cross-bridge mechanism, but the steady-state force-velocity relation in skinned frog fibers is insensitive to these changes.

To examine these effects in greater detail, the isotonic velocity transients of skinned frog fibers (Podolsky et al., 1974) were studied as a function of both calcium ion concentration and ionic strength because the presteady motion is generally a more sensitive measure of cross-bridge properties than the steady-state motion (Civan and Podolsky, 1966). The results show that pCa does not affect the transient, which is direct evidence that calcium regulates force through a switchlike mechanism that controls the number of active cross-bridges without affecting the kinetic or mechanical properties of the active cross-bridges. In contrast, the transient was significantly changed by ionic strength, which implies that the properties of the active cross-bridges are sensitive to this parameter. Additional observations regarding the influence of these parameters on the steady-state contraction properties of skinned frog muscle fibers will be reported in a subsequent publication.

Preliminary accounts of this work have been reported briefly (Gulati and Podolsky, 1974, 1976).

METHODS

Fiber Preparation

Northern frogs, Rana piriens pipiens, and tropical frogs, Rana piriens beriindieri, were used. The former were stored in a cold room at 5°C, and the latter were kept at room temperature. The dorsal head of the semitendinosus muscle was dissected out and mounted in a chamber containing cold Ringer solution of the following composition (millimolar): KCl, 2.5; NaCl, 115; CaCl₂, 1.8; NaH₂PO₄ + Na₂HPO₄, 3 (pH = 7.0). A bundle of about 10 fibers was cut from the muscle, blotted lightly, and laid on a glass cover slip. The cover slip was quickly submerged in ice-cold paraffin oil (viscosity 125/135). The largest fiber was very carefully isolated from the bundle in oil and skinned with fine stainless steel needles. The length of the skinned region varied between 5-10 mm in length.

The displacement and force transducers were those described by Civan and Podolsky (1966). They were adapted for use with skinned fiber preparations by cementing a short attachment wire to the end of each transducer. The fiber was tied to the wires with square knots of 5-0 surgical silk. The fiber length between the ties in different experiments ranged from 0.8 to 3 mm; this was kept short to reduce the compliance of the moving system. The equivalent mass of the displacement transducer was about 3 mg and the natural period for moderate force steps was <5 ms.

Skinned fibers were also prepared from Rana temporaria frogs obtained by air from England. Although the frogs appeared to be healthy, these fibers proved to be unsuitable for the present study because calcium activation in the bathing solution described below caused them to deteriorate after only one or two contractions.
Bathing Solutions

The bathing solutions for the skinned fibers contained 5 mM Na₄ATP, 1 mM MgCl₂, 10 mM imidazole, 5 mM EGTA + CaEGTA, and KCl. The relaxing solution contained 140 mM KCl and no added CaEGTA. The ionic strength of the contracting solutions was controlled by varying the KCl concentration between 20 and 210 mM. The control activation was made in solution containing 140 mM KCl; this KCl concentration was chosen to be consistent with earlier studies from this laboratory. Ionic strength was kept constant in experiments where pCa was varied. The pCa of the activation solutions was adjusted by addition of appropriate amounts of EGTA and CaEGTA. The apparent stability constant of CaEGTA complex at pH 7.0 was taken as 10⁶⁶ M⁻¹. The pH of the bathing solutions was adjusted to be 7.0 at 0°C.

The bathing solutions were contained in rectangular wells (38 mm × 7 mm × 10 mm high) in a solution-changing device similar to that used by Hellam and Podolsky (1969). The solution changer was constructed from an anodized aluminum block which was thermoelectrically cooled using a bipolar controller (Cambion Thermionic Corporation, Cambridge, Mass.); this modification made it possible to reduce the temperature of the test solution to 0°C and to maintain control at a given temperature to within 1°C. Each well had a glass bottom which allowed the preparation to be illuminated from below. The temperature was set at 0°-1°C in low KCl (20-140 mM) experiments, at 4°-6°C in high KCl (140-210 mM) experiments, and at 2°-4°C in experiments where calcium concentration was varied.

