INTRINSIC SHORTENING SPEED OF TEMPERATURE-JUMP-ACTIVATED INTACT MUSCLE FIBERS

Effects of Varying Osmotic Pressure with Sucrose and KCl

JAGDISH GULATI AND ARAVIND BABU
Departments of Medicine, Physiology and Biophysics, and Cardiovascular Research Center, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT Effects of intracellular ionic strength on the isotonic contraction properties of both intact fibers and skinned fibers give insights into the cross-bridge mechanism, but presently there is fundamental disagreement in the results on the two fiber preparations. This paper, which studies the effects on contraction of varying the osmotic pressure of the bathing medium with impermeant and permeant solutes, explains the above controversy and establishes the physiological significance of the previous results on skinned fibers. Fast-twitch fibers, isolated singly from tibialis and semitendinosus muscles of frogs, were activated by a temperature-jump technique in hyperosmotic solutions with either 100 or 150 mM sucrose (impermeant), or 50 or 75 mM KCl (permeant). Intracellular ionic strength was expected to rise in these solutions from the standard value of ~190 to 265 mM. Cell volume and the speed of unloaded shortening both decreased with sucrose and were constant with KCl. On the other hand, isometric force decreased equally with equiosmolar addition of either solute; this is additional evidence that contractile force decreases with ionic strength and is independent of fiber volume. Therefore, for the main cross-bridges, force per bridge is constant with changes in the lateral separation between the myofilaments. The next finding, that at a fixed cell volume the contraction speed is constant with KCl, provides clear evidence in intact fibers that the intrinsic speed of shortening is insensitive to increased ionic strength. The data with KCl are in agreement with the results on skinned fibers. The results suggest that in the cross-bridge kinetics in vivo the rate-limiting step is different for force than that for shortening. On the other hand, the decrease in speed with sucrose is associated with the shrinkage in cell volume, and is explained by the possibility of an increased internal load. A major fraction of the internal load may arise from unusual interactions between the sliding filaments; these interactions are enhanced in the fibers compressed with sucrose, but this does not affect the intrinsic kinetics of the main cross-bridges.

INTRODUCTION

The force of muscle contraction is produced by myosin cross-bridges during their attachment to actin filaments (Huxley, 1969). Contraction transients, steady state force-velocity relations, and the speed of shortening at zero load give insights into the cross-bridge mechanisms and the kinetics of the attachment and detachment processes in force development (Huxley, 1974; Podolsky et al., 1974; Eisenberg et al., 1980). Additional insights into these mechanisms are gained by studying factors that influence the contraction properties. The ionic strength of the intracellular milieu appears to have a direct effect on force and is, therefore, a useful agent for studying the contraction mechanisms in both intact and skinned fibers. Results on skinned fibers from frogs have shown that increasing the ionic strength affects the properties of the transients but does not affect the steady state force-velocity relations (Gulati and Podolsky, 1978). These findings suggested that changes in ionic strength alter the rate constants in the cross-bridge cycle but do not affect the rate-limiting step for the steady speed of shortening.

Data on intact muscle (Howarth, 1958) have suggested that the force-velocity relation and the speed of unloaded shortening are affected by ionic strength, which conflict with the findings on skinned fibers (Edman and Hwang, 1977; Gulati and Podolsky, 1978). The issue should be resolved because of its implications for the cross-bridge mechanisms and also because it questions the physiological significance of work on the contraction of skinned fibers. The experiments on intact cells had employed hypertonic solutions (by the addition of impermeant solutes to the bath), which increased the intracellular ionic strength by decreasing the cell volume. These changes in volume are

associated with compression of the filament lattice (Gulati and Babu, 1982 *b*). On the other hand, in the case of skinned fibers, the desired changes in ionic strength were made directly in the bathing medium. However, the volume and filament lattice of skinned fibers are expanded over those in intact fibers because parameters in the Donnan and osmotic equilibria become altered with the removal of the semipermeable cell membrane, and also because there are differences in the local environment in the two preparations (Matsubara and Elliott, 1972; Rome, 1972). Therefore, the possibility was raised that the previous findings due to ionic strength were complicated by the accompanying changes, particularly those in the lattice. The present study was undertaken to examine this possibility for resolving the above conflicts.

A straightforward way to raise the intracellular ionic strength in intact frog muscles is by a simple addition of KCl (permeant solute) to the bathing medium. In this case the cell volume is held constant. The change in ionic strength is induced at constant volume because Na^+ in the medium is effectively impermeant and K^+ and Cl^- ions distribute across the cell membrane approximately according to their Donnan equilibrium values (Boyle and Conway, 1941; Adrian, 1960; Palmer and Gulati, 1976). This approach had not been well exploited for the contraction studies because the cells become depolarized in high KCl and are, therefore, electrically unexcitable. However, isolated single fibers can be reproducibly contracted in this solution with an instantaneous temperature-jump technique (Gulati and Babu, 1982 *b*), which is believed to act by causing a net release of calcium ions from the sarcoplasmic reticulum at 0°C and reabsorption of the released Ca^{2+} at 25°C (Sakai, 1965). Use of the temperature-jump technique is made here to evaluate the influences of varying the osmotic pressure of the bathing medium, by the addition of sucrose and KCl, on the speed of shortening.

We find that the intrinsic shortening speed is constant in KCl solutions, which is the first evidence on intact fibers that the speed is independent of ionic strength. In explaining the effect in sucrose hypertonicity, where the speed of shortening was decreased, major considerations are given to the possible roles of above two parameters on the cross-bridge dynamics. One is the structural factor of the filament lattice and the second is the influence of intracellular solutes, since both are likely to be simultaneously modified due to decrease in cell volume in the sucrose solution. Preliminary reports of this work are given elsewhere (Gulati and Babu, 1982 *a*; Gulati and Babu, 1983).

GLOSSARY

F_R internal load

L_0 resting fiber length (mm) between tendons, corresponding to a sarcomere length of 2.2 to 2.3 μm

ΔL magnitude of the shortening step during a slack test (expressed as percent L_0)

ΔL_0 intercept of ΔL vs. Δt plot, on the abscissa. Its value in the present study includes contributions from the compliance of cross-bridges and that of tendons at the ends of the fiber

P_1 maximal isometric force per unit area (kN/m^2), with temperature jump in 1.0T solution. The cross-sectional area was calculated from fiber width in 1.0T solution. The cylindrical approximation was used for fiber cross-section

$P_1(T)$ maximal force per unit area (kN/m^2) in hyperosmotic solution of osmolarity T. The cross-sectional area estimated in 1.0T solution was used for this calculation

t_0 time zero at which the slack release is initiated

Δt slack time (from t_0 to the time at which the perid of slack ends)

t_r time for force recovery measured from Δt to time at which instantaneous force becomes 15% of $P_1(T)$

T relative osmolarity or the relative osmotic pressure. We take 1.0T as equivalent to 245 mosM, which is the estimated osmolarity of the standard bathing solution

$V_0(1)$ intrinsic speed of shortening at zero load as determined by the slack test in 1.0T solution

$V_0(T)$ the slack test speed in hyperosmotic solution of relative osmolarity T

V' $V_0(T)/V_0(1)$

$D(T)$ width of the fiber bathed in a solution of given osmolarity. The width is taken as $D(1)$ in the control solution.

METHODS

Single-Fiber Preparation

27-gauge hypodermic needles and a bright-field stereomicroscope (Wild M3; Wild Heerbrugg Instruments, Inc., Farmingdale, NY) were used to isolate fast-twitch fibers from the anterior branch of the tibialis muscle and the dorsal head of the semitendinosus muscle in *Rana temporaria*. The frogs were stored at 4°–7°C. During the isolation procedure the muscle was kept in a cold (4°C) dissection solution (see Solutions section). The isolated fiber, tied to hooks, in the dissection chamber was stimulated electrically with point electrodes ~1 cm apart to test for a vigorous twitch. The preparation was usually stored in this chamber 30 min to 2 h and checked again for the twitch response. It was next retied at both ends, one end attached to a force transducer and the other to the arm of a motor (used for applying length steps during the slack tests, see below). These new ties were made with 8-0 monofilament surgical suture (Ethicon Inc., Somerville, NJ), and were kept close to the tapered ends of the fiber (within ~100 to 300 μm). The loose ends of the tendons were trimmed. At this point, the dissection chamber was removed and the fiber was transferred to the experimental chamber containing a solution with a relative osmolarity (T) of 1.0 (solution B, Solutions section; Table I). A glass plate (1–2 cm long and 0.3–0.5 cm wide) was firmly suspended ~1 mm below the fiber and carried with the fiber during each transfer between chambers. During the transfer the plate retained a small layer of solution surrounding the fiber, which was essential for the survival of the fiber.

Fiber length was adjusted so that sarcomere length in the central region was 2.2–2.3 μm (rest length), as measured using laser diffraction (He-Ne laser model 133, Spectra Physics, Inc., Mountain View, CA). After adjusting the fiber length, the sarcomere lengths were measured near the two ends of the fiber. The sarcomere lengths near the ends, close to the tendons, were usually within 0.1 μm of the measurement at the central region. The diameter was measured at five equidistant points along the length of the fiber in each solution. The diameter measurements were made with a stereomicroscope (with a magnification of 80) for an immediate estimate and permanent records were obtained on photographs with a 35-mm camera attached to the microscope (Kodak film, TriX; Eastman Kodak Co., Rochester, NY). Fiber length was measured to the nearest 0.01 mm between fiber insertions tapered into the tendons, at a magnification of 16.

