Influence of Partial Activation on Force-Velocity Properties of Frog Skinned Muscle Fibers in Millimolar Magnesium Ion

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ABSTRACT Segments of briefly glycerinated muscle fibers from Rana pipiens were activated rapidly by a brief exposure to 2.5 mM free calcium followed by a solution containing calcium buffered with EGTA to produce the desired level of force. Steps to isotonic loads were made using a servomotor, usually 3–5 s after the onset of activation. The relative isotonic forces (P/P0) and velocities from contractions obtained under similar circumstances were grouped together and fitted with hyperbolic functions. Under the condition of 6 mM MgCl2 and 5 mM ATP, there was no significant difference in the relative force-velocity relations obtained at full activation compared with those obtained at partial activation when developed force was ~40% of its full value. Control experiments showed that a variety of factors did not alter either the relative force-velocity relations or the finding that partial activation did not change these properties. The factors investigated included the decline in force that occurs with each successive contraction of skinned fibers, the segment length (over a range of 1–5 mm), the sarcomere length (over a range of 1.9–2.2 μm), the magnesium ion concentration (26 μM and 1.4 mM were tested), the ATP concentration, the presence of free calcium, and the age of the preparation (up to 30 h). Attempts to repeat earlier experiments by others showing a dependence of shortening velocity on activation were unsuccessful because the low ionic strength used in those experiments caused the fibers to break after a few contractions. The main conclusion, that the shortening velocity is independent of the level of activation, is consistent with the hypothesis that the cross-bridges act independently and that activating calcium acts only as an all-or-none switch for individual cross-bridge attachment sites, and does not otherwise influence the kinetics of cross-bridge movement.

INTRODUCTION

A knowledge of the effects of activating calcium on the contractile kinetics of muscle is important for understanding the basic mechanisms of contraction and for evaluating the actions of inotropic agents that influence the contractile system.
strength of the myocardium. In principle, these effects would be defined best in a skinned fiber preparation, where the calcium ion concentration can be controlled directly. Previous experiments designed to examine these effects in skinned fibers have produced contradictory results in spite of apparently adequate techniques (see review by Podolin and Ford, 1983). Podolsky and Teichholz (1970) presented experiments showing that calcium does not affect the maximum shortening velocity of muscle. Julian (1971) published evidence that points to exactly the opposite conclusion, namely, that a decrease in activation decreases the maximum velocity. Each laboratory has published additional evidence supporting its original conclusion (Thames et al., 1974; Julian and Moss, 1981), so the controversy continues. These experiments by others were performed at unphysiologically low magnesium ion concentrations, so that their relation to physiological contraction remains in question. In these earlier experiments, the ATP exceeded the magnesium concentration by four to five times, so that the concentration of the free magnesium ion was very low and there was a substantial amount of free ATP in the solution. More recent analyses of intact muscle using nuclear magnetic resonance indicate that most of the ATP in muscle is complexed to magnesium (Burt et al., 1976) and that the free magnesium ion concentration is somewhere between 0.6 mM (Gupta and Moore, 1980) and several millimolar (Dawson et al., 1978; Cohen and Burt, 1977). The main purpose of the present experiments was to investigate the effects of various levels of calcium activation on the force-velocity properties of the skinned fibers at physiological concentrations of magnesium. A second purpose of the experiments was to attempt to resolve the controversy generated by the earlier studies. To do this, we repeated many aspects of the original experiments.

METHODS

Principle of the Method

Short segments of chemically skinned fibers were exposed to EGTA-buffered activating solutions with dissimilar free calcium ion concentrations. After achieving stable isometric force, the fibers were released to various loads and allowed to shorten isotonically. An initial rapid activation was achieved by including 10 mM caffeine in all solutions and by exposing the fibers to a 2.5 mM free calcium solution for a 0.5–1.5-s period before exposure to the EGTA-buffered solution. This technique permitted the contraction duration to be reduced to several seconds. An electronically controlled solution-changer was used to assure uniform contraction duration (Chiu et al., 1985).

Muscle length was regulated by a servomotor that could function in either of two modes. Length control could be achieved by controlling the servomotor with a signal from its own internal position sensor. Alternatively, the servomotor could be controlled by a signal from a force-transducer, to which the other end of the fiber had been attached (force control). At the time of activation, muscle length was held constant until tension reached its steady state level. This usually occurred 3–5 s after the first exposure to free calcium. The motor was then switched into force control for 160 ms, allowing the muscle to shorten isotonically. The force step was complete within 2–3 ms. During some contractions, three steps to progressively lower isotonic loads were made during the 160-ms period, imitating Julian’s (1971) original experiments. In most experiments, a single isotonic load was used. Shortening velocity was measured over a 15-ms period, beginning 20 ms after the onset of the isotonic step. The 20-ms delay was used to allow time for any
velocity transients to end. At the end of the 160-ms period, the motor was returned to
length control and a sufficient shortening step was imposed to make the muscle go slack,
defining the zero force level. The results from many contractions were grouped together
and fitted with hyperbolic force-velocity curves (Hill, 1938). Relative load (isotonic/
isometric force, P/Po) was used in fitting the curves to normalize for variations in isometric
force. The differences between curves derived under dissimilar conditions were then
evaluated by examining the parameters of the fitted curves.

In some experiments, two conditions could be compared in the same fiber segment.
For example, a single fiber segment could be exposed to both full- and partial-activation
solutions. In such instances, a series of five to eight contractions was performed under
one condition both before and after a series performed under the other condition. In
other experiments, two conditions could not be investigated in the same segment, such as
when short segments were compared with long segments. In these cases, single fibers
were divided into two or more segments and those tested under one condition were
matched by segments of the same fiber tested under the other condition.

