Effect of External Calcium and of Temperature on Contraction in Snake Muscle Fibers

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ABSTRACT The effect of external calcium and of temperature on the contractile responses has been studied in voltage clamped snake twitch muscle fibers. Increasing [Ca++]₀ from 0.2 to 7.0 mM raised contractile threshold by 15–20 mV, the latter coinciding with the appearance of delayed rectification. The duration of contracture, the rates of rise and decay of tension depended on the level of depolarization and [Ca++]₀. The minimum duration of repolarization necessary to restore the contractile response was much shorter in high [Ca++]₀. When the bathing solution was cooled to 10 from 20°C the time-course of contracture was markedly prolonged and the outward current was reduced without significant change in maximum tension. The threshold for contraction tended to be somewhat lower at the lower temperature. The contractile repriming was much slower at low temperature. However, reduction in temperature slowed the rate of recovery much less at low [Ca++]₀ than at normal [Ca++]₀.

INTRODUCTION

The effect of muscle of changing external calcium concentration has been widely studied. In both fast and slow fibers a contracture follows maintained depolarization if this exceeds a certain threshold. Changes in [Ca++]₀ have been shown to affect the threshold and time-course of contracture in both types of fibers. In slow fibers maximum contracture tension is increased by long-term exposure to altered [Ca++]₀ (Lüttgau, 1963; Lännergren, 1967; Kirby, 1970). In twitch-type fibers, in which impulse activity is blocked, a contracture follows suprathreshold depolarization. Here the effect of altering [Ca++]₀ is more complex. Raising [Ca++]₀ prolongs the duration of the contracture response. Prolonged exposure to solutions containing low [Ca++]₀ may also result in a decrease in amplitude of contracture (Frank, 1960; Jenden and Reger, 1963; Lüttgau, 1963).
Another way of altering the time-course and magnitude of the contrac-
tures in twitch muscle fibers is by change in temperature, reduction in tem-
perature causing a prolongation of contracture. Among other effects, reduc-
tion in temperature may decrease the rate of active uptake of Ca++ from sar-
coplasm to sarcoplasmic reticulum (Weber et al., 1966). It was therefore of
interest to compare the changes produced by temperature and changes in
$[Ca^{++}]_o$.

The use of very short twitch muscle fibers in the snake allows rapid and
fairly uniform changes in membrane potential to be imposed by current
passage from an intracellular micropipette (Heistracher and Hunt, 1969 a).
This permits the study of rapid changes of membrane potential on contrac-
tile activation, inactivation, and repriming, which was defined by Hodgkin
and Horowicz (1960) as the restoration of the contractile activation mecha-
nism to subsequent depolarization (also see Heistracher and Hunt, 1969 b).
This preparation has been utilized for a study of the effect of calcium and
temperature on these parameters.

**METHODS**

Isolated short scale muscle fibers (about 1.5 mm in length and 50-100 μm in diam-
eter) of the garter snake (*Thamnophis*) were used. The fibers were impaled with
both recording and current-passing micropipettes. The preparation was studied in a
voltage clamp mode. The membrane potential was fed into a high gain inverting
amplifier, the output of which was connected to the current electrode. Through
summing resistors other potentials could be added to the input of the current ampli-
fier. The current passed through the membrane was recorded in response to step
changes in membrane potential. Tension was detected by a semiconductor strain
gauge. The details of the methods are similar to those used by Heistracher and Hunt
(1969 a). The normal bathing fluid had the following composition (in millimolar):
NaCl 158, KCl 2.15, CaCl$_2$ 3.5, MgCl$_2$ 1.7, Na$_2$HPO$_4$ 2.15, NaH$_2$PO$_4$ 0.85, and glu-
cose 9.8. Variation in $[Ca^{++}]_o$ was made without alteration of other cations. Tetro-
dotoxin (Sankyo, Tokyo, Japan) in a concentration of $1 \times 10^{-7}$ g/ml was usually
used to block impulse activity. Unless otherwise noted, experiments were carried
out at room temperature (20–24°C). Variations in bath temperature were brought
about by a thermoelectric device (Cambridge Thermionic Corp., Cambridge, Mass.),
temperature being sensed by a thermistor.