Solutions

Two typical solutions were used in these experiments. Solution A, used mainly during dissection of the muscle and isolation of single fibers, was of the following composition: NaCl, 115 mM; KCl, 2.5 mM; $CaCl_2$, 0.7 mM; $MgCl_2$, 0.12 mM; dextrose, 5 mM; NaH_2PO_4, 2 mM; and Na_2HPO_4, 1.2 mM. For the temperature-jump experiments, once the fiber was transferred to the experimental chamber, solution B was used: NaCl, 100 mM; KCl, 2.5 mM; imidazole, 10 mM; $CaCl_2$, 10 mM; and caffeine, 1–3 mM. Solution B has a calculated osmolarity (Dick, 1959) of ~245 mosM and is referred to as 1.0T solution. To increase the osmolarity to 1.4T, 100 mM sucrose, 50 mM NaCl, or 50 mM KCl was added; for a 1.6T solution, the addition was made of 150 mM sucrose or 75 mM KCl. The pH of each solution was adjusted to 7.00 ± 0.01 at room temperature just before the experiment. All chemicals were of the best grades available from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific Co., Allied Corp. (Pittsburg, PA).

The calculated and measured osmotic pressures of the various solutions are given in Table I. The measurements of osmotic pressures were made by the method of freezing point depression (Freezing Point Depression Osmometer, Fiske Associates, Burlington, MA). The calculated and measured values in Table I are in good agreement, as expected, since the osmotic coefficients of the various solutes in the present solutions are fairly close to unity, and behavior of the solutions, therefore, remains close to the ideal (Weast, 1972).

Experimental Setup

Four chambers (5.0 cm long, 1.6 cm wide, and 1.5 cm deep) were machined in a 1.5-cm-thick plate with a total area of 12 by 10 cm. The plate was cut in half and rejoined by gluing thermally insulating material (2-mm thick) between the two parts. The plate was placed on four thermoelectric modules (Cambion model 801-3959-01; Cambridge Thermionic Corp., Cambridge, MA) in two pairs; each pair of modules was separately controlled (Cambion bipolar controller model 809-3040-01) so that the temperature in the front and back portions of the plate (each with two chambers) could be individually adjusted to within ± 0.1°C. The temperature was usually 25°C in solutions in the front chambers and 0°C in the two back chambers. One of the front chambers contained a pair of platinum electrodes (each 2 mm wide and 2 cm long) for electrical stimulation.

The force transducer was a semiconductor strain gauge (AE801, Aksjeselskapet Mikro-Elektronikk, Horten, Norway) with truncated beam and a stainless steel attachment made of flattened hypodermic tubing (outer diameter, 0.04 cm; Small Parts Inc., Miami, FL) bent in the shape of an L. The entire length of the L was limited to 2 to 3 mm to minimize the mass on the transducer. The short end of the L was notched for fiber attachment. The transducer, including the attachment but excluding the notch, was completely coated with two to three thin layers of silicon rubber (732-Cl 11, Dow Corning, Midland, MI), applied successively. This coating allowed the device to be submerged in solutions without causing contamination and being electrically shorted. Because breakage of this device is quite common, 11 devices in total were employed for the present study. The natural frequency of the loaded transducer was generally ~1 to 2 kHz and this was adequate for the slack-test experiments in the present study; in some transducers, a value of 5 to 7 kHz was obtained.

TABLE I
OSMOTIC PRESSURES OF DIFFERENT SOLUTIONS

Solution	Measured osmolality	Calculated osmolarity
	mosmol/kg H_2O	*mosM*
Ringer's (solution A)	230	249
1.0T (solution B)	240	245
1.0T + sucrose		
100 mM (1.4T)	351	345
150 mM (1.6T)	401	395
1.0T + KCl		
50 mM (1.4T)	350	345
75 mM (1.6T)	406	395

Experimental Protocol

Temperature-Jump Activation. Isolated frog fibers bathing in a medium containing moderate amounts of caffeine (1–3 mM) are known to be relaxed at 25°C and become fully contracted at 0°C (Gulati and Babu, 1982 *b*), as is the case for whole sartorious muscle (Sakai, 1965). Typical records of force response in the various solutions are shown in Fig. 1. In each case, a fiber was initially equilibrated for 30–60 min in the solution containing caffeine at 25°C (1.0T in Fig. 1 *A*). Next, the chamber was lowered (arrow *a* in Fig. 1 *A*) and moved forward. The fiber was then plunged in the solution in a back chamber at 0°C (arrow *b* in Fig. 1 *A*). The time required for transfer between chambers was 1 to 2 s. The fiber became activated at 0°C the force reaching a plateau. When the fiber was removed from solution at 0°C (arrow *c*) and returned to solution at 25°C (arrow *d*), it immediately relaxed and the force returned to zero. (Note that it was essential that the fiber be surrounded by a small amount of solution during the transfer between chambers for repeated contractions. As pointed out above, this was accomplished in the present setup by suspending a glass plate just below the fiber.) The rest period between activations was at least 12 min (usually 15–30 min). The minimum concentration of caffeine needed to give maximal activation (force) was used. This concentration varied from fiber to fiber, and in many fibers the caffeine concentration needed for maximal force in hyperosmotic solutions was different from that in the solution of standard osmolarity (usually 2 mM caffeine in 1.0T and 1 mM in hyperosmotic solution). The minimum concentration of caffeine needed for maximal contraction in each solution was determined at the outset in each experiment.

In Fig. 1, *B* and *C*, the force response to the applied jump in temperature in solution of 1.0T is compared with the responses in solutions of higher osmotic pressures used in this study. The general pattern of the force response is the same in each case except that the maximal contractile force is lower in hyperosmotic solutions (Gulati and Babu, 1982 *b*). Note that when the fiber was transferred from 1.0T to a hyperosmotic solution, there was a 30-min preequilibration at 25°C, and during this period resting tension of the fiber was also monitored continuously on a chart recorder (not shown).

KCl. Additional precautions were necessary when the fiber was transferred from 1.0T solution to the solutions high in KCl. The fiber was damaged if exposed directly to high KCl in a solution with caffeine. Even the residual caffeine in the fiber (from the preceding incubation in 1.0T) had to be washed out with a 10- to 15-min presoak in the caffeine-free Ringer's solution (solution A) before it was placed in a caffeine-free KCl solution at 0°C. There was an immediate contraction on exposure to 50 mM KCl, presumably due to depolarization (Hodgkin and Horowicz, 1960). This contraction lasted several seconds. Transfer from the KCl hyperosmotic solution without caffeine (solution A + KCl) to one with caffeine (solution B + KCl) was made ~10 min after the fiber had fully relaxed from the depolarization contraction. For experiments in 1.6T solution, the transfer to 75 mM KCl was made serially, having the first equilibration in 50 mM KCl (Palmer and Gulati, 1976). Note also that once the fiber was transferred to any high KCl solution it was never returned to a solution with lower KCl. This was because, while the entry of K^+ and Cl^- into the cell in high KCl solutions was expected to be fairly rapid (Palmer and Gulati, 1976), the reversal would have taken several hours (Adrian, 1960). The practice of 12- to 15-min rest intervals between activations was followed in each case.

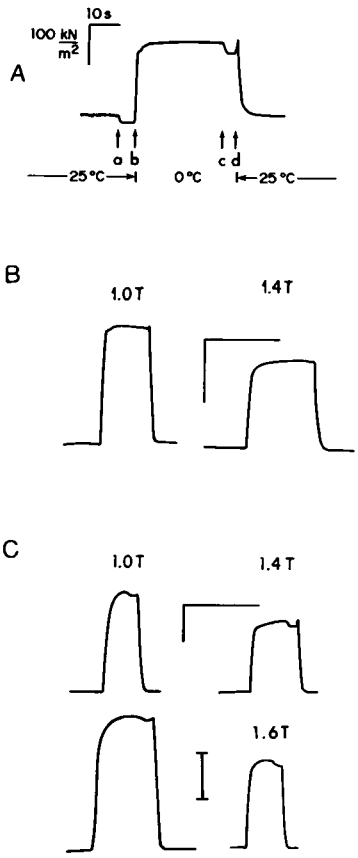

FIGURE 1 Temperature-jump activation of isolated frog fibers in solutions of various osmolarities containing either sucrose or KCl. The typical force records from four fibers are shown. Tracings of actual records from the chart recorder are given. (A) The procedure of instantaneous temperature-jump activation. The fiber is equilibrated in 1.0T solution at 25°C for 30 min. At *a* the fiber is removed from this chamber, at *b* the fiber is submerged in solution at 0°C, at *c* the fiber is removed from 0°, and at *d* it is brought back to 25°C. Depressions in the trace between arrows are artifacts arising at the time of fiber transfer between chambers. Note that force is developed practically instantaneously at 0°C and that the plateau is maintained for more than 20 s. The activation response is completely reversible with reproducible plateau. [Experiment 30 i 81, $D(l) = 76\ \mu m$, and $P_1 = 180\ kN/m^2$.] (*B*) Comparison of activation in 1.0T with that in hyperosmotic solutions with 50 mM NaCl or 100 mM sucrose. [Experiment 7 v 81, $D(l) = 111\ \mu m$, $P_1 = 190\ kN/m^2$.] (*C*) Comparison of activation in 1.0T with that in 50 mM KCl (1.4T, *upper* traces) and 75 mM KCl (1.6T, *lower* traces). [For 1.4T, Experiment 24 iv 81, $D(l) = 72\ \mu m$, $P_1 = 268\ kN/m^2$; for 1.6T, 19 iv 81, $D(l) = 57\ \mu m$, $P_1 = 305\ kN/m^2$.] The activation response in each case was the same in semitendinosus and tibialis fibers.

Electrical Stimulation for Tetanic Contraction

Although the use of the temperature-jump technique provided a unique way to determine the contraction properties in KCl solutions, the effects in sucrose solutions were studied by both this technique and electrical stimulation. In the experiments where electrical stimulation was used to produce tetanic contractions in 1.0T and 1.4T (sucrose) solutions, the solution type A was used (no caffeine). The stimulation was achieved with 1-ms pulses (duration 1–2 s; frequency 20 Hz) of 15–26 V amplitude that was 2–3 times above rheobase (Rudel and Taylor, 1969). The rest period between tetanic stimulations, as for the temperature jump, was at least 12 min.