Preparation
The posterior head of the semitendinosus muscle from Rana pipsens was suspended in a
cold solution containing 200 mM sucrose, 8 mM NaCl, 1.65 mM NaH2PO4, adjusted to
pH 7.2 (Ford and Podolsky, 1972). Small bundles of muscle fibers were cut out, soaked
in a 5 mM EGTA relaxing solution for 20 s, blotted, and placed in cold silicone oil (20
centistokes, Sigma Chemical Co., St. Louis, MO). Single fibers were isolated from the
bundles and cut into multiple segments, each 3–4 mm long. After chemical skinning, the
ends of each fiber were gripped with aluminum foil clips (Ford et al., 1977). The fibers
were attached to the apparatus by passing holes in the clips over hooks on the apparatus.

Chemical skinning. A brief glycerination technique similar to that described by Julian
(1971) was used. The fibers were rinsed in a cold relaxing solution containing 140 mM
K-propionate, 5 mM Na2ATP, 6 mM MgCl2, 5 mM K2EGTA, and 10 mM imidazole,
adjusted to pH 7.0 at room temperature. Fiber segments were then placed for 30–60 min
in a cold solution containing 50% (vol/vol) glycerol and 50% aqueous solution of 2 mM
K2EGTA and 10 mM NaH2PO4, adjusted to pH 7.0 with NaOH. During this treatment,
the solution was allowed to warm to room temperature. The fibers were then transferred
to a cold relaxing solution similar to that described above, to which 0.5% Triton X-100
had been added. They remained in this solution for 30 min. At the end of this treatment,
the fibers were stored in relaxing solution at 0°C for 30 min to 30 h before being used.

Experimental solutions. All solutions used in making fibers contract and relax con-
tained 10 mM caffeine, 5 mM Na2ATP, either 6 or 1 mM MgCl2, and 10 mM imidazole,
adjusted to pH 7.00 ± 0.02 at room temperature. The pH rose to ~7.4 when the
temperature was lowered to the working range of 3–5°C. The major ions were K+ and
propionate. Their concentration was 140 mM in the solutions with 1 mM MgCl2 and 151
mM in the solutions with 6 mM MgCl2. Each solution also contained one of the following:
0.1 mM K2EGTA, 5 mM K2EGTA, 2.5 mM CaCl2 (free calcium), or 5 mM EGTA,
together with various amounts of calcium (buffered calcium solutions). The buffers are
described in terms of the CaEGTA/EGTA ratio as well as pCa, calculated using a CaEGTA
stability constant of 6.39 at pH 7.4 and 5°C. The values used to derive this stability
constant were taken from Sillen and Martell (1971) and corrected for changes in hydrogen
ion activity that occur with increasing ionic strength (see Tsien and Rink, 1980, p. 627,
for discussion).

Apparatus

Force-transducer. A dual-phototransistor transducer (Chiu et al., 1982) was used. It
had a resonant frequency of 6 kHz and a compliance of ~1 mm/N.
Length control. A moving-coil, linear, loudspeaker-type "motor" was used to control length. A diode switching network (Ford et al., 1977) allowed the motor position to be controlled by a signal from either its own internal position sensor (length control) or by the force-transducer (force control). Steps to a constant isotonic force were complete within 2–3 ms.

Fiber chamber. A specially made chamber enabled precooled solutions to be injected into a trough that contained the fiber. A cooling solution circulated through the chamber. The temperature of this solution was continuously monitored to maintain a trough temperature of 3–5°C. In addition to the fiber trough, the chamber contained four channels, each of ~1 ml volume, used to precool the experimental solutions. New solutions were forced into the cooling channels by vacuum-driven syringe pumps controlled by solenoid valves. The valves were driven automatically by timers.

The fiber trough was 2 mm wide and 1 mm deep. The fiber was positioned ~10 mm downstream from the solution entry ports, leaving ~20 μl dead volume. Approximately 400 μl of cold solution was pushed through the trough with each solution change, which took ~400 ms.

Fiber trough. The fiber trough was covered so that solution could be forced through under pressure. At the end where solution entered the trough, the fiber was connected to the motor by a piece of 0.5-mm-o.d. hypodermic tubing passing through a seal consisting of a 25-mm-long piece of polyethylene tubing of 0.75 mm i.d. The end of the trough opposite the motor was not covered, and solution injected through the trough was removed by suction from this open portion. The vertical arm of the force-transducer projected into the open portion of the trough. A 5-mm-long hook projected horizontally from the end of the force-transducer arm, under the trough cover, where it was attached to a clip on the fiber.

Timing. The timing of all events, including solution changes, switching between tension and length control, and triggering the recording devices, was controlled by discrete digital logic circuits made by BRS/LVE, Beltsville, MD.

Recording. Records of length and force signals were made digitally and stored on a floppy disk by a PDP 11/10 computer (Digital Equipment Corp., Maynard, MA). Two sets of records were made for each contraction. A slower set, usually lasting 10 s but sometimes longer, was obtained with a digital input/output interface board in the computer. This slow set recorded the entire contraction-relaxation cycle. A more rapid set, usually lasting 200 ms, was made in early experiments using a transient recorder (513A, Physical Data, Beaverton, OR). The digital data stored in the transient recorder were transferred to the computer after the contraction. This technique permitted the timing of the isotonic steps in the contraction to be altered without altering the computer program. When the protocols for the experiments became well developed, both fast and slow digital records were made with the computer and the transient recorder was not used. In the earlier experiments, 1,018 data samples were taken for each of the four records—fast and slow length and force. In the later records, 506 data samples were taken for each record. Both the computer and the transient recorder had 10-bit analog-to-digital converters.