**RESULTS**

*Effect of Calcium on Contraction Threshold*

Contractile threshold was reached when the membrane was depolarized
to a particular level, in keeping with previous findings (Hodgkin and Horo-
wicz, 1960; Orkand, 1962, and others). The contraction threshold in 3.5
mM Ca was found to be $-36.4 \pm 3.3$ mV (SD of mean) in 16 fibers. This
was determined by applying graded depolarization steps until a just-detect-
able contraction appeared on the strain gauge record. In accordance with earlier findings (Costantin, 1968; Kao and Stanfield, 1968; Heistracher and Hunt, 1969 a), the threshold for contraction usually coincided with the appearance of delayed rectification, evident in studies of the inactivating outward current (Heistracher and Hunt, 1969 a and b).

The contractile threshold varied with changes in $[\text{Ca}^{++}]_o$, as shown in Fig. 1. Thus an increase in $[\text{Ca}^{++}]_o$ from 0.2 to 7 mM shifted the contraction threshold from $-47$ to $-28$ mV. A similar relation has been observed by Lüttgau (1963) and by Costantin (1968) in frog muscle fibers and by Frankenhaeuser and Lännergren (1967) in Xenopus muscle fibers.

![Figure 1. Effect of $[\text{Ca}^{++}]_o$ on the contractile threshold. Vertical bars represent the standard deviation of the mean. Figures in parenthesis give number of examined fibers. Abscissa: $[\text{Ca}^{++}]_o$ in millimoles per liter. Ordinate: membrane potential in millivolts.](image)

Fig. 2 shows some examples of the responses to graded depolarization steps at two different $[\text{Ca}^{++}]_o$, 0.2 and 7.0 mM. In the upper series of records taken in 0.2 mM Ca, the appearance of an outward current which inactivated with time was associated with a small contracture. During smaller depolarization steps the current remained constant. Increasing the amplitude of the depolarization step, which lasted approximately 1 s, caused a progressive increase in contractile response up to a maximal value. The lower series of records were taken in 7.0 mM Ca and the depolarization steps are approximately comparable to those above. Threshold for both contraction and delayed rectification occurred at a more depolarized level of membrane potential. The maximal contractures were approximately the same in both. Fig. 3 B shows a plot of the relation between tension and membrane potential determined in the same fiber at $[\text{Ca}^{++}]_o$ of 0.2 and 7.0 mM. The upper plot (Fig. 3 A) shows the relation between membrane current and amplitude of
Figure 2. Tension \( (T) \) and current \( (I) \) changes in a muscle fiber in 0.2 (upper series) and 7.0 mM (lower series) Ca solutions. The lower records show the responses to a roughly comparable series of depolarizing steps. At the left of each record, the upper trace is current \( (I) \); middle trace, potential \( (V) \); lower trace, tension \( (T) \).

The depolarization step. Both the peak and final currents are shown, the difference indicating the amount of the current which was inactivated. The current associated with small depolarization steps, below the threshold for delayed rectification, was smaller in high \([\text{Ca}^{++}]_o\) than in low, indicating that the membrane resistance is raised as \([\text{Ca}^{++}]_o\) is increased. The threshold for delayed rectification shifted by approximately the same amount as did the threshold for contractile activation in agreement with earlier work by Costantin (1968). Also the initial amplitude of inactivating current was found to be reduced by high \([\text{Ca}^{++}]_o\).

**Effect of Calcium on Contracture**

\([\text{Ca}^{++}]_o\) is known to affect the time-course of contractures in twitch muscle fibers in which impulse activity has been blocked (Lüttgau, 1963; Foulks and Perry, 1966; Caputo and Gimenez, 1967; Frankenhaeuser and Lannergren, 1967). In such fibers, the contractile response to a prolonged depolarization decays with time. Elevating \([\text{Ca}^{++}]_o\) prolongs the duration of such a contracture.