Experimental Procedure

The contraction kinetics of the skinned fiber preparations were studied using a quick release technique (Podolsky et al., 1974). The mounted skinned fiber segment was transferred from oil to the relaxing solution. The sarcomere length was set between 2.2 and 2.3 μm, as indicated by the diffraction pattern from a helium-neon gas laser (Spectra-Physics model 133; Spectra-Physics Inc., Mountain View, Calif.; λ = 6,320 Å). At this length the fibers usually made 1-2 mg force in the relaxing solution. A stop, mounted on an electromagnetic relay, was next placed in front of the displacement level so that the fiber could be activated isometrically. The segment was then transferred to the control-activating solution (140 mM KCl, pCa 5). The load on the lever was adjusted manually to a predetermined level (P₀) and when the force became steady (P₀), the electromagnetic relay was activated which quickly changed the load from P₀ to P₂. The force step and the resulting displacement response of the fiber were recorded on a dual beam storage oscilloscope (Tektronix 5031, Tektronix, Inc., Beaverton, Oreg.). The fiber was quickly thereafter returned to the relaxing solution. As soon as the fiber was relaxed, the force level in the relaxing solution was traced on the oscilloscope. The record on the oscilloscope screen containing the three traces was photographed with a Polaroid camera (Polaroid Corp., Cambridge, Mass.). Several records of this kind were obtained for a variety of loads. Next, releases were made in either low free calcium at fixed 140 mM KCl or with high calcium (pCa 5) and low (50, 20 mM) or high (210 mM) KCl. The force steps were varied so that it was possible to find records in both the control and the test activating solutions which were at relative loads differing by < 0.01 P₀. A few control releases were made in the control solution at the end of the test releases. The results discussed below were from experiments in which the isotonic displacement responses and the resting tension were essentially the same in the two sets of control records. In addition, the fiber was observed continually with a microscope (magnification ×40) during the isometric contraction, and the experiment was discarded if the fiber
contracted nonuniformly in any of the first 20 contractions, as indicated by the development of one or more opaque bands along the length of the fiber. These criteria eliminated about 30% of the fibers tested. The remaining 70% of the fibers gave reproducible results, which suggests (but does not prove) that the distribution of sarcomere length within each preparation was relatively uniform. This was assumed to be the case in scaling the motion of the displacement transducer to the motion of the half sarcomere. Specifically, the half sarcomere motion was taken to be (lever motion) (sarcomere length)/2(fiber length).

The variation in the cross-sectional area of the fibers used in the present study was about threefold, as indicated by the force developed in the control-activating solution (Hellam and Podolsky, 1969).

Analysis of the Isotonic Velocity Transients

Because the contraction kinetics of skinned fibers are qualitatively the same as those of intact fibers (Podolsky et al., 1974), analysis methods that have proved useful for the motion of intact fibers were adopted for the present study. Typical records and the way in which they were analyzed are shown diagrammatically in Fig. 1. These are traces of actual records obtained earlier (see Fig. 1 of Podolsky et al., 1974). The records were enlarged photographically threefold for analysis. $P_o$ is the isometric force, $L_o$ is the fiber length during isometric contraction, and $P_L$ is the load during isotonic contraction. The relative load $(P_L/P_o)$ is 0.83 on the left and 0.63 on the right. For the purposes of the present study it is appropriate to divide the motion of the fiber (upper traces) into three phases. In the first phase, which takes several milliseconds to complete, the fiber appears to shorten instantaneously as the force is changed from $P_o$ to $P_L$. The small upward deflections in the force and displacement traces at the start of this phase are artifacts produced by the release mechanism. The second phase, part of which contains high frequency oscillations that appear simultaneously in both the force and the displacement records, is the velocity transient. Finally, there is the steady phase during which the fiber shortens with a steady speed. The shortening behavior of the fiber during the transient is such that at first the fiber shortens with speed greater than the steady value. Then the shortening speed decreases and slowly oscillates about the steady value (Podolsky et al., 1974).