Sarcomere Length Adjustment

Sarcomere length was measured from the diffraction pattern of a helium/neon laser beam projected through the fiber onto a calibrated translucent screen. Before each contraction, the fibers were stretched to a sarcomere length of 3.0 μm and shortened to 2.0 μm several times, in an effort to realign any inhomogeneities of striation spacing that developed during the preceding contraction (Gordon et al., 1966a). Unless otherwise stated, all contractions began from a sarcomere length of 2.6 μm.
Procedure

The typical protocol for most experiments and typical force and length responses are illustrated in Fig. 1. The resting fiber was bathed in a solution containing 0.1 mM EGTA. The initial sarcomere length was set to 2.6 µm, determined by laser diffraction. Slow recording (Fig. 1, A–C) was begun a few hundred milliseconds before the activating sequence was started. After this brief delay, which was intended to permit baseline recording, a precooled solution containing 2.5 mM free calcium was flushed through the trough. For full activation (Fig. 1, A and C), the exposure to the free calcium solution lasted 1.0–1.5 s. For partial activation, the exposure time was 0.5–0.7 s. At the end of this period, a solution containing calcium buffered with EGTA was injected into the trough. Steps to isotonic loads were applied as soon as the steady force level was achieved, usually 3–5 s. These were followed by a large shortening step applied after 160 ms of isotonic shortening. The redevelopment of force at the shorter length was interrupted by

![Figure 1](image)

**Figure 1.** Records of force and length during three contractions of a single skinned fiber. The upper records (A–C) were made on a slow time base to show the whole contraction. The lower records (D–F) were made on a rapid time base to resolve the events associated with a step to an isotonic load followed by a large shortening to make the muscle go slack and define the zero force level. The full activation solution had a CaEGTA/EGTA ratio of 15 (pCa = 5.21); the partial activation solution had a ratio of 2.5 (pCa = 5.99). The relative isotonic loads in the three experiments were 0.13, 0.13, and 0.09, respectively. Fiber length, 3 mm. Records 33, 40, and 45 from the experiment of 21-vi-83.

the infusion of a relaxing solution containing 5 mM EGTA, 0.7–1.5 s after the onset of isotonic shortening. The muscle was stretched to its original length several seconds after the initiation of relaxation.

Rapid recording was initiated 20 ms before the step to the isotonic load (Fig. 1, D–F). The level of isotonic force was a preselected fraction of the difference between the resting force and the isometric force measured immediately before the step. In practice, there was considerable inaccuracy in the value of isometric force used for this determination since there was drift in the force-transducer output during the 3–5 s of activation that preceded the step. The large shortening step applied after the period of isotonic shortening was therefore used to define the zero force level.

The inaccuracy in the electronic estimate of isometric force created by force-transducer drift made it impossible to reproduce exactly a given isotonic load from one contraction to the next. Since direct comparison of shortening records in response to a given isotonic
load was not feasible, comparisons were made using force-velocity curves.

**Data analysis.** Force values were measured by averaging the records over a 15-ms period, from 20 to 35 ms after each step to an isotonic load. Isotonic velocities were measured as the slope of a least-squares linear regression fitted to the length record over the same period when the force was averaged. The 20-ms delay was used even though the steps were complete within 1–2 ms, to ensure that the steady state shortening had been achieved. Under the conditions used here, velocity transients were very inconspicuous. The zero reference for force was taken in the last 15 ms of the fast recording, after a large, rapid shortening step, which caused the fiber to go slack. Isometric force was measured as the difference between the force in the first 15 ms of fast recording, while muscle length was held constant, and the subsequent zero level.

The force-velocity values obtained under similar conditions were grouped together and fitted by a Newton-Raphson least-squares routine to the hyperbolic Hill (1938) equation. The equation used was

\[(P/P_0 + a)(V + b) = c,\]

where \(P/P_0\) is the relative force, \(V\) is the velocity, and \(a, b,\) and \(c\) are constants. The fitting routines also returned the standard errors and the covariances of three constants. The constants were then transformed into the more physiological parameters, isometric force \((P_0)\), maximum velocity \((V_{max})\), and maximum power \((P \cdot V_{max})\), using the equations

\[P_0 = c/b - a;\]  \(2\)
\[V_{max} = c/a - b;\]  \(3\)
\[P \cdot V_{max} = ab + c - 2\sqrt{abc}.\]  \(4\)

The standard errors of these physiological parameters were derived using standard formulae, and statistical analysis was performed by comparing these parameters obtained under different conditions, using the unpaired Student's \(t\) test. A \(P\) value of < 0.05 was considered significant.

**RESULTS**

The isometric force was frequently higher in the second contraction than in the first, but it diminished with each subsequent contraction. To compensate for any progressive changes in shortening ability associated with the fall in isometric force, control sets of force-velocity relations were measured before and after contractions under the test condition. The results under the test condition were then compared with the combined values obtained from the "pre" and "post" control experiments. The effects of the decay in isometric force were evaluated directly in a separate analysis.

**Partial Activation**

The effects of partial activation on the relative force-velocity curves are shown in Fig. 2. The calcium level required to obtain a given fraction of maximum force varied from fiber to fiber. Thus, a predictable level of force was difficult to achieve. The eight fibers in Fig. 2 had an average isometric force in the partial-activation solution that was half or less of the average force produced in the full-activation solution for both the "pre" and "post" groups of control contractions. A minimum of five partially activated and nine fully activated
contractions were obtained from each fiber. The total numbers of partially and fully activated contractions were 51 and 103, respectively. The number of fully activated contractions in the "pre" and "post" series was approximately the same. During the first set of fully activated contractions, the average isometric force was 1.02 mN (inset, Fig. 1A). The average force in the partially activated contraction was 37.5% of the initial control values. The average isometric force in the "post" control series was 72% of the initial value.

Separate hyperbolic curves (Eq. 1) were fitted to the combined data from the control contractions (Fig. 2A) and to the data from the partially activated contractions (Fig. 2B). The two curves are almost identical (Fig. 2C). The P values for t tests comparing the three parameters of the fitted curves were all >0.2. This suggests strongly that a variation in calcium had no effect on the kinetic properties of the unloaded cross-bridges, so that the maximum velocity at zero force was unchanged.

Control Experiments

Fiber deterioration. The decline of isometric force with successive contractions was not associated with any deterioration of the resting laser diffraction pattern or with any fiber damage visible under the dissecting microscope. To determine whether the decline of force was associated with any deterioration of kinetic properties, the data from fully activated contractions before and after partial activation were plotted separately in Fig. 3A. None of the parameters of the hyperbolic fits to the two sets of data were significantly different (all P values > 0.2).