A similar effect was found in snake fibers and here the relationships between inactivation, membrane potential, and calcium concentration could be studied in more detail. Fig. 4 shows the response of a fiber to supramaximal depolarization steps of varied amplitude. In both 0.2 mM Ca (upper records) and 7.0 mM Ca (lower records) the fiber was initially clamped at \(-100 \, \text{mV}\). In the upper series of records the fiber was depolarized by 83 mV in A, 97 mV in B, and 109 mV in C, the duration of depolarization being about 6 s. The maximal tension produced in the three cases was essentially the same. As the amplitude of the depolarizing step increased the rate of decay of tension also increased. There was, as well, an increase in the rate of
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Figure 3. Plot of the relation between current and membrane potential (A) and between tension and membrane potential (B) in 0.2 and 7.0 mM Ca solutions. In A open circles and open triangles are measurements of peak current near the beginning of a depolarizing step. Filled circles and filled triangles are the current values at the end of the step.

tension rise as depolarization step increased. In all of the records tension had completely disappeared by the end of the depolarization step. The inactivation of contraction shows a dependence on membrane potential as noted previously (Heistracher and Hunt, 1969 a and b). The lower series of records were taken in 7.0 mM Ca. The depolarization steps were 96, 108, and 122 mV in D, E, and F. In record D inactivation was not complete by the end of the depolarization step. With a larger depolarization, inactivation proceeded more rapidly but at a slower rate than the same level of depolarization produced with the fiber in low [Ca++]o. (Compare C and E.) Still further depolarization, however, could further increase the rate of inactivation as
seen in F. Thus the rate of inactivation was slower in high \([\text{Ca}^{++}]_0\) at the same levels of depolarization. Finally, it may be seen that the rate of inactivation was almost the same at comparable levels of depolarization when the two series of records are compared (A to D, B to E, etc.).

The effect of \([\text{Ca}^{++}]_0\) on contractile activation and inactivation was studied further using double step voltage changes. An example is shown in Fig. 5. Left-hand series were taken in 7.0 mM Ca and those on the right in 0.2 mM Ca. In all cases the fiber was clamped at -100 mV and then initially stepped to +20 mV in 7.0 mM Ca (A–D) or to +5 mV in 0.2 mM Ca (E–G). After being held at these levels for about 0.6 s, the potential was either kept the same or was shifted to a new level until the end of the depolarization step, which had total duration of about 7 s. In B the fiber was held at +20 mV throughout. The tension nearly disappeared by the end of the depolarization step, but a small residual contracture persisted. In A the second step brought the membrane potential to +35 mV, and inactivation was more rapid and complete midway through the depolarization pulse. The second step was to +7 mV in C and to +3 mV in D. This reduction in size of the depolarization step diminished the rate of contractile inactivation, and a substantial amount of tension persisted throughout the total duration of depolarization step in D. In 0.2 mM Ca, maintaining the clamp potential at a steady level of +5 mV (F) caused a contracture which inactivated nearly completely by the middle of the depolarization step. In E a second step brought the membrane to +30 mV and accelerated the inactivation. In G the second step was to -15 mV causing a decrease in the rate of inactivation so that a slight amount of tension persisted until the end of the depolarization step.
Fig. 6 shows the relation between maximum rate of inactivation and membrane potential clamped at the second step in Fig. 5 with two different values of $[Ca^{++}]_o$. This clearly showed that the maximum rate depends on the membrane potential. Also different $[Ca^{++}]_o$ may shift the relation along the membrane potential. Although a linear relation between them was found over the range of membrane potential tested, it is probable that the rate is part of an S-shaped curve as has been shown in potassium contractures in frog toe muscles (Foulks and Perry, 1966).

**Effect of Calcium on Repriming**

Following the contractile inactivation which occurs after prolonged depolarization, twitch muscle fibers show a restoration of contractile response after a subsequent period of polarization, repriming, (Hodgkin and Horowicz, 1960). $[Ca^{++}]_o$ was found to modify the rate of repriming.
FIGURE 6. Relation between maximum rate of inactivation and membrane potential clamped after maximum contracture as shown at the second step in Fig. 5. The open and filled circles represent the results obtained in [Ca++] of 0.2 and 7.0 mM, respectively. The maximum rate is plotted as the ordinate, the membrane potential as the abscissa. Data obtained from Fig. 5.

An example may be seen in the records of Fig. 7. The upper record was taken with a fiber in 0.2 mM Ca, the lower record in 7.0 mM Ca. In both cases the fiber was clamped at -100 mV and depolarized to 0 mV. Each depolarization step was sufficient in duration to produce complete inactivation. Periods of repolarization to -100 mV, of varying duration, were given and the contractile response on subsequent depolarization recorded. It may be seen that the briefest periods of repolarization were not followed by a detectable contractile response when the fiber was again depolarized, but, as the duration of hyperpolarization increased, the restoration of contractile response appeared and then grew to a maximum. A comparison of the responses in 0.2 and 7.0 mM Ca shows that the minimum duration of repolarization necessary to restore the contractile response is much shorter in high [Ca++]o. It may also be noted that the rate of contractile inactivation is less rapid in high [Ca++]o, that is, the contracture response to prolonged depolarization lasts longer. It may also be seen in Fig. 7 that the decay of current accompanying a depolarization step fails to occur after brief periods of repolarization but with longer repolarization shows a restoration which appears together with the restoration of contractile response.