Slack Tests

For the determination of the speed of unloaded shortening, length releases (ΔL) of various magnitudes producing slack in the fiber were applied during the force plateau. Only one release was used per activation. To obtain a slack release of the desired magnitude, a square pulse from a stimulator (model S88, Grass Instrument Co., Quincy, MA) was applied to the motor (G-100PD, General Scanning Inc., Watertown, MA). The calibration of the motor arm at the point where the fiber was attached was 1.25 mm/V. The amplitude of the pulse could be adjusted to within 0.4% L_0. Although the amplitude of the length release step was somewhat variable because of the limitations of the stimulator, it was accurately recorded on the oscilloscope during the slack test. This measurement was used in the calculation of the speed of shortening. The fiber response during the slack test was also recorded on a storage oscilloscope (model 5103N; Tektronix, Inc., Beaverton, OR), and each set of records was separately photographed (Polaroid film 667; Polaroid Corp., Cambridge, MA). Slow time-base records of force were made simultaneously on a chart recorder (Physiograph model DMP-4B; Narco Bio-Systems, Inc., Houston, TX). The force response was monitored continuously during the experiment, and if the plateau of force before the release had dropped by more than 10% during the successive contractions, or if in any contraction the force had decreased 20% or more from the first contraction in the same solution, the experiment was discontinued. Usually 15–20 contractions were made in a successful preparation. Reversibilities of force and slack time were checked by returning the fiber from a hyperosmolar solution with sucrose to 1.0T solution. With KCl, criterion for viability of the fiber was that the plateau of force was constant in successive contractions.

Check for Fibrillation in Fibers

When the fiber was transferred from the solution of standard osmolarity (1.0T) to a hyperosmotic solution at 25°C (where the fiber remained relaxed), it was examined under a microscope (magnifications of 32 and 80) continuously for a period of time. There was no visible effect in the 1.4T solution of sucrose except in one fiber out of a total of 21 studied, where the fiber erupted in fibrillation (Sato, 1954). This particular fiber was not used for the slack test experiment, although the experiment was included in the force data. Fibrillation consisted of nonpropagating contractile activity at multiple nodes along the fiber's length, lasting 1–3 min, and was accompanied by a slight rise in resting tension. In the 1.6T solution of sucrose at 25°C, fibrillation was observed in every fiber and lasted up to 3 min; therefore, slack test experiments were not performed in this solution. In contrast to the behavior in hyperosmotic solutions with sucrose, none of the fibers studied so far fibrillated in the KCl solutions of either 1.4T or 1.6T osmotic pressure.

Sarcomere Uniformity During Activation

This was checked under high-power objectives (magnifications of 270 and 430) with a Zeiss microscope (model ACM; Carl Zeiss, Inc., Thornwood, NY) using a 50X objective (Leitz UMK, numerical aperture equals 0.6, long working distance; E. Leitz, Inc., Rockleigh, NJ). The micromanipulators holding the fiber were mounted on a movable stage that was separate from the microscope stage. In this way, the fiber could be moved for examination along its length while attached at its ends. The sarcomeres in several regions were photographed (Polaroid film 667; Polaroid Corp.) in each solution in the relaxed fiber (25°C) and in the central region during the force plateau in each solution. A 60-W strobe light was used for illumination and was triggered simultaneously with the camera shutter to photograph the sarcomere pattern.

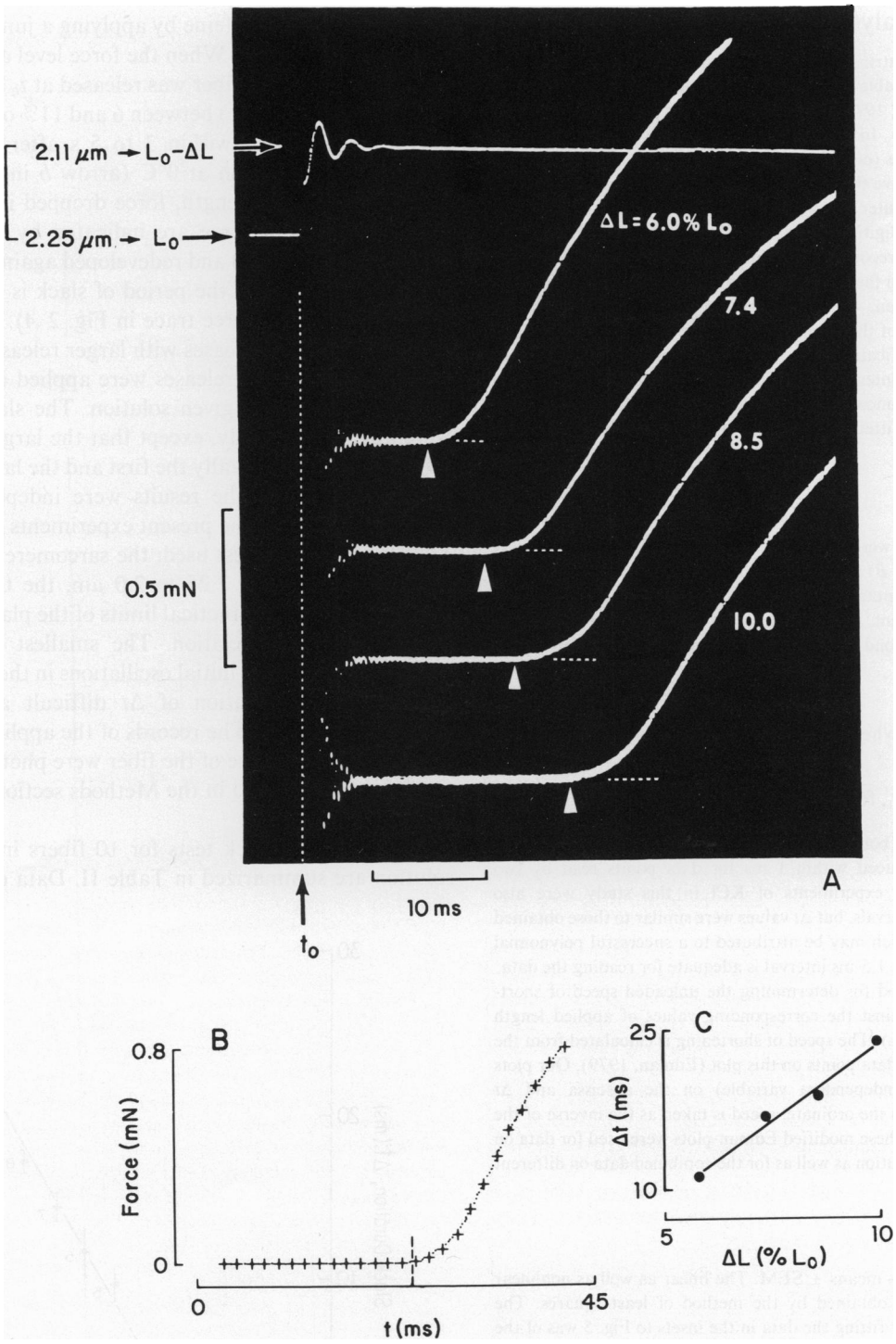

FIGURE 2 Slack test of a single fiber activated by applying an instantaneous jump in temperature from 25° to 0°C in standard solution (osmotic pressure, 245 mosM and 2 mM caffeine). (*A*) Shortening steps of various magnitudes were applied at the force plateau (not shown) on maximally activated fibers. The photographs of actual traces are shown. The *top* trace is a record of the applied length step ($\Delta L = 0.06\ L_0$), and the next four traces are records of force corresponding to various length releases as indicated. The upward direction of the length trace indicates the decrease in fiber length. The time of the application of the step is indicated by (vertical dashed line) at which time the fiber becomes slack and the force drops to zero (the zero of force is indicated by the horizontal dashed line). The end of slack is indicated by ▲. Note the increment in slack duration Δt with larger releases. (*B*) Method of computer analysis of the data. The force traces were digitized (+) and fitted with a polynomial function. The time at which the force deviated from the baseline was computed from the fit. This trace corresponds to the actual force record in *A* for $\Delta L = 10.0\%\ L_0$. (*C*) The modified Edman-plot. The slope of this ΔL vs. Δt plot gives the inverse of the speed of shortening (V_0) at zero load.

Data Analysis

The estimation of the intrinsic speed of shortening at zero load (V_0) from slack tests requires reliable measurements of slack duration Δt for each applied step (A.V. Hill, 1970, pp. 42–45). This has previously been done by eye (Edman, 1979). In our records we found that such estimation introduced uncertainties (of 2–4 ms) that were unacceptable for the data at smaller steps. We have therefore developed an algorithm, for use with the HP-85 microcomputer (Hewlett-Packard Co., Palo Alto, CA), to determine Δt from the digitized force traces. For digitization, the Polaroid pictures of actual data records were photographically enlarged threefold. A hairline was drawn on the enlargement parallel to a horizontal grid line of the oscilloscope screen, ~2–3 cm below the zero of force. The actual distances at midpoints of the force trace from the hairline were carefully measured (at a magnification of 2.5) to the nearest 0.1 mm along the entire trace, at 1.5-ms intervals. (The initial 1–4 ms of the trace was not used, to minimize the uncertainties due to equipment oscillations.) The measured points were fitted (Bevington, 1969) with a polynomial of the form

$$y = \sum_{n=0}^{10} b_n x^n. \quad (1)$$

The fitted parameters were used next to compute new data points at 0.1-ms intervals (Fig. 2 *B*) and these points were used to compute Δt. In the computation, instantaneous change in force ($\Delta y_i = \bar{y} - y_i$) was determined for each point, and the following two criteria were applied to determine the point beyond which the vertical excursion of the force trace increased continuously:

$$\Delta y_i \geq \sigma_i, \text{ where } \sigma_i = \sqrt{\frac{(\bar{y} - y_i)^2}{i - 1}}, \bar{y} = \frac{\Sigma y_i}{i}$$

$$y_{i+k} > y_{i+k-1}, \text{ for each } k \text{ (where } k = 1 \ldots 50).$$

The smallest i for which both inequalities were met gave the slack time Δt. Δt values were reproduced within 1 ms for data points read by two different readers. The experiments of KCl in this study were also measured at 0.5-ms intervals, but Δt values were similar to those obtained at 1.5-ms intervals, which may be attributed to a successful polynomial fit. This indicates that a 1.5-ms interval is adequate for reading the data.