The effect of a greater decline in developed force was examined in a second group of 10 fibers. These had been used in various protocols until their isometric force, developed at full activation, had decreased by >50%. Force-velocity curves derived from the initial, fully activated contractions of these fibers were compared with those from later contractions, after the fibers had "fatigued." The average isometric force in the second group of contractions was 37% of that in the first. The force-velocity curves for the sets of contractions are plotted in Fig. 3B. The difference in the two curves is somewhat greater than in the two previous graphs, but the curves cross in the middle. The maximum velocity for the later contractions is actually higher than that for the initial contractions. This difference in maximum velocities is significant (P < 0.01), but the other two parameters of the curves are not significantly different (both P values > 0.1). These findings suggest that the decline in isometric force generation is not associated with a decline in the shortening capability of the elements in the muscle that remain active.

The cause of the fiber deterioration was not found. Its rate was increased in series of long-duration contractions. In Fig. 4, the isometric force in each of a series of contractions is plotted against the contraction number. As shown, the rate of force decline was substantially greater when the duration of the contraction was increased from 4-6 to 16 s. In Fig. 4B, the isometric force is plotted against the contraction number for the eight fibers used in the partial-activation experiment. The slope for the second group of fully activated contractions ap-
THE JOURNAL OF GENERAL PHYSIOLOGY · VOLUME 87 · 1956

**A**

**B**

**C**

![Graphs showing relative force and velocity](image)

| Parameter   | Full       | Partial  |
|-------------|------------|----------|
| $P$ (min)   | 0.88±0.008 | 0.40±0.046 |
| Rel. $P_0$  | 1.06±0.125 | 0.97±0.130 |
| $V_{max}$   | 2.63±0.170 | 2.89±0.160 |
| P $V_{max}$ | 0.21±0.009 | 0.20±0.010 |
FIGURE 3. Force-velocity relations obtained after decline of isometric force compared with initial data from the same fibers. (A) The fully activated contractions from the eight fibers used to obtain the data in Fig. 2. The fully activated contractions obtained before and after partial activation are compared. (B) Fully activated contractions obtained after force had declined by at least 50% compared with an initial set of contractions in 10 fibers. In this and all subsequent figures, the solid circles are fitted by the solid curves and the open circles are fitted by the dashed curves.

FIGURE 2. (opposite) Force-velocity relations for fully (A) and partially (B) activated skinned fibers. The fitted curves (C) superimpose almost exactly, such that the parameters of the fitted curves (inset) do not differ significantly. Three sets of contractions were performed in each of the eight fibers. A set of five to eight partially activated contractions both preceded and followed a set of fully activated contractions. The force-velocity data from the fully activated contractions are combined in A. The means and standard errors for the average isometric forces in each set are plotted in the inset of A. The values of the relative $P_0$ values are dimensionless, whereas the values of $V_{max}$ have the same dimensions as the ordinate scales in the graphs. Thus, the values of $P \cdot V_{max}$ have the units of velocity ($\mu m$/half-sarcomere-s). To obtain $P \cdot V_{max}$ in units of power, it is necessary to multiply the values of $P \cdot V_{max}$ in the units given by the absolute value of $P_0$ in millinewtons.
pears to be an extension of a line fitted through the first group of points, as if the force decline continued unabated during the partial activation. Thus, partial activation did not appear to spare the fibers from a decline in force.\(^1\)

*Free calcium activation.* Fresh fibers often would develop tension nearly as rapidly in the full-activation, buffered calcium solution as in the free calcium solution. With successive contractions, however, the rate of force development in the buffered calcium solutions declined, unless the fibers were initially stimulated with free calcium. Force development in the partial-activation solution alone was much slower, requiring several tens of seconds to achieve a steady level. Contractions of this duration were associated with an accelerated decline in isometric force. For this reason, free calcium was used to keep the contraction duration to a minimum.

Control experiments were performed to determine whether exposure to free

\(^1\) The data in Fig. 4 cannot be subjected to any rigorous statistical analysis because the fibers did not all undergo the same number of contractions in each series. In spite of this lack of uniformity, the data do illustrate the trend for isometric force to decline systematically with each succeeding contraction, even when activation is partial.

**Figure 4.** Progressive decline of isometric force with successive contractions. (A) A group of four fibers studied with 16-s contractions compared with control contractions of 4–6 ms duration before and after the longer contractions. The force-velocity data for these fibers are given in Fig. 5B. The different fiber segments were subjected to different numbers of contractions in each group. (B) The isometric forces in the same series of eight fibers used in Figs. 2 and 3A are plotted as a function of contraction number. The minimum number of contractions in each group is five, but some fibers underwent as many as eight contractions. The numbers above the symbols indicate the number of fibers when it is not four in A or eight in B. Error bars indicate standard errors of the mean.
calcium or the duration of isometric contraction had any influence on the force-velocity properties of the preparation. A set of contractions in free calcium was preceded and followed by sets of control contractions in the full-activation, buffered calcium solution. The force-velocity curves obtained (Fig. 5A) were not significantly different from the controls (all $P$ values for fitted parameters $> 0.05$). In a different set of fibers, contractions with isotonic steps imposed at 15 s were preceded and followed by a set of control contractions with steps imposed at 3–5 s. The force-velocity curves obtained later in the contraction showed depressed velocities for intermediate loads, such that the maximum power values were slightly but significantly depressed ($P < 0.02$). Although the difference is not great, the observation does emphasize the importance of performing tests as early as possible in the contraction.