For analysis, the $P_L$ trace is back extrapolated with a horizontal dashed line and its intersection with the instantaneous force trace during the step change from $P_o$ to $P_L$ marks time $t_o$. A vertical dashed line is drawn through time $t_o$. The intersection of this vertical line with the displacement trace above marks the end of the quick shortening phase and the beginning of the transient. The sloping dashed line is back extrapolation of the steady shortening. The first intersection of the instantaneous motion with the sloping dashed line is labeled with a vertical arrow; the time $t_r$ between $t_o$ and this intersection, is the null time of the transient. Null time is one of the parameters of the transient that is evaluated in the present study as a function of changes in the chemical milieu.

Another relevant parameter is the maximum amplitude $A$ of the transient from the sloping dashed line. The high frequency oscillation during the initial part of the isotonic transient, which was especially marked at lower relative loads, limits the accuracy of the

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1 This phase corresponds to phase 1 and the initial parts of phase 2 in the tension transient that occurs after a considerably more rapid length step (text-Fig. 7 in Huxley, 1974; see also Ford et al., 1977). Therefore, phase 2 in the velocity transient corresponds to the remaining parts of phase 2 as well as phase 3 in the tension transient, and phase 3 in the present study corresponds to phase 4 in the tension transient.
amplitude measurements to about 20%. The natural period and the damping of these oscillations depend on the equivalent mass of the displacement transducer as well as the overall combined compliance of the force transducer and the fiber preparation (Civan and Podolsky, 1966). As most of the compliance is due to the fiber, the fiber length was kept small (between 0.8 and 3.0 mm) in the present study. In records where these oscillations were prominent, the “true” displacement of the muscle fiber (dotted segment of displacement trace) was taken as the motion which kept the ratio of the displacement oscillation and the force oscillation constant, so that the dynamic compliance (Civan and Podolsky, 1966) remained the same during the transient.

![Figure 1](image)

**Figure 1.** Analysis of isotonic velocity transient. The upper trace is displacement, the middle trace is force, and the bottom trace is the force base line. $L_o$ and $P_a$ are the length and steady force before release; $P_l$ is the force of the load. In the record on the left, $P_l$ is 0.83 $P_o$; in the record on the right, $P_l$ is 0.63 $P_o$. The interrupted sloping line is the back extrapolation of the steady shortening; $\tau$ marks the time at which the instantaneous motion first intersects this line; $A$ marks the maximum amplitude of the transient relative to this line. After the release, the actual force oscillates about $P_l$ due to the inertia of the displacement transducer. An estimate of the instantaneous motion during this oscillation is given by the dotted segment of the displacement trace. This was constructed to make the dynamic compliance remain constant throughout the oscillation.

**Results**

**Effect of Calcium at 140 mM KCl**

Isotonic contraction kinetics of fully activated (pCa = 5) and partially activated (pCa = 6.2-6.4) fibers were determined at fixed 140 mM KCl on nine fibers, and typical results at 3°C-4°C on one fiber are shown in Fig. 2. The displacement response of the skinned fibers to the application of a quick (2-4 ms) force step consists of a presteady phase and a steady phase (see Analysis part of Methods). In agreement with earlier studies (Podolsky and Teichholz, 1970; Thames et al., 1974), the relative force-velocity relation was the same in high and low calcium.
The ratio of the steady speeds of shortening in pCa 5 to those in pCa 6.2–6.4 in these fibers for paired relative loads ($P_{rel}$ range = 0.4 to 0.8 $P_o$) was found to be 0.94 ± 0.15 (mean ± SD).

**DURATION OF THE PRESTEADY MOTION**  The time $\tau$ between the completion of the force step and the first intersection of the instantaneous motion with the line back extrapolating the steady speed of shortening is the null time of the transient and is a measure of the duration of the transient. The null times are sensitive to changes in the load step and the temperature of the activation solution (Podolsky et al., 1974). The effect of calcium was studied using force steps between 0.5 and 0.83; null times can be measured accurately in this range, and previous experiments showed that they increased from 20 and 60 ms over this range of relative loads. With a 3°-4°C change in temperature, the null times at a given relative load changed by about twofold.