Fig. 8 shows the outward current and the recovery of contractile response as a function of duration of repolarization. It may be seen that the minimum time required for contractile repriming is approximately halved by shifting from 0.2 to 7.0 mM Ca. There is a comparable shift in the duration of the hyperpolarization required for the onset of delayed rectification when [Ca++]o is changed from 0.2 to 7.0 mM. A comparison of the results of a number of experiments on the repriming in 0.2 and 7.0 mM Ca is shown in Table I. The time-course of recovery of contraction was examined at -100 and -80 mV. The minimum times for repriming and the half recovery times are com-
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**Figure 7.** Effect of $[\text{Ca}^{++}]_o$ (0.2 mM Ca in the upper record, 7.0 mM Ca in the lower record) on the contractile repriming which were obtained from the same fiber. The fiber was clamped at 0 mV except for periods of polarization to $-100 \text{ mV}$. At the end of each record, the upper trace is current; the middle trace, potential; the lower trace, tension.

pared. Both are approximately halved on shifting from 0.2 to 7.0 mM Ca. These findings are in keeping with the observations of Frankenhaeuser and Lannerengren (1967) on the effect of $[\text{Ca}^{++}]_o$ on the recovery of twitch responses following a preceding contracture produced by 190 mM potassium in *Xenopus* muscle fibers. They found that increasing $[\text{Ca}^{++}]_o$ decreased the time required after the contracture for the twitch response to recover.

**Effect of Cooling on Contracture**

When the bathing solution was cooled from 20 to 10°C the time-course of contracture was markedly prolonged without significant change in the maximum tension developed. An example is shown in Figs. 9 and 10. In Fig. 9 the left-hand records were taken at 20°C, the right-hand records at 10°C. In all cases the fiber was initially clamped at $-100 \text{ mV}$. In the upper records the fiber was depolarized to 0 mV. At this depolarization a contracture developed which inactivated so that tension became negligible after about 4.5 s at 20°C. In the lower left the fiber was depolarized to $+10 \text{ mV}$ and inactivation was more rapid, the tension disappearing in about 3 s. The upper right figure shows that depolarization to 0 mV at 10°C caused a very prolonged contracture which was maintained for more than 30 s. In the lower right a step depolarization to $+10 \text{ mV}$ produced a contracture which lasted about 12 s. Thus, although the rate of inactivation was greatly slowed by this change in temperature, an increase in the level of depolarization was still effective in increasing the rate of inactivation. In Fig. 9 it may also be noted that the rate of rise of tension in the contracture was considerably slowed at low temperature.
Figure 8. Rate of repriming at -100 mV in 0.2 and 7.0 mM Ca solutions. Lower graph shows the contractile response when the fiber was depolarized to 0 mV following varying durations of polarization to -100 mV. Upper graph shows peak current (open circle and open triangle) and final current (filled circle and filled triangle) to depolarizing pulses.

Table I

Repriming times at membrane potentials of -80 and -100 mV in 0.2 and 7.0 mM Ca solutions. Depolarization to 0 mV. All times are means. Number of experiments in parentheses.

| Membrane potential | 0.2 mM Ca | 7.0 mM Ca |
|--------------------|-----------|-----------|
|                    | Minimum time | Half recovery time | Minimum time | Half recovery time |
| mV                 | s           | s          | s            | s            |
| -80                | 0.71 (3)    | 1.34 (3)   | 0.37 (3)     | 0.78 (3)     |
| -100               | 0.54 (5)    | 0.92 (5)   | 0.25 (7)     | 0.52 (7)     |
Another striking effect of cooling was a marked decrease in the delayed current which accompanied a depolarization step. Thus, in the experiment of Fig. 9 the peak current following depolarization to 0 mV was $4 \times 10^{-7}$A at 20°C whereas the current associated with the same depolarization step was only $1.3 \times 10^{-7}$A at 10°C. The reduction in the amount of outward current may be related to the decrease in potassium conductance. It has been shown that the magnitude of the outward current attained at any voltage decreases at low temperature in giant axons (Hodgkin et al., 1952). However, the rate constant for decline of potassium conductance decreases much more, at 3°C being about one-fifth of that at 19°C (Adrian et al., 1972).