**Long vs. short sarcomere length.** All of the experiments described above were done at a starting sarcomere length of 2.6 μm, determined by laser diffraction. Sarcomere length generally decreased to ~2.1–2.2 μm as force developed. Thus, the force-velocity data were usually measured at a sarcomere length of 2.0–2.2 μm. The laser patterns indicated that if the fibers were activated from an initial sarcomere length of 2.0–2.2 μm, the sarcomeres shortened below 1.9 μm. Force-velocity relations measured from these short lengths were compared with those

![Figure 5](https://via.placeholder.com/150)

**Figure 5.** Force-velocity relations for fibers studied with 2.5 mM free calcium ion vs. buffered calcium (A) and with isotonic steps applied at 15 vs. 3–5 s (B).
obtained from contractions starting at 2.6 μm in the same fibers. The experiment was done in two groups, one in June 1983 and the other in May 1984 (Fig. 6). There was no difference in the force-velocity curves obtained at different initial lengths in either group of fibers (P values for comparing parameters of the fitted curves within each group were all > 0.2). This suggests that there is very little internal load impeding the initial shortening of these skinned fibers over the range of lengths studied. There was, however, a significant and substantial difference between the maximum velocity and maximum power (both P values < 0.001) in the control curves for the two sets of fibers. This difference emphasizes the importance of comparing data taken from fibers that are closely matched.

Observations of striation pattern. The laser diffraction pattern was observed during many contractions. The first-order pattern always became broader and less distinct as force developed and the sarcomeres shortened. When the sarcomeres shortened below 1.9 μm, either because contractions were started from a short sarcomere length or because of the large shortening step applied at the end of each period of isotonic shortening, the first-order pattern disappeared entirely. The pattern instantly returned in a very distinct form when the fibers

![Figure 6. Force-velocity relations obtained with 2.6 and 2.0–2.2 μm initial sarcomere length. As with the preceding experiments, the experimental contractions, starting at 2.0–2.2 μm, were both preceded and followed by control contractions starting at 2.6 μm sarcomere spacing. The data in A are for eight fibers studied in June 1983. In B, the data are for seven fibers studied in May 1984.](image-url)
were restretched during relaxation. In some preliminary experiments, the fibers were not stretched and released between each contraction. The sarcomere patterns obtained from these fibers tended to become progressively less distinct with each successive contraction. The pattern did not lose much of its sharp image when the fibers were stretched and released several times between contractions, and so this method of restoring the uniformity of sarcomere spacing (Gordon et al., 1966a) was used routinely in all the experiments described here.

**Lack of resistance to stretch.** The large shortening step that was applied after isotonic shortening usually caused the sarcomere spacing to decrease to \(<1.9 \mu m\). This was observed even in fibers that began contracting at 2.6 \(\mu m\). As shown in Fig. 1A, force rose very little in response to restretching the relaxing fiber. Thus, it is unlikely that there were any lasting bridges between the filaments. The observations that there was little resistance to stretch and that maximum velocity did not decrease as developed force declined argue strongly against the type of internal load that Thames et al. (1974) have described in their preparation.

**Long vs. short segments.** The observation that the sarcomeres shortened during isometric force development suggests that there were compliant elements in series with the contractile elements. This compliance might have arisen from damage at the ends, where the fibers were gripped, or from less complete activation at the ends, because of slower diffusion around the clips. In either case, this compliant tissue might have continued to shorten after the initial series elastic recoil, during isotonic contraction. Mechanisms that might account for this continued shortening include damped recoil of the damaged tissue or a higher relative force in stretched or less well-activated sarcomeres. Thus, the method of attachment of the fibers to the apparatus might have introduced unknown quantities of extra shortening into the system. If this extra shortening were significant, it would be expected that its effects would be greater in short fibers than in long fibers, since the amount of abnormal tissue at the sites of attachment would be proportionately larger in short fibers. To evaluate the influence of the abnormal tissue, the force-velocity curves obtained from long and short fiber segments were compared. The eight fibers used to obtain the partial activation data in Fig. 2 were conveniently divided into two groups of four fibers each, whose average length differed by a factor of 2.4. In Fig. 7A, full activation is compared with partial activation in the four long segments. Fig. 7B shows the results obtained from the short fibers. The parameters of the fitted curves for full and partial activation did not differ significantly from each other at either segment length (\(P\) values for comparing fitted parameters of fully activated contractions with partially activated contractions > 0.1).

The second comparison (Fig. 7C) was made in a separate group of nine fibers studied at full activation only. In this experiment, fibers were cut into long (>3.0 mm) and short (<1.25 mm) segments. Each short segment was matched by a long segment of the same fiber. As shown, the force-velocity curves are not greatly different, although the difference between the maximum power values is significant (\(P < 0.02\)). The substantial differences between the maximum velocities and maximum powers of the curves fitted to the data in Fig. 7C and
Figure 7. Force-velocity relations in long and short fiber segments. The eight segments used to obtain the data for partial activation were evenly divided into four segments longer than 2.7 mm (mean, 3.1; range, 2.7–3.5) (A) and four segments shorter than 1.6 mm (mean, 1.3; range, 1.0–1.6) (B). The effects of partial activation on these two groups are shown separately in A and B. (C) Comparison of force-velocity relations obtained at full activation in a separate group of long and short segments. Nine fibers were cut into long and short segments so that each long segment is matched by a short segment from the same fiber.
the corresponding values for the curves in Fig. 7, A and B, again illustrate the importance of matching the fibers as closely as possible.

**Effect of low ATP concentrations.** An ATP regenerating system, such as creatine phosphate and creatine kinase, was not used in these experiments because the inclusion of these constituents in the large volumes of solution required by the solution-changers would have greatly increased the cost of the study. Instead, experiments were done at various ATP concentrations to determine whether substrate limitation might have affected the results. When ATP was reduced to 60% of the standard concentration, there was a small (18%) but significant ($P < 0.01$) decrease in maximum power ($P \cdot V_{\text{max}}$ in Fig. 8A). When ATP was 20% of the standard values (Fig. 8B), maximum power declined by 58% ($P < 0.001$). The differences in maximum velocities and relative isometric forces at both low ATP concentrations were not significantly different from controls. The maximum power might be expected to be the most sensitive index of substrate limitation, since the maximum energy liberation occurs near the intermediate loads, where maximum power is generated (Hill, 1964).