![Figure 2](image)

**Figure 2.** A typical experiment showing isotonic quick release records at 2°–3°C of a skinned fiber (*R. pipiens p. pipiens*) as a function of free calcium concentration. Top trace, displacement; middle trace, force; bottom trace, zero of force. Fiber length = 1.4 mm; isometric force is 63 mg in pCa 5 and 32 mg in pCa 6.4. The relative load, $P_{rel}$ ($P_L/P_o$) is 0.7. The dashed lines are back extrapolations of the steady motion. The slope of this line gives an estimate of the steady speed of shortening. The arrow, $\tau$, marks the null time of the transient. The ratios of the values of the null times at high and low calcium for a variety of relative loads are summarized in Table I. *Inset:* Juxtaposed tracings of the displacement records; the top trace is the displacement response in pCa 6.4 and the lower trace is that in pCa 5.

To quantitatively evaluate the effect of calcium concentration on the presteady motion, null times of the transients were measured for matching relative loads ($P_{rel} = P_L/P_o$) in the high and low free calcium activating solutions. Records of matching relative loads for each measurement were selected from the same fiber. In the experiment in Fig. 2, the null times for $P_{rel} = 0.7$ are about 25 ms in both high and low calcium. Table I summarizes these results on nine skinned fibers, each from a different frog. The second column in Table I compares the level of isometric force in the activating solution with low free calcium and that
in the control maximal free calcium solution for each fiber. The range of this force ratio is 0.3 to 0.8. The relative loads placed on the fibers during isotonic shortening are indicated in the third column. The null time ratios (fourth column) are close to unity. This means that the null time at a given relative load is insensitive to changes in the concentration of free calcium that cause the isometric force to change by as much as threefold. Statistical analysis of the results indicated that with 95% confidence the ratio of the null times at the two calcium levels was between 0.96 and 1.15.

AMPLITUDE OF THE PRESTEADY MOTION A second way of characterizing the isotonic velocity transient is to measure the maximum amplitude of the presteady motion around the back extrapolation of the steady contraction speed. Like the null time, this parameter depends on both relative load and temperature. In normal activating solution, the amplitude of the transient increased from 0 to about 20 Å per 1/2 sarcomere when the relative load decreased from \( P_o \) to 0.75 \( P_o \). Further decrease in relative load to 0.5 \( P_o \) caused the amplitude to decrease to a value close to zero. When the temperature was increased at a given relative load, the amplitude decreased. For example, increase in temperature from 0.5°C to 4.5°C caused a twofold decrease in the amplitude of the transient after a force step from \( P_o \) to 0.77 \( P_o \) (Fig. 3 in Podolsky et al., 1974).

The ratios of the transient amplitude in the low and high calcium solutions

### Table 1

| Experiment | \( P_{n0} \) | \( P_{n1} \) | \( P_{n0} - P_{n1} \) | \( P_{n0} \) | \( P_{n1} \) |
|------------|-------------|-------------|-------------------|-------------|-------------|
| 6 xii 73   | 0.34        | 0.64        | 0.88              | 0.88        |             |
| 7 xii 73a  | 0.48        | 0.73        | 1.17              | 0.56        |             |
| 7 xii 73b  | 0.36        | 0.67        | 1.14              | 0.36        |             |
| 8 xii 73   | 0.57        | 0.77        | 1.03              | 1.09        |             |
| 14 xii 73  | 0.47        | 0.75        | 0.98              | 1.08        |             |
| 20 xii 73  | 0.40        | 0.75        | 1.20              | 1.13        |             |
| 22 xii 73  | 0.30        | 0.50        | 1.14              | 0.85        |             |
| 13 xii 73a | 0.45        | 0.66        | 0.76              | 1.40        |             |
| 13 xii 73b | 0.80        | 0.76        | 1.01              | 1.16        |             |