An acceptable method for determining the unloaded speed of shortening is to plot Δt against the corresponding values of applied length releases (slack steps, ΔL). The speed of shortening is calculated from the slope of the line fitting data points on this plot (Edman, 1979). Our plots are made with ΔL (independent variable) on the abscissa and Δt (dependent variable) on the ordinate; speed is taken as the inverse of the slope of the linear fit. These modified Edman-plots were used for data on each fiber in a given solution as well as for the combined data on different fibers in a batch.

Statistics

All data are reported as means ± SEM. The linear as well as nonlinear fits in this study were obtained by the method of least squares. The exponential function for fitting the data in the insets to Fig. 5 was of the form $\Delta t = A_1 \cdot \exp(A_2 \cdot \Delta L) + A_3$. Throughout the study, statistical significance was assumed when student's t-test gave a P value of 0.05 or less.

RESULTS

Intrinsic Shortening Speed with Temperature-Jump Activation Using Slack Tests

The fiber in Fig. 2 was activated at rest length (sarcomere length: 2.2–2.3 μm) in a solution of standard osmolarity (1.0T) and 2 mM caffeine by applying a jump in temperature from 25° to 0°C. When the force level at 0°C reached a stable plateau, the fiber was released at t_0 to slack length with a magnitude (ΔL) between 6 and 11% of L_0. The slack was usually applied within 3 to 5 s after the fiber was transferred to solution at 0°C (arrow *b* in Fig. 1). With release of the fiber length, force dropped immediately to zero (the zeros of force are indicated by the horizontal dashed lines in Fig. 2) and redeveloped again after a period of slack. (The end of the period of slack is marked by an arrowhead in each force trace in Fig. 2 *A*). Note that the duration of slack increases with larger releases.

Three to six slack releases were applied during successive activations in a given solution. The slacks in length were applied randomly, except that the largest releases of 10 to 11% L_0 were usually the first and the last applied (see Methods section). The results were independent of the slack-step order. In the present experiments a slack step of 11% L_0 was the largest used; the sarcomere length in this case decreased from 2.25 to 2.0 μm, the two sarcomere lengths that are the practical limits of the plateau region in the length-tension relation. The smallest releases were usually 5 to 6% of L_0; initial oscillations in the force records made the determination of Δt difficult at releases of magnitude <5% L_0. The records of the applied length step and the force response of the fiber were photographed and analyzed as described in the Methods section (Figs. 2 and 3).

The results of slack tests for 10 fibers in 1.0T control solution are summarized in Table II. Data obtained from

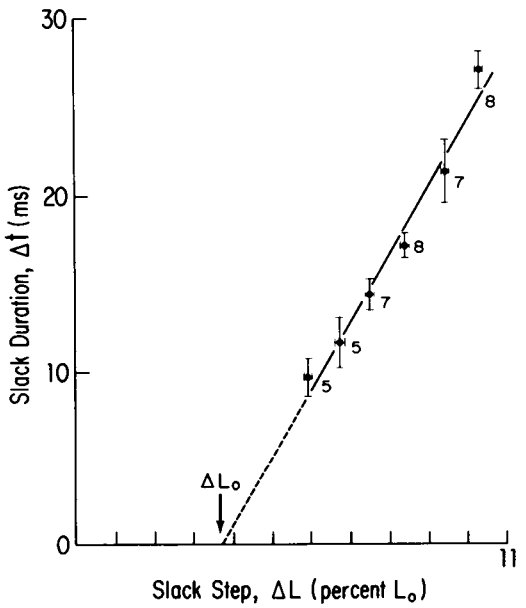

FIGURE 3 Contraction speed by slack test in 1.0T solution from data combined from 10 fibers. Numerals next to the data points indicate the number of releases of different fibers. The vertical bars are standard errors of the means in Δt. The solid line through the points is the best fit between the means, each weighted according to its SEM and the number of determinations. V_0 is 2.6 ± 0.2 L_0/s, ΔL_0 = 3.7% L_0.

TABLE II
INTRINSIC SPEED OF SHORTENING OF FROG TIBIALIS MUSCLE FIBERS AS DETERMINED BY THE SLACK TEST

$n = 10$	Method 1	Method 2
V_o (1) (L_o/s)	2.72 ± 0.14	2.56 ± 0.24
Range of values	1.88–3.3	—

the above records were used in two different ways to determine the speed of shortening. In the first of these (Fig. 2 *C*), V_0 was determined for each individual fiber and was found to range between 1.9 and 3.3 L_0/s (mean value = 2.7 L_0/s). This mean value is close to the value (2.6 L_0/s) obtained by the second method, in which the pooled data from all fibers were used for a single plot (Fig. 3); the small differences in the values obtained from the two methods were determined to be statistically insignificant.

The force level reached in steady state following the largest slack releases (9% L_0 to 11% L_0) was measured on a number of temperature-jump-activated fibers (12 fibers). This level was usually lower than the steady force value before the release, and the mean difference in steady levels before and after the release was 9.3 ± 1.2% P_1. Similar length dependent effects are known also for electrical stimulations under special conditions (Edman, 1980), but the reason for the observed depression in the force-plateau following the release was not examined further.

Contraction in Solutions of Increased Osmotic Pressure.

To evaluate the effects of intracellular ionic strength and cell volume on the intrinsic speed of shortening, various properties (speed of shortening, force, and cell volume) of isolated fibers were studied in hyperosmotic solutions. The osmotic pressure of the solution was increased by the addition of sucrose or KCl.

Sucrose

1.4T. Typical effects of sucrose hyperosmolarity in slack tests are shown in Fig. 4 *A*. For such experiments, a set of slack records was first obtained in 1.0T control solution. A similar set was then obtained in the test 1.4T solution containing 100 mM sucrose. Reversibility was checked after washing out the sucrose by returning the fiber to 1.0T solution and repeating one to three slack releases (usually of 10% L_0 and 30 min washout). Reversibility was complete in this solution.

A major effect of 1.4T sucrose solution, illustrated in Fig. 4 *A*, was that the duration of slack for a given release was greatly increased over that in the control solution. In the present fiber, slack time for the 10% L_0 step in 1.0T

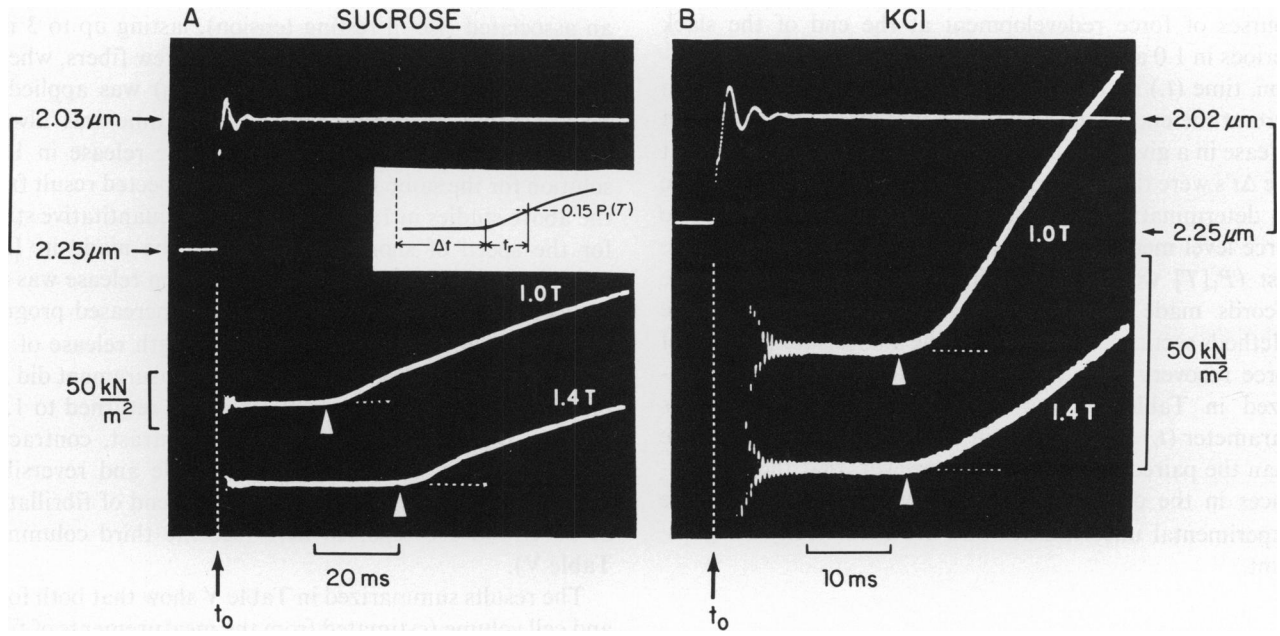

FIGURE 4 Effect of osmotic strength on the slack time at 0°C. (*A*) Effect of sucrose hyperosmolarity. The fiber is released by 9.8% L_0 and the force responses in 1.0T and 1.4T are shown. (Experiment 24 ix 81, tibialis fiber, L_0 = 7.50 mm, fiber width, 159 μm in 1.0T and 139 μm in 1.4T, P_1 = 175 kN/m² and $P_1(T)$ = 131 kN/m²). Note that the slack time in 1.4T is nearly twice that in 1.0T. (*B*) Effect of KCl. ΔL = 10.2% L_0. (Experiment 20 x 81, tibialis fiber, L_0 = 6.19 mm, $D(1)$ = 153 μm, P_1 = 210 kN/m², $P_1(T)$ = 157 kN/m²). Note that, in contrast with the nearly twofold prolongation of the slack times in sucrose, the slack times are practically unaffected by KCl of osmolarity equal to that in the case of sucrose. *Inset*, copy of the third trace in *A* at a reduced time scale, indicates measurement of the time (t_r) for the force recovery from the end of the slack to 0.15 $P_1(T)$. t_r values are 20.4 ms and 20.1 ms for slack responses in *A* for 1.0 and 1.4T, respectively. The corresponding values for traces in *B* for a different fiber are 17.5 and 17.3 ms.

most trace in Fig. 4 *A*) was 42.2 ms. Similar (nearly twofold) prolongation of the slack time with sucrose hyperosmolarity was seen in paired studies on all fibers with temperature-jump activation (mean Δt values for 10% L_0 releases in 1.0T with five fibers: 23.5 ± 1.7 [SEM] ms; in 1.4T: 50.3 ± 7.6 ms).