![Figure 8](image_url)

**Figure 8.** Effect of lowering ATP concentration to 3 mM in 10 fiber segments (A) and to 1 mM in 7 segments (B). The data in the two graphs were obtained from separate batches of frogs, which may account for the differences in the control curves.
The observation that maximum power decreased only slightly when ATP was decreased to 60% of the standard value suggests that there is very little, if any, substrate limitation at the standard concentration. Furthermore, substrate limitation is much less likely during partial activation, when total power is reduced. This consideration suggests that our main conclusion about the effects of partial activation on the relative force-velocity relation was not influenced by the rate of supply of ATP to the contractile elements.

Comparison of fitted curves. The maximum velocity obtained in the lowest ATP solution (Fig. 8B) was substantially greater than that for the control curve, even though the velocities at intermediate loads lay well below the control values. This increased maximum velocity in the fitted curve was due to a few high-

![Figure 9](image_url)

Figure 9. Omission of low force points. Two fits to the data for fully activated fibers in Fig. 2 are shown. The solid lines represent a fit to all the points. The dashed lines show a fit to the points for relative forces >0.2.

velocity points at very low loads. These high-velocity points also increased the standard error at the extrapolated maximum velocity, so that it was not significantly different from the control value ($P > 0.05$). Because the fitted curves are nonlinear, it is possible for a few aberrant points at low loads to distort the estimates of maximum velocity, despite the absence of any substantial difference in the curves over the rest of their range. This suggests that maximum velocity as determined here may not be as reliable as some other parameter. To further assess the influence of low-force data on the fitted parameters, the data from the fully activated fibers in Fig. 2 were refitted, excluding points with relative loads of <0.2, and the results were compared with the fits to the data for all loads. As shown in Fig. 9, the maximum velocities for the two fitted curves differ by more
than a factor of 2 ($P < 0.001$), whereas the relative isometric force and maximum power differ by 10 and 4%, respectively, and neither difference is significant (both $P$ values $> 0.4$).

One of the major reasons for the greater sensitivity of maximum velocity to small differences in the data is that it is determined by extrapolation of the fitted curves to an axis that it approaches nearly tangentially. A similar criticism can be made of the relative isometric force, determined by extrapolation of the curves to zero velocity, and especially of the values $a$ and $b$ in the Hill (1938) equation, which are asymptotes determined by a larger extrapolation. For comparing force-velocity data, it is much more reliable to use a parameter that is determined by interpolation rather than by extrapolation. Maximum power is such an interpolated parameter. The standard errors of maximum power were a much smaller fraction of the fitted values than the standard errors of either the maximum velocity or the isometric force. For the 31 force-velocity curves shown here, the standard errors of maximum power averaged 0.055 of the mean value, whereas the standard errors of maximum velocity and relative isometric force averaged 0.110 and 0.218, respectively, or two and four times greater.

Fiber Storage. Some of the experiments required the use of multiple segments of the same fiber. In all experiments, it was more convenient to finish the dissections early in the day. Frequently, fibers were stored for up to 8 h in relaxing solution at $0^\circ$C. To determine whether the storage had any influence on fiber performance, six segments were stored overnight and tested the next day. Each of the segments was matched by another segment of the same fiber tested on the day of dissection. The force-velocity curves obtained from the two groups of fibers are shown in Fig. 10. There is little difference between the two curves, although the maximum velocity is just significantly higher ($P < 0.02$) in the fibers stored overnight. This observation suggests that storage had no adverse effect on fiber performance.

The method of chemically skinning the fibers required that they be immersed in relaxing solution for 30 min while being treated with detergent. An additional period of immersion was required during mounting in the apparatus, so the skinned fibers could not be tested until after they had been immersed in a relaxing solution for nearly 1 h. Thus, the influence of the initial 1 h of storage on fiber performance could not be assessed.

Attempts to Repeat Earlier Experiments

The experiments reported above differed in several ways from earlier experiments used to assess the effects of activating calcium levels (Podolsky and Teichholz, 1970; Julian, 1971; Thames et al., 1974; Julian and Moss, 1981). The magnesium ion concentration was higher and probably more physiological (Endo, 1975; Dawson et al., 1978). Propionate was used instead of chloride as the major anion, and the ionic strength was higher. In addition, a single-step protocol was used instead of the triple-step technique developed by Julian. Attempts were therefore made to determine the effects of these factors on the main conclusion about the relationship between calcium and shortening velocity.

Chloride vs. propionate. Our early experiments were done in KCl rather...
than K-propionate solutions. Soon after the change to propionate, the decline in isometric force with each succeeding contraction was greatly diminished. From that point on, nearly every contraction yielded usable data. It is possible that this marked improvement was due to seasonal variation in the quality of frog muscle fibers, our increased experience, or the substitution of K-propionate for KCl. Thus, no firm conclusions about chloride can be made. We did not repeat the experiment in chloride because Podolsky and Teichholz's (1970) experiments had been done in chloride and their conclusions were similar to ours. Further-

more, the difference between their results and those of Julian (1971) could not be attributed to the use of chloride, since Julian used chloride also.

Effects of magnesium ion. In all of the experiments described above, the magnesium was 1 mM in excess of the ATP concentration, so that the free magnesium ion concentration was \( \sim 1.4 \) mM. The effects of a much lower magnesium ion concentration were assessed in solution containing 1 mM MgCl\(_2\) and 5 mM ATP. In these solutions, the K-propionate concentration was lowered to 140 mM to compensate for the increased ionic strength of the free ATP. The protocol for these experiments was the same as that for the experiments in Fig.