Each experiment consists of two matched releases, one at low (pCa 6.2–6.4) and the other at high (pCa 5.0) calcium ion concentration; temperature 2°C–4°C. \( P_{n0} \) is the steady isometric force developed in the low calcium solution, \( P_{n1} \) is the corresponding value in the high calcium solution, \( P_{n0} \) is the ratio of \( P_{n0} \), the value of the load, and \( P_{n1} \), the steady isometric force for each solution. The 95% confidence interval for the mean null time ratio is between 0.95 and 1.15; for the mean amplitude ratio it is between 0.75 and 1.15.
for matching records are shown in the last column of Table I. The mean of these ratios from nine experiments is again close to unity, indicating that this parameter is also insensitive to the variation in the concentration of free calcium. The uncertainty in this measurement as determined from the 95% confidence intervals is close to 20%. This uncertainty is twice as great as that in the null time measurement because of the damped mechanical oscillations that are present in the initial part of the transient. These oscillations, which do not affect the null time measurement, become even more prominent in the low calcium solutions because the lower isometric forces produced in these solutions make the fibers proportionately more compliant.

Effect of Ionic Strength at pH Ca = 5

High KCl: To study the effect of raising the ionic strength on the transients, isotonic quick release records were made on skinned fibers activated in high calcium solutions containing 140 mM (control) or 210 mM KCl (test). The typical results on one fiber, with \( P_{rel} = 0.65 \) at 5°C–6°C, are shown in Fig. 3.

![Figure 3](image)

**Figure 3.** Influence of high KCl on the response of a skinned fiber (\( R. pipiens \) berlandieri) to a step change in load at 5°C–6°C. Top trace, displacement; middle trace, force; bottom trace, zero of force. Fiber length = 2.2 mm; isometric force is 93 mg in 140 mM KCl and 50 mg in 210 mM KCl. Relative load is 0.67. The dashed line in the right panel is the back extrapolation of the steady motion. It is drawn to emphasize the effect of ionic strength on amplitude of the transient.

Analysis of this and of records from five additional fibers for null time and maximum amplitude of the transient was made as described in Methods. The results (Table II) show that the isometric force developed in the high KCl solution in these experiments is on the average about half that in the control solution. The null times of the transients for a variety of loads (range of \( P_{rel} = 0.56 \) to 0.77) are seen to be unaffected by raising the ionic strength. However, KCl has a large effect on the amplitude, which is two to three times greater in the high KCl in comparison to that in the control solution. The transient amplitude appeared to be more sensitive to high KCl in fibers from the tropical frog (\( R. pipiens \) berlandieri) than in those from northern frogs (\( R. pipiens \) pipiens).
Activating the frog skinned fibers at 5\(^\circ\)–7\(^\circ\)C in solutions made with KCl below 140 mM has been shown to cause the fibers to develop irreversible resting tension as well as retard the shortening speeds (Thames et al., 1974). However, activation at still lower temperatures eliminates both these effects (Gulati and Podolsky, 1974). For this reason, investigation of the effects of low ionic strength on the steady and presteady contraction properties was made at 0\(^\circ\)–1\(^\circ\)C. Under these conditions, the force developed by the skinned fibers increased progressively as the ionic strength of the activating solution was decreased. Also, the fibers did not develop additional resting tension and the relative force-velocity relation was found to be unaffected by ionic strength. These effects ionic strength at 0\(^\circ\)–1\(^\circ\)C will be discussed in greater detail in a subsequent publication. The effects on the presteady motion are described below.