![Figure 9. Effect of cooling on contracture. 20°C in the left column, 10°C in the right column. The fiber was depolarized to 0 mV (in upper records) and to +10 mV (in lower records). At the beginning of each record, the upper trace is current; middle trace, potential; lower trace, tension.](image)

Further details of the effect of temperature are shown in Figs. 10 and 11. The responses to graded depolarization steps are shown at 21°C (left column) and at 9°C (right column) in Fig. 10. In both cases the initial clamped potential was −80 mV. As the depolarization step was increased at 21°C, the contracture at first lasted the duration of the depolarization step. As the depolarization step became larger the development of tension was more rapid, and the contracture became briefer, so that with a step to +15 mV the contracture had completely inactivated in about 1 s. In all records at 21°C in which contraction threshold was exceeded, there was a component of the delayed current. At 9°C increasing the depolarization step above threshold increased the magnitude of contraction but there was no significant increment of outward current which inactivated with time for this duration depolarization step even when the membrane was clamped at +15 mV in this fiber. Furthermore, the amplitude of the steady-state current was considerably less at the lower temperature (Fig. 11 A).

Fig. 11 shows plots of the relation between current and membrane potential (A) and between tension and membrane potential (B) in the same fiber.
FIGURE 10. Tension (T) and current (I) changes in a muscle fiber in the standard saline at 21°C (left column) and 9°C (right column). At the beginning of each record, the upper trace is current (I); middle trace, tension (T); lower trace, potential (V). Calibration markers: 2 × 10^{-7}A and 64 mg for both columns.

FIGURE 11. Plot of the relation between current and membrane potential (A) and between tension and membrane potential (B) at 21 and 9°C. In A open circles and open triangles are measurements of peak current at the beginning of a depolarizing step at 21 and 9°C, respectively. Filled circles are the current values at the end of the step at 21°C. The current values at the end of the step at 9°C are not shown. See text.

at 21 and 9°C. The potentials for half-maximum tension at 21 and 9°C were -20 and -29 mV, respectively. When the contractile threshold was determined by applying graded depolarization steps just as described before, it was -36.4 ± 3.3 mV (SD of mean) at 20°C in 16 fibers and -38.4 ± 5.2 mV (SD of mean) in 13 fibers at 10°C. Measurements at both temperatures were made with [Ca++]o of 3.5 mM. In these experimental conditions the threshold for contraction at the two temperatures was not significantly different. However, there was a tendency for the threshold to be lower with a decrease in temperature. Recently Caputo (1972a) has found that the contractile threshold for potassium contractures in single frog muscle fibers is about 10 mV lower at 3°C than at room temperature.

Cooling had a rapid effect on the time-course of contracture as shown in Fig. 12. In the upper record the temperature in the bathing fluid recorded by a thermistor near the muscle was initially 18.4°C (left arrow in the upper record). By the end of the record it had been lowered to 12.3°C (right arrow).
Figure 12. Effect of cooling on the membrane current and contractile tension. In the upper record, the temperature in the bathing solution was changed from 18.4 to 12.3°C (arrows at the beginning and end of the record); in the lower record, from 10.5 to 10.0°C (arrows at the beginning and end of the record). The fiber was clamped at -100 mV and depolarized to +15 mV. At the beginning of each record, the upper trace is current; middle trace, potential; lower trace, tension; except the trace for temperature.

Effect of Cooling on Repriming

Although the contracture at low temperatures was maintained far longer than at room temperature the contractile response finally inactivated with a depolarization of sufficient duration. Repolarization could then restore the contractile response. The repriming was much slower at low temperatures. Similar results were found in earlier works on potassium contractures in frog skeletal muscles (Milligan and Edwards, 1965; Caputo, 1972 a). Fig. 13 compares the repriming in a fiber at 16.5°C in the upper record and at 10°C in the lower record. The fiber was held at 0 mV except for periods of polarization to -100 mV.