**Figure 10.** Force-velocity relations from fiber segments stored overnight compared with similar data from other segments of the same fibers studied on the day of dissection.
2. Several partially activated contractions of each fiber were both preceded and followed by fully activated contractions. The criterion for including a partially activated contraction in the analysis was that its isometric force was <50% of the average isotonic force of the "pre" and "post" fully activated contractions. For all fibers, the average force in the partially activated contractions was 43% of that in the control contractions. As shown in Fig. 11A, partial activation did not decrease the shortening velocity. The ATP and magnesium concentrations and the ionic strength were identical to those of the Podolsky and Teichholz (1970) study. The ATP and magnesium ion concentrations were also similar to those of Julian's (1971) experiments. The major differences between his conditions and those of the present experiments were his use of a lower ionic strength and a triple-step protocol.

**Ionic strength.** In some preliminary experiments, we attempted to work with solutions containing 120 mM KCl instead of 140 or 151 mM K-propionate.
Fibers exposed to these solutions frequently broke during the first contraction and never survived more than a few contractions. The fibers survived much longer when the KCl concentration was raised to 151 mM, so as to bring the ionic strength to the values used by Podolsky and Teichholz (1970).

**Triple-step protocol.** To minimize the number of contractions in his original experiments, Julian (1971) used steps to three progressively lower isotonic loads during each contraction. Shortening at the lowest loads was preceded, therefore, by two periods of prior shortening. To assess the effect of the earlier shortening on the velocity at the lower loads, we modified our apparatus to produce the three steps, according to the protocol illustrated in Fig. 12. The last two steps were made 60 and 110 ms after the first step. Force and velocity were measured over a 15-ms period beginning 20 ms after the onset of each of the three steps. As with the single-step experiments, partially activated contractions were both preceded and followed by fully activated control contractions. In addition, the same fibers that were used for the single-step experiments shown in Fig. 11A were used for the triple-step experiments in Fig. 11B. Thus, the results from the

**FIGURE 12.** Triple-step protocol used for low magnesium experiments. (Left) Records made on a slow time base to show the whole contraction. The activation sequence was identical to that used for high magnesium ion experiments (Fig. 2). (Right) Records made on a fast time base to show that the isotonic shortening was divided into three periods of shortening at progressively lower loads, followed by a large step to make the muscle go slack and define zero force. Segment length, 1.8 mm. Record 33; experiment 24-vii-84.

two graphs can be compared to assess the effects of the triple step at each level of activation. As shown, the velocities at the lower loads are very much reduced in the triple-step protocol. As expected, the greatest difference was seen at the lowest loads, which, in the triple-step protocol, had been preceded by two periods of prior shortening. The velocities measured at intermediate loads, after only one period of prior shortening, were not very much reduced. Thus, the maximum power values are not significantly lower ($P > 0.5$ for full activation and $P > 0.1$ for partial activation). No significant differences were found between full and partial activation, when the force-velocity curves were determined using the triple-step procedure. It appears, therefore, that the use of triple steps in each contraction was not, by itself, responsible for Julian's (1971) observation that maximum velocity is dependent on activation.

**DISCUSSION**

The results show that the maximum shortening velocity of skinned muscle fibers is not influenced by the ambient calcium ion concentration, at least when the
level of calcium ion is sufficient to produce ~40% of maximum force. This observation is consistent with the theory that calcium acts only to facilitate the attachment of cross-bridges to the thin filaments, and specifically that it does not influence the contractile performance of the cross-bridges once they have attached. The results also support the theory that the cross-bridges function as independent units. The possibility that calcium has two or more competing effects on the contractile function of the cross-bridges cannot be excluded, but such an exact cancellation seems unlikely.

The Huxley (1957) model of muscle contraction attributes both force generation and shortening to the activity of independent, thick-filament cross-bridges. According to the model, the isometric force developed at any level of activation is proportional to the number of attached cross-bridges. The maximum shortening velocity of the unloaded muscle, in contrast, is determined by the intrinsic kinetic properties of the cross-bridges, and is independent of the number of cross-bridges. This was supported by the observation that isometric force is proportional to filament overlap, while maximum velocity is independent of that parameter (Gordon et al., 1966b). The discovery that contraction is activated by the binding of calcium to thin-filament regulatory proteins led to the hypothesis that calcium acts as a simple switch, releasing the inhibition of actin binding sites so that myosin cross-bridges can attach. Calcium was not expected to alter the kinetics of the actin-myosin interaction once binding had occurred. Thus, according to this hypothesis, the shortening velocity of the unloaded fiber should be independent of activation, just as it was independent of filament overlap in the experiment of Gordon et al. (1966b).

**Comparison of Force-Velocity Parameters**

According to the cross-bridge theory, the force of contraction is determined by the number of attached bridges and by the average force per bridge. At higher velocities, both the number of attached bridges and the force per bridge decrease. Recent experiments have shown that at maximum velocity, the number of attached bridges is reduced to ~30% of that in the isometric state (Ford et al., 1985). The average force per bridge is zero. The bridges that are attached and generating force in the shortening direction are exactly balanced by bridges that have pulled through their range of useful work, but remain attached and resist further shortening. The maximum velocity is therefore determined by the ability of the cross-bridges to pull through their useful range and detach in the absence of an external load. While this parameter has been considered to be the most sensitive indicator of changes in the kinetics of attached cross-bridges, it is also very sensitive to small errors in measurements. Because the force-velocity curves are steepest in the region of zero load, small errors in the estimation of load, or small internal loads, can have a large effect on the estimation of maximum velocity. For this reason, we prefer to use maximum power to compare force-velocity curves under different conditions. As described, the error of the maximum power estimates was less than half that of the maximum velocity estimates.

Another advantage of using maximum power to assess changes in cross-bridge kinetics is that it reflects changes in the entire cross-bridge cycle. For example,
if some intervention increased the time required for the detached bridges to recover and reattach, the number of attached cross-bridges at all velocities would be diminished. This would reduce maximum power but would not influence maximum velocity, since the latter parameter is not influenced by the number of attached cross-bridges.