**Table II**

| Experiment | \(P_\text{max}/P_\text{rest}\) | \(P_{\text{null}}/P_\text{null}\) | \(P_{\text{null}}/P_\text{rest}\) | \(\Delta_{\text{null}}/\Delta_{\text{rest}}\) | \(\Delta_{\text{null}}/\Delta_{\text{rest}}\) |
|------------|------------------|------------------|------------------|------------------|------------------|
| 24 iv 74   | 0.65             | 0.70             | 1.17             | 2.06             |
| 2 v 74     | 0.62             | 0.72             | 1.08             | 1.71             |
| 3 v 74     | 0.55             | 0.64             | 0.97             | 1.42             |
| 10 v 74a   | 0.56             | 0.77             | 0.93             | 4.00             |
| 10 v 74b   | 0.50             | 0.72             | 0.98             | 3.75             |
| 14 v 74    | 0.58             | 0.61             | 0.90             | 2.80             |
| 14 v 74a   | 0.48             | 0.56             | 1.12             | 3.90             |

Range of \(P_{\text{null}}\): 0.56\(\pm\)0.06

Mean \(\pm\) SD: 0.56\(\pm\)0.06 1.01\(\pm\)0.1 1.7 3.2\(\pm\)0.7

Each experiment consists of two matched releases, one in high KCl (240 mM) and the other in the control KCl (140 mM) activating solution; temperature 4\(^\circ\)–6\(^\circ\)C. \(P_{\text{null}}\) is the ratio of \(P_{\text{null}}\), the value of the load, and \(P_\text{null}\), the steady isometric force for each solution. The 95% confidence interval for the mean null time ratio is between 0.93 and 1.09. The first three experiments were made with fibers from *R. petersi* and the last three with fibers from *R. petersi berlandieri*; the steady speeds at a given relative load were not significantly different in fibers from the two species.

Fig. 4 shows the effect of activation in 50 mM KCl. The isometric force at the lower ionic strength is 1.7 times greater than that in the control. The steady speed is the same as in the control but the transient is markedly different. The amplitude of the transient is suppressed to the extent that the motion appears to be nearly steady from the start. It is difficult in these cases to make reliable quantitative measurements of the null time, but it appears to be unchanged.

Fig. 5 shows the effect of still lower KCl (20 mM) on the motion of the skinned fiber. In this case the speed of shortening decreased continuously as the motion progressed. Although this behavior makes it difficult to separate the motion into a transient and a steady phase, the record suggests that the transient seen in 50 mM and higher KCl concentrations was completely suppressed in 20 mM.
50mM KCl  140mM KCl

Figure 4. Influence of low KCl on the presteady motion at 0°-1°C (R. pipiens pipiens). Top trace, displacement; middle trace, force; bottom trace, zero of force. Fiber length = 1.6 mm, isometric force is 128 mg in 50 mM KCl and 77 mg in the control solution (140 mM KCl). Relative load is 0.62. As in Fig. 2, the dashed line is drawn to emphasize the effect of ionic strength on the transient. The amplitude of the transient is very much reduced in 50 mM KCl.
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**FIGURE 5.** Suppression of the transient amplitude in a skinned fiber (*R. pipiens pipiens*) with 20 mM KCl at 0°-1°C. Identification of the different traces is the same as described in the earlier figures. Fiber length = 1.7 mm, isometric force is 53 mg in 20 mM KCl and 31 mg in the control solution. Relative load is 0.68.

KCl. When the initial isotonic motion in 20 mM KCl was estimated by means of the interpolation technique shown in Fig. 1, the initial velocity appeared to be close to that of the steady motion in 140 mM KCl. The velocity decreased continuously as the motion continued. The reason for this curvature in the shortening trace was not investigated further.

There is a possibility that the increase in force in the skinned fibers in low KCl is accompanied by the utilization of ATP at a rate faster than it can be replenished from the bathing medium and that this change in ATP concentration affected the contraction kinetics rather than the change in KCl per se. A number of control experiments in which the influence of fiber diameter on the isotonic velocity transients was examined were made to rule this out. Also, because in 140 mM KCl the rate of ATP utilization is closely related to the calcium level (Levy et al., 1976), the observation that the transients are the same in both high and low free calcium solutions indicates that under our conditions...