The results of the unloaded shortening speed in the same five fibers are given in Table III A and Fig. 5 *A*. The speed of shortening decreased from 2.3 L_0/s in 1.0T solution to 1.3 L_0/s in 1.4T sucrose solution. The decrease in speed in the hyperosmotic solution by a factor of ~1/2 half is in agreement with the effect seen in Fig. 4 *A* on the slack times for the 10% L_0 release. This suggests that the slack response to a 10% L_0 release is a good indicator for the effects of osmotic pressure on the speed of shortening.

The linear least-squares fits through the two sets of data points (weighted in proportion to the standard errors of the means and the number of determinations) yield $V_0(1) = 2.4$ L_0/s and $V_0(T) = 1.4$ L_0/s (Fig. 5 *A*). To check if the result depends on the type of the fit, the data were also subjected to a nonlinear analysis by using a function of the form: $A_1 \cdot e^{A_2 \cdot \Delta L} + A_3$, which is that used by Gulati and Podolsky (1981) for fitting the traces in quick-release experiments on skinned fibers. The exponential fits are shown in the inset. Evaluation of speeds $1/(A_1 \cdot A_2 \cdot e^{A_2 \cdot \Delta L})$ at various ΔL's in the data range show a twofold difference between test and control, which is consistent with results of linear fits.

Results such as in Fig. 4 *A* give information on the time courses of force redevelopment at the end of the slack periods in 1.0 and 1.4T solutions. To evaluate this information, time (t_r) required for force to reach 0.15 $P_1(T)$ from zero at the end of slack (Δt) was measured for the largest release in a given solution (see inset in Fig. 4 *A*). Note that the Δt's were the same as those measured previously for the V_0 determination, and the value of $P_1(T)$ used was the force level measured before the release for the slack time test ($P_1[T]$ was measured from the the slow time base records made simultaneously on a chart recorder; see Methods section). The results of the analysis for the time of force recovery after the 10% length release are summarized in Table IV and show that the values of this parameter (t_r) are 6% more in KCl and 10% less in sucrose than the paired controls. Note, however, that these differences in the control and test activations are within the experimental uncertainties and are statistically insignificant.

1.6T. Experiments similar to those in 1.4T solution were also made on five tibialis fibers in a 1.6T solution containing 150 mM sucrose. However, in this series, isometric force levels [$P_1(T)$] and cell volume were the only parameters measured (Table V). Systematic study of slack tests was not made for 1.6T. The reasons for omitting the slack test were that (*a*) each fiber examined in 1.6T solution (sucrose) at 25°C exhibited fibrillation (and an associated rise in resting tension), lasting up to 3 min (see Methods section), and that (*b*) in a few fibers, when a selected length release (usually 10% L_0) was applied in 1.6T solution, the value for the slack time was always greater than that obtained for the same release in 1.4T solution for the same fiber. This is the expected result from the above studies in 1.4T solution. But a quantitative study for the speed of shortening could not be made in 1.6T solution because the slack time for a given release was not reproducible in this solution; its value increased progressively in each successive test with a length release of the same magnitude. Also the slack time measurement did not indicate reversibility when the fiber was returned to 1.0T solution from the 1.6T solution. In contrast, contractile force and fiber width were found stable and reversible; these parameters were measured at the end of fibrillation at 25°C and are reported here (see the third column of Table V).

The results summarized in Table V show that both force and cell volume (estimated from the measurements of fiber width and sarcomere length) decrease in these hyperosmotic solutions. The effects were greater in 1.6T solution than in 1.4T solution. Table V shows also the results of force, volume, and speed of shortening for four tibialis fibers activated by electrical stimulation in 1.0 and 1.4T solutions. The effects of sucrose are similar in this case to those obtained with temperature-jump activation.

TABLE III
SPEED OF SHORTENING. EFFECT OF HYPEROSMOLARITY WITH SUCROSE AND KCl

A. Effect of 100 mM sucrose solution

Experiment	Speed (L_o/s) 1.0T control $V_o(1)$	1.4T $V_o(T)$	$V' = V_o(T)/V_o(1)$
24 viii 81	2.90	1.81	0.62
26 viii 81	2.10	1.49	0.71
27 viii 81	2.45	1.02	0.42
2 ix 81	1.85	0.80	0.43
24 ix 81	2.10	1.48	0.70
Mean ± SEM	2.28 ± 0.19	1.32 ± 0.18	0.58 ± 0.06

B. Effect of 50 mM KCl solution

21 vii 81	1.66	1.79	1.07
19 viii 81	2.63	2.87	1.09
14 x 81	2.79	1.20	0.43
16 x 81	2.21	2.58	1.16
19 x 81	3.05	2.49	0.82
22 x 81	3.64	2.55	0.70
20 xi 81	2.70	2.59	0.96
28 xi 81	2.22	1.91	0.86
Mean ± SEM	2.61 ± 0.21	2.25 ± 0.20	0.89 ± 0.08

All the experiments in these tables were made on fibers from tibialis muscles.

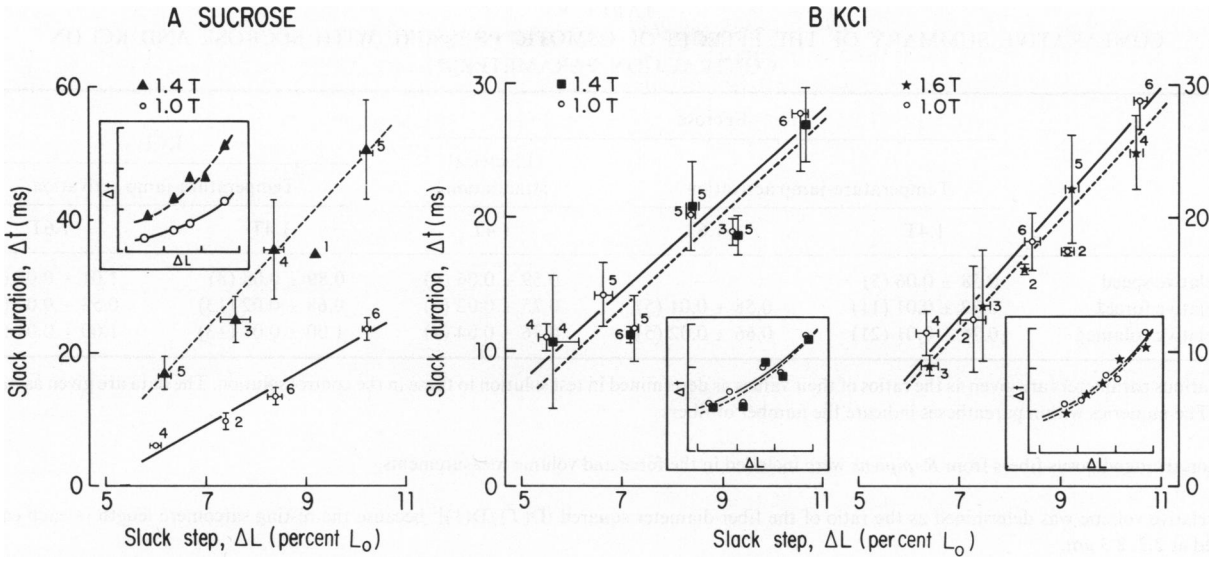

FIGURE 5 Effect of osmotic strength on V_0. (A). Effect of 100 mM sucrose. Control points in 1.0T are shown as open circles (○); data in 1.4T are shown as closed triangles (▲). The solid and the dashed lines are those of best linear fits, respectively, to the control and the test data determined by using the least-squares analysis and weighting the data points according to the number of determinations. Results are on five fibers [range of L_0 = 6.87–7.81 mm; range of D(l) = 145–217 μm]. V_0(l) is 2.4 ± 0.2 L_0/s, $V_0(T)$ is 1.2 ± 0.3 L_0/s. ΔL_0 intercept values are 4.9% L_0 and 4.1% L_0. The *inset* gives the exponential fits to the two sets of data with the function: $\Delta t = A_1 \cdot \exp(A_2 \cdot \Delta L) + A_3$, where Δt is in milliseconds and ΔL is in percent L_0 (see text). The various fitted parameters were as follows: (1.0T): A_1 = 1.16, A_2 = 0.30, A_3 = −0.63; (1.4T sucrose): A_1 = 3.52, A_2 = 0.26, A_3 = 0. The speeds estimated at 5% L_0 and 10% L_0 from these fits are about twofold less in 1.4T than in control, and this factor is similar to that found with the linear fits. (*B*) Effect of 50 mM (1.4T) and 75 mM (1.6T) KCl. The solid line in each case is the best linear fit through the weighted data points for controls, and the dashed lines are similar fits for the test cases. The data illustrated are collected on 13 fibers (range of L_0 = 6.06–7.81 mm, range of D[l] = 57–187 μm). For 1.4T, V_0(l) = 2.9 ± 0.5 L_0/s, ΔL_0 = 2.8% L_0; $V_0(T)$ = 2.8 ± 0.6 L_0/s, ΔL_0 = 3.2% L_0. For 1.6T, V_0(l) = 2.3 ± 0.3 L_0/s, ΔL_0 = 4.1% L_0; $V_0(T)$ = 2.4 ± 0.6 L_0/s, ΔL_0 = 4.2% L_0. The insets show exponential fits through the data. The various parameters for the exponential fits are as follows. 1.0T in the left-hand panel of *B*: A_1 = 3.96, A_2 = 0.18, A_3 = 0; 1.4T KCl: A_1 = 3.38, A_2 = 0.20, A_3 = 0; 1.0T in the right-hand panel of *B*: A_1 = 2.7, A_2 = 0.22, A_3 = 0; 1.6T KCl: A_1 = 1.92, A_2 = 0.25, A_3 = 0.85. The speeds of shortening can be estimated from the fits for any given ΔL (see text) and are essentially the same for the test and control cases in KCl.