**Relationship to Earlier Experiments**

The results and conclusion of the present experiments are identical to those of Podolsky and Teichholz (1970) but different from those of Julian (1971), although both were obtained at a much lower magnesium ion concentration. We have repeated the experiment using conditions of ATP, magnesium, and ionic strength identical to those of Podolsky and Teichholz (Fig. 11A) and again we found that the relative force-velocity curves are identical at full and partial activation. The differences between ATP and magnesium ion concentration in the early experiments were very slight; Julian used 4 mM ATP, Podolsky and Teichholz used 5 mM ATP, and both groups used 1 mM total magnesium. Thus, it seems highly unlikely that the differences in the results from the two laboratories are due to any difference in ATP or magnesium concentrations.

In several respects, Julian's methods appear to be superior to those of Podolsky and Teichholz. In particular, the chemically skinned fibers are much less likely to be damaged in preparation than mechanically skinned fibers. Additionally, the use of a servomotor to control length and force greatly facilitates quick changes in load and produces quieter traces. Because of the technical superiority of these methods, we adopted them in our experiments. We can thus conclude that the differences between the results of the earlier experiments were not due to these differences in techniques.

**Fiber damage.** The major difference between the conditions used by Julian (1971) and those of Podolsky and Teichholz (1970) is that Julian used a somewhat lower ionic strength. He used 120 KCl, whereas Podolsky and Teichholz used 140 mM. Although this difference seems small, the lower ionic strength is associated with substantially higher force generation (Thames et al., 1974; Julian and Moss, 1981). In our hands, the fibers were pulled apart after a few contractions in the lower ionic strength solution. It is possible that with more experience we could have made the fibers last longer, but Julian and Moss' (1981) observation that the fibers undergo substantial structural damage when fully activated under these conditions persuaded us to avoid the lower ionic strength solutions.

If Julian's lower velocities at partial activation were due to fiber damage, it might be difficult to reproduce the damage exactly. Julian's data were obtained from just two contractions in each of seven fibers. One contraction was at full activation and the other was at partial activation. The order in which the two contractions were generated was not described, but if the fully activated contractions were done first in each case, it is possible that there was systematic damage to the fibers before the effects of partial activation were studied. The present technique of both preceding and following experimental contractions with contractions obtained under control conditions and then comparing the "pre" and "post" control contractions should have both revealed the effect of any damage
caused by the experimental contractions and minimized these effects on the observed results. On the basis of these control observations, we feel safe in concluding that our interpretations are not influenced by any progressive damage.

**Excess series compliance.** In order to reduce fiber damage in the present experiments, the contraction duration was minimized by briefly exposing the fibers to a free calcium solution. In separate control experiments, exposure to free calcium and short contraction times were shown to have no effect on maximum velocity. The one place where significant fiber damage could not be avoided was at the points of attachment of the fiber to the apparatus. If this local damage resulted in significant confounding effects, these effects should have been most pronounced in short fibers, in which the damaged regions would have constituted a greater proportion of the total fiber length. When fibers with lengths differing by an average of 240% were examined, however, the congruency of the relative force-velocity curves at full and partial activation was maintained.

Damage to the fibers at the points of attachment may have introduced a substantial compliance in series with the sarcomeres. It was observed that sarcomere length at peak isometric tension was considerably smaller than that at rest. Podolsky and Teichholz (1970) stimulated their fibers to contract from resting sarcomere lengths of 2.0–2.2 μm. In the present experiments, fiber activation from those lengths generally resulted in sarcomere lengths below 1.9 μm at the outset of isotonic shortening. If the sarcomere length–velocity relationship demonstrated in intact fibers is applicable to skinned preparations, these fibers would have been working over a range where the maximum shortening velocity declines precipitously with decreases in sarcomere length (Gordon et al., 1966b). Julian (1971) used a resting sarcomere length of 2.6 μm, which is identical to that used here. In the present experiments, resting sarcomere lengths of 2.6 and 2.0–2.2 μm were examined in the same fibers. No differences were found between the relative force-velocity curves obtained. Thus, it is unlikely that the difference in resting sarcomere length accounts for the contradictory findings of the earlier experiments.

**Other mechanisms.** In an earlier review (Podolin and Ford, 1983), it was suggested that the difference between Julian’s (1971) findings and those of Podolsky and Teichholz (1970) might have been due to Julian’s use of the triple-step protocol. Prior shortening at the higher loads might have reduced the velocity in the second and third steps. It was further suggested that this apparent slowing might have been due to fiber inactivation caused by the prior shortening. If the fiber was partially inactivated by shortening, then in subsequent steps the value of \( P_0 \), the isometric force, would be inappropriately high, and the relative force (\( P/P_0 \)) would be correspondingly reduced. It was suggested that this effect would be most prominent at low loads, in partial activation, pulling that portion of the curves downward. In the present experiments, lower velocities were observed at low loads when triple steps were used. This effect was similar in fully and partially activated fibers, however, and so cannot account for Julian’s finding.

It was also suggested (Podolin and Ford, 1983) that the difference might have
arisen from some activation-dependent biochemical effect, such as phosphorylation. A biochemical change that depended upon activation might occur more completely in fully activated fibers. Such a mechanism would confound the interpretation of the results, since it would result from a secondary effect of the activating calcium. The short contractions used in the present experiments should have minimized the possibility of such a change. Nevertheless, the maximum velocity of fully activated fibers was not altered when the duration of contraction, before the isotonic step, was three to five times longer (Fig. 5B).

Although our control experiments have eliminated a number of possible explanations of the differences in the earlier experiments, we are unable to account for those differences because we were unable to reproduce Julian's (1971) results. We did not, however, imitate his experimental conditions exactly because these conditions are associated with fiber damage. Since the earlier experiments using skinned fibers were performed at unphysiologically low magnesium ion concentrations, perhaps the questions raised by these experiments need not be pursued so vigorously. The results obtained in the present experiments are very similar to those in intact muscle fibers, where various levels of activation were achieved by measuring force-velocity properties at different times during the rise of tetanic force (Cecchi et al., 1978). This favorable comparison supports the main conclusion that under physiological conditions calcium serves only as a switch to turn on the contractile process.

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