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2 This was a matter of concern because in experiments where the ATP concentration of the bathing solution was lowered to 0.5 mM (Mg = 0.1 mM), the transient amplitude in 140 mM KCl was reduced. We checked for an effect of diffusion in the presence of the standard ATP concentration (5 mM) in two ways. First, the effect of low ionic strength (50 mM KCl) was compared on a large fiber with another fiber, from the same fiber bundle, of half the diameter. The effect of low ionic strength on the transient was found to be similar in the two fibers. In another experiment, a skinned fiber isolated in the usual manner was carefully split to about 1/3 the initial size. The effect of low KCl on the transients in the split fiber was the same as that in Fig. 4. These experiments showed that reducing the diffusion pathway for ATP by a factor of 2 or 3 has little influence on the effects of ionic strength on the isotonic motion. Finally, the fact that the effects of low KCl on the transients are similar in the rabbit psoas fibers to those in the frog (Gulati, 1975) also argues against the possibility that suppression of the amplitude of the transient by low ionic strength is due to depletion of ATP within the fiber. At the same temperature (5°-6°C) the unloaded shortening velocity (*V*<sub>max</sub>) of the rabbit fibers is 1/10th that of frog fibers (Gulati, 1975), suggesting (Barany, 1967) a proportionately reduced utilization of ATP in the mammalian fibers.
the ATP gradient within the fiber does not influence the motion. This argument implies that the increased amplitude of the transient seen in high KCl is an ionic strength rather than an ATP effect, because high KCl probably causes the ATP utilization within the fiber to decrease. The decrease in transient amplitude seen in low KCl is consistent with the increase in amplitude produced by high KCl, although some other factor associated with the increased level of isometric force can not be excluded.

In summary, the maximum amplitude of the transient increased in high KCl and decreased in low KCl. Ionic strength had no effect on the null time of the transient.

**DISCUSSION**

The most significant findings of the present study are that (a) the isotonic presteady contraction kinetics at a given relative load are independent of the calcium concentration and (b) these kinetics are affected by ionic strength. This is important because previous studies (Thames et al., 1974) have shown that under appropriate conditions (ionic strength > 190 mM) changes in calcium and KCl concentration affect the steady-state response of skinned frog muscle fibers in the same way. The fact that the transient response is affected differently is the first direct evidence that calcium and KCl modulate the isometric force developed by the contractile mechanism through intrinsically different mechanisms.

**Effect of Calcium**

Measurements of the null time as well as the amplitude of the transient at a given relative load indicate that these parameters are insensitive to changes in calcium concentration of the activating medium (Table I). Because the velocity transients reflect properties of the cross-bridge mechanism (Civan and Podolsky, 1966; Huxley, 1974; Podolsky et al., 1974), these findings provide additional evidence that the effect of calcium on force is mediated by modulating the number of switched “on” cross-bridges without influencing either the rate functions for cross-bridges that are turning over or the force developed by a given cross-bridge (Podolsky and Teichholz, 1970). The recent measurements of force transients in tortoise iliofibularis (Heinl et al., 1974) and rabbit psoas (Ruegg et al., 1975) also appear to be consistent with the idea that calcium acts through a simple recruitment process. It is worth pointing out that these observations imply that the steady force at a given calcium level is directly proportional to cross-bridge number and that the cross-bridges work independently.

Levy et al., (1976) recently reported a good correlation between relative tension and relative ATPase when the calcium ion concentration was varied in glycerinated frog fiber. However, there appeared to be a discrepancy of 20-25% between the two parameters at low calcium, from which the authors concluded that calcium ions affect both the kinetics of the cross-bridge turnover as well as the number of force generators. Our results do not rule out differences of this magnitude because these could be masked by the 10-20% uncertainty of
measurements in the null times and the amplitudes of the velocity transients (Table I).