KCl

The effects of the addition of 50 and 75 mM KCl were determined for a number of isolated fibers. The typical response in slack tests to ΔL of 10% L_0 for one fiber is shown in Fig. 4 *B* for 1.4T solution. As in similar experiments with sucrose, the fibers were first activated in 1.0T solution and the force response to a slack release was recorded on a fast time-base (second trace in Fig. 4 *B*). The response to a release of the same magnitude in KCl was recorded next (third trace, 50 mM KCl solution). The slack times for these records are found to be 20 and 22 ms, respectively. These values in 50 mM KCl are nearly the same, indicating that slack times for a given release are practically unaffected by the increased osmotic pressure in 50 mM KCl solution.

Additional results of slack times for a number of slack releases in 50 mM KCl solution (on eight fibers) and in 75 mM KCl solution (on five fibers) are given in Fig. 5 *B*. The data points in the control solution on the same fibers in each set are also given. As described before, the control measurements in each case were made before exposing the fiber to the desired hyperosmotic solution. Separate least-squares fits were obtained for the individual sets of data in 1.4 and 1.6T solutions, and the values for the speed of shortening as found from the slopes of these fits were 2.8 ± 0.5 L_0/s (1.4T) and 2.9 ± 0.5 L_0/s (1.0T) in paired measurements on eight fibers; 2.4 ± 0.6 L_0/s (1.6T) and 2.3 ± 0.3 L_0/s (1.0T) in paired measurements on five different fibers. In each case the difference in the value in

TABLE IV
EFFECTS OF SUCROSE AND KCl HYPEROSMOLARITIES (1.4T) ON THE TIME COURSE OF FORCE RECOVERY IN SLACK TESTS*‡

Solution	Duration for force recovery to 0.15 $P_1(T)$, ms(t_r)	Relative duration [t_r in test/t_r in control]§
KCl		
1.0T	19.1 ± 1.9 (4)	1.06 ± 0.05 (4)
1.4T	19.9 ± 1.3 (4)	
Sucrose		
1.0T	22.0 ± 1.1 (4)	0.90 ± 0.07 (4)
1.4T	19.4 ± 0.8 (4)	

*Data are given as means ± SEM. The numerals within parentheses indicate the number of fibers. Only paired observations on a given fiber (for test and control) were used in each case.
‡The slack steps (ΔL) in these tests were close to 10% L_0
§The ratio of t_r in test to t_r in control was determined for each pair.

TABLE V
COMPARATIVE SUMMARY OF THE EFFECTS OF OSMOTIC PRESSURE WITH SUCROSE AND KCl ON CONTRACTION PARAMETERS*

	Sucrose			KCl	
	Temperature-jump activation		Electrical stimulation‖	Temperature-jump activation	
	1.4T	1.6T	1.4T	1.4T	1.6T
Relative speed	0.58 ± 0.06 (5)	—	0.59 ± 0.06 (4)	0.89 ± 0.08 (8)	1.01 ± 0.06 (5)
Relative force‡	0.72 ± 0.01 (11)	0.58 ± 0.01 (5)	0.75 ± 0.02 (4)	0.68 ± 0.02 (13)	0.53 ± 0.02 (7)
Relative volume§	0.76 ± 0.01 (21)	0.66 ± 0.02 (5)	0.76 ± 0.04 (4)	1.00 ± 0.01 (13)	1.00 ± 0.01 (7)

*The various parameters are given as the ratios of their values as determined in test solution to those in the control solution. The data are given as means ± SEM. The numerics within parentheses indicate the number of fibers.

‡Data on semitendinosus fibers from *R. pipiens* were included in the force and volume measurements.

§The relative volume was determined as the ratio of the fiber diameter squared $\{D(T)/D(1)\}^2$ because the resting sarcomere length in each case was adjusted at 2.2–2.3 μm.

‖ Experiments of electrical stimulation were made in solutions (1.0T and 1.4T) that contained no caffeine.

the test solution was statistically not significantly different from the control value. This indicates that, in contrast to the effect with sucrose, increasing the osmotic pressure with KCl has practically no effect on the speed of unloaded shortening.

The speeds calculated from paired data for 50 mM KCl in individual experiments are also shown in Table III B to evaluate the reproducibilty of the effect of KCl from fiber to fiber. In most cases the ratio of the unloaded shortening speed in KCl to that in control range from 0.8 to 1.1 and the mean of all values is 0.89. Therefore, the lack of effect with KCl on the speed of shortening is quite reproducible in different experiments. There was one exception, in which case (the third experiment in Table III B) KCl hyperosmolarity appeared to have an effect similar to that of sucrose, but the reason for this was not clear.

As with sucrose, the data in Fig. 5 *B* were also fitted with an exponential function; the fits are shown in the insets. The form of the function in each case was the same as that used in the inset of Fig. 5 *A*. The exponential fits are close for the test and control data for both 1.4 and 1.6T solutions, which are consistent with the results of the linear fit.

Relation Between Force and Cell Volume

The present results on speed, force, and cell volume in 1.4 and 1.6T KCl and sucrose solutions on tibialis and semitendinosus fibers from frogs are summarized in Table V. The results on these fibers from two different muscles were indistinguishable. The force was decreased in the hyperosmotic solutions with KCl and with sucrose. The effect on force depended on the osmotic pressure but was independent of the solute; the results are additional evidence that force development is independent of cell volume. Because the fiber width in hypertonic solutions seems to be well correlated with lateral filament separation (Gulati and Babu, 1982 *b*), the results strongly support the idea that force per cross bridge is constant with varying distance of interfilament separation in intact fibers.[1] The implications of this finding with isolated frog fibers for interpreting the length-tension relations and for evaluating the structural constraints on cross-bridge movements during the isometric contractions have been discussed previously (Gulati and Babu, 1982 *b*; see also, T. L. Hill, 1970; Schoenberg, 1980). Our one specific suggestion was that rocking of the entire cross bridge (between the "90°" or a random orientation, in the relaxed state, to a final orientation of "45°" preferred in the rigor state; see Huxley, 1969; also, Poulson and Lowy, 1983) is not an essential aspect of the cross-bridge cycle in the contracting state, and the results of Cooke et al. (1982), monitoring the electron paramagnetic resonance spectra of contracting glycerinated rabbit psoas fibers, provided additional support for this idea (Gulati and Babu, 1982 *b*). Similarly, with highly sophisticated structural studies measuring the x-ray diffraction patterns of contracting muscles, with fast-time resolution, Huxley et al. (1983) have made the additional suggestion that significant cross-bridge movements during the working stroke are in the axial direction, in the domain of myosin head near the S1-S2 junction.

Sarcomere Uniformity During Contraction in Hyperosmolar Solutions

To check the possibility that the differences in the effects of KCl and sucrose hyperosmolarities on the speed of

[1]Note that these results of force with isolated fibers over a wide estimated range of filament separation (Gulati and Babu, 1982 *b*) differ from the published results on compressed skinned fibers (Maughan and Godt, 1981); the latter studies showed that force depended on fiber volume, but, as we previously mentioned, there were certain complications associated with those studies of skinned fibers that remain to be clarified.

shortening are the result of differences in the uniformity of sarcomeres during activation, a number of fibers were examined with a light microscope under high power magnification (270 and 430). This was done both at 25°C, when the fiber was relaxed, and at 0°C, during the force plateau with temperature-jump activation. Typical results with a hyperosmolar sucrose solution are shown in Fig. 6 and with a hyperosmolar KCl solution in Fig. 7.

The fiber in each case was photographed in the control solution at an overall magnification of 270 and 430 (control pictures in 1.0T not shown). When transferred to hyperosmotic sucrose at 25°C, the fiber shrank in diameter from 158 μm (not shown) to 135 μm (Fig. 6 *A*). For analysis, these pictures were partitioned into nine rectangular blocks (four lengthwise and five widthwise), and the mean sarcomere length in each block was measured. The sarcomere pattern was completely unchanged in hyperosmolar KCl solutions at 25°C; indeed no visible changes were uncovered in this solution by inspecting the length of the fiber and by using a range of focal planes. In hyperosmolar sucrose solutions the transparency of the fiber was reduced, making it more difficult to obtain photographs during activation than was the case with hyperosmolar KCl. This property was consistent with our laser diffraction observations, in which the intensity of the first-order pattern was found to be weaker (but no effect on the separation between the zeroth and first orders) on transferring the relaxed fiber from the control solution to hyperosmolar sucrose solution at 25°C with caffeine and at 0°C without caffeine (Rudel and Zite-Ferenczy, 1980). No such effect on the diffraction pattern was seen in hyperosmolar KCl solutions.