A plot of the relation between the recovery of contractile response and the duration of repolarization is shown in Fig. 14. At 16.5°C (curve a) the
Figure 13. Effect of temperature (16.5°C in the upper record; 10°C in the lower record) on rate of contractile repriming. The fiber was clamped at 0 mV except for period of polarization to -100 mV. A separate record in the lower part of the figure was taken in 3 min after the polarization. At the beginning of each record, the upper trace is current (I); middle trace, potential (V); lower trace, tension (T).

Figure 14. Rate of repriming at different temperatures in 3.5 and 0.2 mM Ca solutions. The fiber was depolarized to 0 mV following varying duration of polarization to -100 mV. a, 16.5°C in 3.5 mM Ca; b and b', 10°C in 3.5 mM Ca; c, 17°C in 0.2 mM Ca; d, 11°C in 0.2 mM Ca; e, 23°C in 0.2 mM Ca. See text.

minimum time for repriming was 0.95 s and the curve was quite steep. At 10°C (curve b and b') the minimum time was about 3.0 s and the curve had a much smaller slope. The result was roughly comparable to that found in potassium contractures in frog muscle fibers by Caputo (1972 a). Curve b was plotted for the first few contractile responses and b' was for later responses. At this lower temperature these responses were gradually reduced by continuous depolarization pulses and consequently the curve moved to the right direction with slower recovery. However, almost complete recovery of repriming was observed in 3 min after the repolarization as shown in a separate figure in Fig. 13. In Fig. 14 control responses (100%) were taken as the first contractile response to the same depolarization with sufficient duration.

Also Fig. 14 shows the results of a single experiment to determine at low
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[Ca++]o, the effect of varying the temperature on contractile repriming. Curves c and d represent the relation between the recovery of contractile response and the duration of repolarization in 0.2 mM Ca at 17 and 11°C, respectively. The fiber was held at 0 mV except for periods of polarization to -100 mV. At this calcium concentration lowering temperature also prolonged the time of polarization required for contractile repriming. However, reduction in temperature slowed the rate of recovery much less at low [Ca++]o than at normal [Ca++]o.

DISCUSSION

The effect of changes in [Ca++]o and in temperature have been examined on voltage-clamped twitch-type snake muscle fibers treated with tetrodotoxin. Contractile threshold is appreciably shifted by [Ca++]o and contracture produced by suprathreshold depolarization is prolonged by increasing [Ca++]o. The prolongation of contracture that accompanies an increase in [Ca++]o may result from enriched stores in sarcoplasmic reticulum. However, it would seem likely that the effect of increased [Ca++]o is due to an effect on a calcium conductance of the sarcoplasmic reticulum membrane suggested by Ebashi and Endo (1968) and Heistracher and Hunt (1969 a). Increase in [Ca++]o hastens the rate of repriming at room temperature. This again might be due to an increased storage of Ca in sarcoplasmic reticulum. The effects of changing [Ca++]o on outward current which accompanies depolarization are similar to the changes found in squid axon. There, Frankenhaeuser and Hodgkin (1957) found that a reduction in [Ca++]o caused a large increase in the potassium current which accompanied moderate depolarizations. The increase in fiber resistance found with increase in [Ca++]o, noted in the present study, appears to have a similar explanation.

Contractile threshold is little affected by change in temperature (between 20 and 10°C) but the contracture produced by suprathreshold depolarization is much prolonged by lowering temperature. If relaxation is due to the uptake of Ca from sarcoplasm to sarcoplasmic reticulum, the prolongation of contracture at low temperature may depend in part on the decrease in the rate of Ca uptake by sarcoplasmic reticulum that occurs with reduction in temperature (Weber et al., 1966). The reduced rate of repriming seen on lowering temperature might be explained if repriming depended on a potential and time-dependent reactivation of a calcium conductance in the sarcoplasmic reticulum membrane since such a process is likely to be temperature dependent. This would be in keeping with the suggestions that contractile activation results from depolarization of the sarcoplasmic membrane causing release of Ca (Ebashi and Endo, 1968) and that repriming results from a reactivation of this conductance mechanism (Heistracher and Hunt, 1969 b). It seems likely that the rate of inactivation of the
conductance would be influenced by temperature. This might then be a major reason for the slower fall of tension at low temperature, equally important as a slowing down of a calcium pump. Recently Caputo (1972 b) has shown a prolonged release of Ca in the cold in the experiment on the interruption of potassium contracture in frog single muscle fibers.

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