**Effect of Ionic Strength**

Changes in the calcium-activated force of skinned frog muscle fibers due to changes in ionic strength are accompanied by changes in the presteady phases of the isotonic displacement. High KCl decreases the force and increases the amplitude of the transient; low KCl increases the force and decreases the amplitude of the transient. The null times at the moderate relative loads used in the present study are not affected. Similar effects of ionic strength on isometric force and on the velocity transients have been found in chemically skinned rabbit psoas fibers, where the resolution of the presteady motion is greater (Gulati, 1975, and unpublished experiments). These results provide evidence that ionic strength affects force by changing the kinetic parameters of the cross-bridge mechanism. Therefore, although both calcium and ionic strength have the same effect on the steady-state behavior of skinned frog muscle fibers (both modulate the isometric force but do not change the steady shortening speed in a significant way), the mechanisms of action are apparently different.

The changes in isometric force that accompany changes in ionic strength could be due to (a) a change in the force produced by a given cross-bridge and/or (b) a change in cross-bridge number. As regards the first mechanism, a change in the cross-bridge force function is necessarily associated with a change in the rate functions for cross-bridge turnover (Hill, 1974). In this case one would expect to see changes in the contraction transients with ionic strength. As these do occur, an influence of ionic strength on the force produced by a given number of cross-bridges is consistent with the present results. On the other hand, it is also possible that the total number of active cross-bridges could be affected by ionic strength. However, the fact that the contraction transients are modulated by ionic strength implies that this would have to come about through a change in the properties of the cross-bridge mechanism rather than through the switchlike mechanism underlying the calcium effect. For example, ionic strength could change (a) the rate functions per se and/or (b) the "reach" of a myosin projection (Huxley, 1957; Squire, 1974) and thus the number of projections that are close enough to actin sites to form cross-bridges.

An effect of ionic strength on the kinetic properties of the cross-bridge mechanism in the organized system is consistent with the behavior of the actomyosin ATPase system in solution. Stimulation by calcium requires the presence of the regulatory proteins. On the other hand, ionic strength affects the actomyosin ATPase even in the absence of regulatory proteins. In particular, increasing ionic strength (in the range between 18 and 61 mM) decreases the apparent affinity constant of the actin-activated heavy meromyosin (HMM) ATPase, without changing the maximum ATPase activity at infinite actin (Rizzino et al., 1970). It should be noted that the steady-state ATPase measurement (in which HMM is kept constant and actin is varied) do not exclude the possibility that ionic strength has a direct effect on the number of HMM binding
sites on actin. The skinned fiber data make this mechanism unlikely, however, because in this case a change in ionic strength would be equivalent to a change in calcium, and the isotonic velocity transient would not have been affected.

In view of the biochemical evidence that ionic strength changes the kinetic properties of the contraction mechanism, the insensitivity of the skinned frog fiber relative force-velocity relation to ionic strength is unexpected. However, this finding simply indicates that the steady motion of these fibers is less sensitive to changes in the kinetic properties that result from variation in ionic strength than are the rapid contraction kinetics. In short, the steady-state properties of the fiber give less information about cross-bridge kinetics than is given by the contraction transients. Calculations based on a specific model of the cross-bridge mechanism provide an example of this behavior (e.g., compare the motion in Podolsky et al., 1969, with that in Podolsky and Nolan, 1973). In this case, cross-bridge parameters were found that changed the characteristics of the transients but not the steady speed of isotonic contraction.

It is interesting to note that in intact fibers ionic strength appears to have the expected effect on relative force-velocity relation (Howarth, 1958; Edman and Hwang, 1977). The reason this is seen in intact fibers but not in skinned fibers is not known, but it may be due to differences in the intracellular chemical milieu in the two preparations. Some of the normal cellular constituents may be lost when skinned fibers are perfused, and under our experimental conditions the concentrations of certain metabolites and ions (magnesium, for example) are probably not physiological. However, inasmuch as the intracellular milieu is under experimental control in the skinned fibers, it is possible to study directly the influence of various chemical parameters on the basic cross-mechanism. In the present study we have done this for the case of ionic strength and calcium for a frog muscle fiber preparation. The same approach can be used to study the influence of other chemical parameters in this and other skinned fiber preparations.

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