The fibers in Figs. 6 *A* and 7 *A* (as well as eight other fibers, similarly) were rephotographed at 0°C during contraction with temperature-jump activation when the force was at a steady level. The photographs of activated fibers are reproduced in Figs. 6 *B* and 7 *B*. The mean sarcomere lengths in these photographs were similar to the mean lengths of the corresponding regions in the relaxed fibers. One major change in activation was that the sarcomere pattern became somewhat misaligned, but this effect was quite similar in 1.4T solutions using both sucrose (Fig. 6 *B*) and KCl (Fig. 7 *B*). This effect was also observed in 1.0T solution and is similar to that noted previously by Gulati and Podolsky (1981) with calcium-activated frog skinned fibers (for a similar result on rabbit psoas fiber, see Brenner, 1983; on hamster, J. Gulati, manuscript submitted for publication).

The similarities in the sarcomere patterns in the sucrose and KCl solutions (1.4T; identical results in 1.6T not

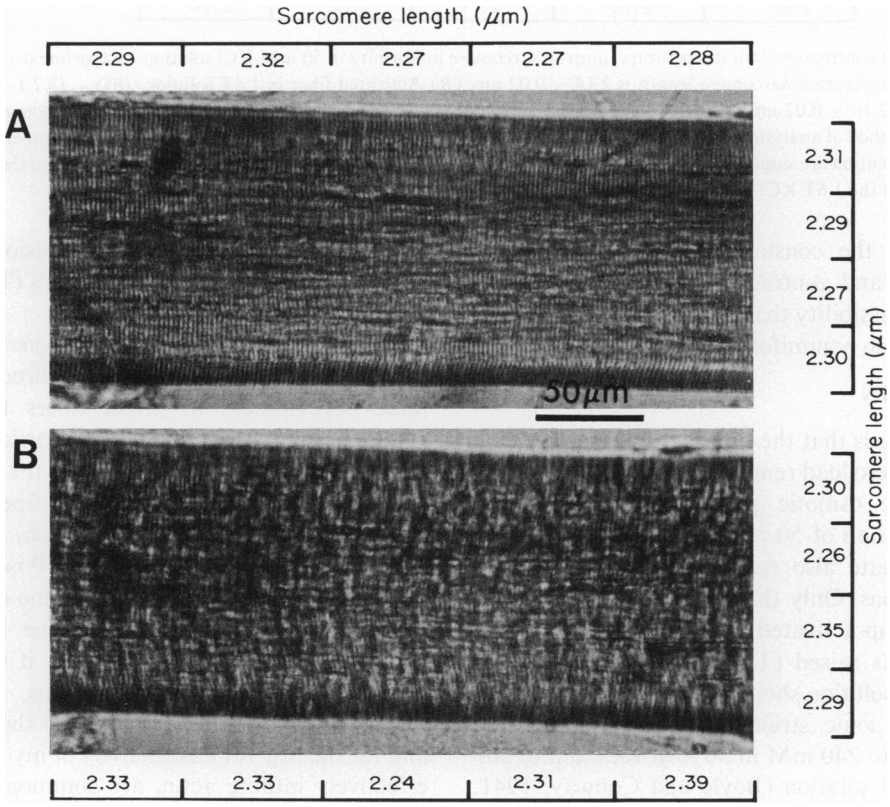

FIGURE 6 Effect of activation on sarcomere uniformity in 100 mM sucrose solution. (Experiment 8 ii 82). (*A*) Relaxed fiber in 1.4T solution. Fiber diameter $D(T)$ is 135 μm [$D(l)$ was = 158 μm]. Average sarcomere length is 2.29 ± 0.01 μm. (*B*) Fiber under steady activation. Average sarcomere length is 2.30 ± 0.02 μm. For analysis of sarcomere lengths, the photographs were divided into nine rectangular blocks. Magnification in both *A* and *B* was 270. Note that there is a slight alteration in the alignment of sarcomeres in different regions of the fiber during contraction, but that the mean sarcomere length is unchanged.

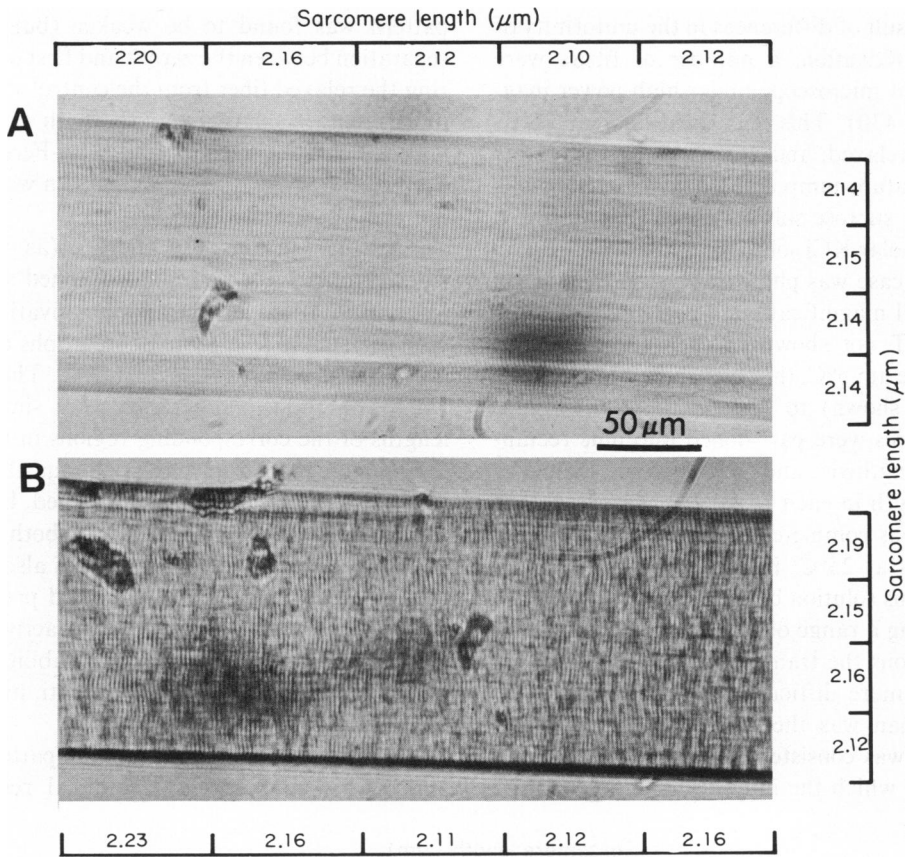

FIGURE 7 Effect of contraction with temperature jump on sarcomere uniformity in 50 mM KCl solution. (Experiment 1 ii 82) (*A*) Relaxed fiber in 1.4T solution. Average sarcomere length is 2.14 ± 0.02 μm. (*B*) Activated fiber in 1.4T solution. $D(l) = D(T) = 132$ μm. Average sarcomere length is 2.16 ± 0.02 μm. Magnification in both *A* and *B* was 270. Note that the location of the fiber photographed was shifted slightly in *B*. The method of analysis was the same as that used in Fig. 6. The effects on sarcomere uniformity and alignment with activation in hyperosmolar KCl solution are similar to the effects with activation in hyperosmolar sucrose solutions. Results similar to those described above were also observed in the 1.6T KCl.

shown), as well as the constancy of mean sarcomere lengths, for relaxed and contracted fibers in the present study, rule out the possibility that the contractile effects of these agents are due to nonuniformity in the sarcomeres.

DISCUSSION

Our principal finding is that the intrinsic shortening speed of isolated fibers at zero load remained practically constant with increasing the osmotic pressure of the bathing medium by the addition of 50 and 75 mM KCl. The cell volume at steady state also remained constant in such hyperosmotic solutions. Only the isometric force levels of the temperature-jump-activated fibers were depressed as osmotic pressure was raised (Table V). The addition of KCl to the bathing solution should produce a net increase in the intracellular ionic strength, from ~190 mM in standard conditions to 240 mM in 50 mM KCl and to 265 mM in 75 mM KCl solution (Boyle and Conway, 1941; Palmer and Gulati, 1976). Therefore, the present finding, that the unloaded shortening speed is relatively unaffected by KCl, is conclusive evidence that the contraction speed of isolated fibers is independent of intracellular ionic strength and supports the previous conclusion based on force-velocity relations with skinned fibers (Thames et al., 1974; Gulati and Podolsky, 1981).

Similarly, the effect of KCl on force is additional evidence that ionic strength is a direct parameter in the force development in intact fibers (Gulati and Babu, 1982 *b*) as in the skinned fibers (Gordon et al., 1973).

The lack of effect of KCl on the unloaded speed is consistent with the biochemical properties of acto-heavy meromyosin and acto-S1 in solution, in which the maximum rate for the hydrolysis of ATP is constant with ionic strength (from 18 to 61 mM, Rizzino et al., 1970; Mornet et al., 1981). On the basis of the biochemical results combined with skinned fiber data, it was previously concluded that the rate-limiting steps, in the cross-bridge turnover, for the speed of unloaded shortening in the fiber and for the Mg-ATPase activity of myosin in solution, with effectively infinite actin, are common and unaffected by ionic strength (Podolsky, 1980). Our present finding confirms and extends this conclusion for intact fibers. Furthermore, note that, since force is decreased with increasing the ionic strength, the results suggest that the rate-limiting

steps in the cross-bridge mechanism are different for force and shortening speed.

Sucrose Hypertonicity

Mechanisms of the Volume Effect on Speed of Shortening. Our finding that the slack speed of unloaded shortening of the temperature-jump-activated fibers decreases in 1.4T sucrose solution is in agreement with data on electrically tetanized fibers (Table V). Intracellular ionic strength is increased in the sucrose solution, but in this case (unlike the effect with KCl) the change in ionic strength is associated with shrinkage in cell volume. Therefore, the combined results in sucrose and KCl solutions indicate that the decrease in speed of shortening with sucrose hyperosmolarity is a consequence of the change in volume rather than an effect of ionic strength per se.

The force redevelopment following the 10% slack release reached a slightly lower level in the new steady state than in the force level before the release, and a part of this effect may be due to length induced deactivation of cross bridges (Edman, 1980; Ekelund, 1983). Therefore, one should consider that the cross-bridge activation properties are different in sucrose and KCl, and that the decrease in speed in sucrose is an effect of this difference. But the available results argue against this possibility. First, a given increment in osmotic pressure had the same effect on steady force level with sucrose and KCl (Table V), and the same effect was expected from the accompanying increase in ionic strength (Gulati and Babu, 1982 *b*). This result argues against a significant difference in the cross-bridge activation properties in the two solutions in the steady state before the applied length release. Second, the time courses of force redevelopment at the end of equal slacks in sucrose and KCl are similar to the time course in control solutions. This was suggested by the measurements of duration t_r for the force to rise from zero at the end of slack to 0.15 $P_1(T)$ (Fig. 4 and Table IV) and is a good argument against any major differences in deactivation following the applied length changes, between sucrose and KCl, under the present experimental conditions.

The simplest explanation for the decrease in shortening speed is that the internal load in the fiber increases in hyperosmolar sucrose. According to this explanation, the intrinsic properties of the cross-bridge turnover mechanism are unaffected, but presence of the load creates resistance to shortening. A quantitative prediction for the amount of internal resistance (F_R) necessary to reduce the speed of shortening to the known value may be obtained from the characteristic force-velocity relationship and assuming that the internal load acts as would an equivalent amount of external load. Using the simplified form of Hill's equation (Huxley, 1974), taking $V_0(l)$ as the approximate V_{max} with no internal load and $V_0(T)$ as the speed with F_R as the internal load, we get

$$\frac{F_R}{P_1(T)} = \frac{1 - V_0(T)/V_0(l)}{1 + \frac{P_1(T)}{a} \cdot \frac{V_0(T)}{V_0(l)}} . \quad (2)$$

Assuming that $P_1(T)/a$ is constant with osmotic pressure and taking this constant as 4 (according to A. V. Hill, 1970, p. 28), and using $V_0(T)/V_0(1) = 0.58$ from our Table III *A*, $F_R/P_1(T)$ is found to be 0.13 in the above equation. Therefore, according to this explanation, the average internal load in the fibers in 1.4T sucrose solution must be close to 13% of the isometric force level in the same solution. An interesting implication of this treatment is that the speed of shortening measured by slack tests for externally unloaded fibers equals the intrinsic unloaded speed only when the internal load is also zero.

Several mechanisms may be considered for the increase in internal load in shrunken fibers. One is that intracellular viscosity is increased (the internal viscosity due to soluble proteins is estimated at ~1.7 cP, rising to 2.0 cP in 1.4T sucrose solution),[2] and this raises the viscous drag on myofilaments during shortening. But a simple calculation of viscous drag (Huxley, 1980, pp. 66, 67), under the present conditions, indicates that this effect amounts to $<1\%P_1$, which is too small to explain the present results.

A parallel effect of the decrease in fiber volume in hyperosmotic sucrose solutions is the compression of myofilament lattice (Millman et al., 1981; Gulati and Babu, 1982 *b*), and one should consider that the internal load increases in this solution as a result of new or enhanced filamentary interactions due to the above compression. Also, increasing the tonicity of the bathing medium (in the range of 1.9 to 2.5T) causes swelling of the T-tubules (Franzini-Armstrong et. al., 1978; Davey and O'Brien, 1978), but it is unlikely that this change in the extracellular compartment inhibits the speed of filament sliding. Regarding the effects associated with the compression of filament lattice, it is interesting to recall Hill's observation on frog sartorious muscle; he observed that the filamentary resting tension (FRT) derived from the step rise in force in the response of resting muscle to a steady rate of stretch

[2]Following Huxley (1980), the cell viscosity was estimated by using the Huggins Formula (p. 392, Tanford, 1967), $\eta_{sp}/c = [\eta] + k[\eta]^2c$, where η_{sp} and $[\eta]$ are specific and intrinsic viscosities, respectively; c is concentration of soluble proteins and k a constant. By assuming that 20% of the dry mass of muscle represents soluble proteins, and that these are concentrated twofold, due to compartmentalization and other factors, $c \approx 0.1$ g/ml in 1.0T and 0.13 g/ml in 1.4T solution. Using data on serum albumin (Table I in Tanford and Buzzell, 1956; data at pH = 7.3, ionic strength = 0.1-0.5 M and 25°C), $k = 2$ and $[\eta] = 3.7$ ml/gm. The values for η_{sp} are found to be 0.65 (in 1.0T) and 0.94 (in 1.4T). Since the viscosity of water is ~1 cP, the absolute viscosity of the cell comes to ~1.7 cP in 1.0T solution and 2 cP in 1.4T solution. (Note that Huxley used a higher value of c and assumed $k = 1$, but these differences do not change the estimate of viscous drag by a substantial amount to affect the present conclusions.)

was increased several fold in solutions made hyperosmotic with impermeant solutes (see Fig. 2, Hill, 1968). Therefore, it is possible that the underlying mechanism, both for FRT in the relaxed fibers and for a significant fraction of the internal load during active shortening is similar.[3]

It was suggested that filamentary resting tension in unstimulated muscles arises by unusual interactions between actin and myosin filaments, which have the properties expected of long-lived cross-bridges (Hill, 1968). This is an attractive idea; the attempts to characterize these interactions in active muscle have been limited by the presence of force, however, and their influence on the contractile behavior of the main bridges remains uncertain at present. There is evidence for an unusual kind of cross-bridge attachment in relaxed skinned fibers from rabbit muscles too; these bridges appeared to have rapid attachment-detachment rates and were measurable only in low ionic strength (20 mM; Ca^{2+}-free; 5°C) (Brenner et al., 1982). When the fibers were allowed to make force under slightly different, but Ca^{2+}-free, conditions (frog muscle, 40–50 mM ionic strength, 13°–16°C), turnover of the bridges was found to be slower than normal (Gulati, 1983). Because in the present study the resistance during shortening for intact frog fibers was a fraction of the total force, if either of the above two (or some other) types of cross-bridges are involved in producing that resistance, the number of such bridges may be a minor fraction of the main bridges also. (The cross-bridge heterogeneity of this kind in active fibers can be envisioned, for instance, as resulting from the isoforms of sarcomeric myosin coded by a multigene family [Nguyen et al., 1982; Masaki et al., 1982].) For such a mechanism to operate in vivo, the preent results would suggest that acto-myosin interactions are not completely blocked in the relaxed muscle and that this residual activity in the relaxed condition is enhanced by sucrose hypertonicity.

One question is whether the factors associated with the increase in internal load in compressed fibers arise simply due to the closer packing of myofilaments or whether they require the combination of packing and other unspecified effects, associated with decreased volume (e.g., due to concentrated enzymes, nonionic, and ionic solutes, etc.). Because the speed of unloaded shortening is practically constant at long sarcomere lengths (Gordon et al., 1966; Edman, 1979), while the filaments are more closely packed laterally, it would appear that the first possibility is unlikely. But in those experiments there were changes in filament overlap as well.[4] Because this question of the influence of filament separation on the contraction kinetics is important for the cross-bridge mechanism, additional studies on this point would be worthwhile. One useful approach, suggested by the present study, is to extend the effects of varying the osmotic pressure from resting length to longer sarcomere lengths; this would augment the range of filament separations for measuring its effect on the speed of shortening and the results would be helpful also in evaluating the current ideas on the nature of cross-bridge movements during active shortening.

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[3]To examine the relation between FRT and internal load more closely, we measured the characteristic force of isolated relaxed fibers under conditions similar to those for studies in the text. The details of these measurements will be reported in a separate publication. FRT values were as follows: in 1.0T, 0.01 ± 0.001 P_1; in 1.4T with sucrose, 0.09 ± 0.01 P_1; in 1.4T with KCl, 0.013 ± 0.001 P_1 (all measurements made at 0°C without caffeine, and the velocity of stretch in each case was equal to the corresponding V_0 in that particular solution). Note that Ford et al. (1977, their Fig. 17) had also measured FRT for isolated fibialis fibers at 10 V_0 (in a 1.0T solution) and accordingly their value is somewhat higher. Conclusions are (*a*) FRT is increased in the compressed fibers, but is constant by change in ionic strength alone (*b*) FRT of 0.09 P_1 in 1.4T sucrose solution amounts to 0.125 $P_1(T)$, by taking the factor of 0.72 from the observed $P_1/P_1(T)$ in Table V. This amount is the same as the estimate of internal load to shortening in the same solution (see text).

[4]Data on sarcomere-length dependence of FRT in relaxed fibers from frog tibialis muscles allow an estimate of the expected effect of filament packing, at stretched sarcomere lengths, on the speed of shortening. The value of FRT in 1.0T solution increased with sarcomere length, from 0.01 P_1 at 2.2 μm to 0.02 P_1 at 2.7 μm (J. Gulati and A. Babu, unpublished results; for results on semitendinosus fibers, Haugen and Sten-Knudsen, 1981). If the internal load to shortening had increased in the stretched fiber by an amount similar to FRT, substitution of these values in Eq. 2 in the text, with the appropriate factor of 0.83 for force at the two sarcomere lengths (from the standard length-tension relation, Gordon et al., 1966), indicates that the speed of shortening at 2.7 μm is expected to decrease by ~5% from its value at 2.2 μm. The 5% effect is within the measurement errors of all studies of this kind.

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