Effect of Osmotic Compression on the Force–Velocity Properties of Glycerinated Rabbit Skeletal Muscle Cells

LINCOLN E. FORD, KENT NAKAGAWA, JOHN DESPER, and CHUN Y. SEOW

From the Cardiology Section, Department of Medicine, The University of Chicago, Chicago, Illinois 60637

ABSTRACT The force–velocity relations of single glycerinated rabbit psoas muscle fibers at 5°C were studied at maximum and half-maximum activation in the presence of 0 (control) and 39–145 g/liter dextran T-70. Resting fiber diameter decreased progressively to ~70% of the nondextran control as the dextran concentration was increased. Isometric force at full activation increased to a maximum of 136% of control at 111 g/liter dextran and then fell to 80% of control in 145 g/liter dextran. Maximum velocity, which fell to 49% of the control value in the highest concentration of dextran, was nearly constant at ~65% control over the range of 58–111 g/liter dextran. Relative maximum power, which gives an estimate of changes in intermediate velocity, was not significantly reduced by dextran concentrations up to 76 g/liter, but then fell progressively to 62% of control in the highest concentration of dextran. At half-maximum activation, maximum velocity and relative maximum power were not significantly different from the values at full activation. The results obtained at partial activation indicate that the decline of velocity seen in the presence of dextran is not due to a passive internal load and that the dextran does not cause a viscous resistance to shortening. The increased velocity in the absence of dextran can be explained by the reduced ability of cross-bridges to resist shortening, as proposed by Goldman (1987. *Biophys. J.* 51:57.).

INTRODUCTION

Muscle cells swell when they are skinned (Godt and Maughan, 1977) and this swelling is caused by swelling of the myofilament lattice (Matsubara and Elliott, 1972), as is expected by a change in the balance of osmotic Donnan and other forces (Boyle and Conway, 1941; Maughan and Godt, 1980). The swollen lattice can be compressed to varying extents by changing the concentration of large polymers that do not penetrate the lattice (Godt and Maughan, 1977). Recently, Goldman (1987) has shown that shortening velocities at very low loads are increased substantially by skinning, and that these high velocities are diminished when high molecular weight

Address reprint requests to Dr. Lincoln E. Ford, Box 249, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637.

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polymers are included in the bathing medium. He attributed this increased velocity to buckling of cross-bridges during rapid shortening. According to the cross-bridge theory of contraction (Huxley, 1957), shortening velocities at very low loads are determined by a balance between positively and negatively strained cross-bridges. Bridges that remain attached after they have pulled through their useful range resist the shortening motion of more recently formed bridges. Goldman (1987) postulated that in swollen skinned fibers these spent cross-bridges would not resist shortening as effectively, possibly because of an unfavorable angle between bridge and filaments.

Goldman's findings raise two important issues. The first is the question of whether the polymer acts partly by imposing a passive load that increases the resistance to shortening, perhaps by diffusing into the lattice to increase viscosity. Second, and more important, is the question of the appropriate amount of shrinkage for skinned fiber studies. In this study we have attempted to address both of these issues by studying the force–velocity properties of skinned fibers at different concentrations of polymer and different levels of activation. The experiments at different levels of activation were designed to detect a passive internal load not related to cross-bridge mechanisms. Such an internal load would be expected to reduce maximum shortening velocity at partial activation. The experiments at different polymer concentrations were designed to determine whether there is some range of lattice spacing over which the force–velocity properties of the fibers are fairly constant.

METHODS

Tissue Preparation

Young adult rabbits weighing ~2 kg were anesthetized with intramuscular injections of 50 mg ketamine and 20 mg xylazine and their hearts were excised. Small pieces of psoas muscle 20 mm long and 2 mm in diameter were tied to glass rods and kept overnight at 0°C in a skinning solution containing 150 mM K-propionate, 1 mM $MgCl_2$, 5 mM Na_2ATP, 5 mM K_2EGTA, 0.1 mM phenylmethylsulfonylfluoride, and 10 mM imidazole adjusted to pH 7.0 at 25°C. After the overnight soak the tissue was transferred to a solution containing equal portions of glycerol and a relaxing solution containing 150 mM K-propionate, 6 mM $MgCl_2$, 5 mM MgATP, 5 mM K_2EGTA, 10 mM Na_2ATP, and 10 mM imidazole and stored at −20°C. The pH of the storage solution was set to either 6.2 or 6.5 to provide a pH close to 7 at the storage temperature.

At periodic intervals small pieces of tissue were removed from the stored tissue tied to the rods, and segments of single fibers were isolated in the glycerol relaxing solution at 0°C. Aluminum foil clips (Ford et al., 1977) were attached to the fibers so that there was 1.5 ± 0.2 mm of fiber between the clips. In some cases the clipped fibers were stored in the glycerol relaxing solution at 0°C for several hours and used the same day. In other cases the clipped fibers were stored at −20°C in the glycerol solution for several days before use.

Apparatus

During experiments the skinned fibers were held horizontally in a covered trough into which precooled solutions could be injected (Chiu et al., 1985). The solutions were injected into the closed, upstream end of the trough and drawn away from the open, downstream end by suction. Muscle length was controlled by a linear, loudspeaker-type, servo motor which had a 50-mm length of hypodermic tubing projecting through a seal into the upstream end of the trough. A 1 cm, horizontal, stainless steel wire hook projected from the vertical arm of a

force-transducer through a vertical meniscus at the open, downstream end of the trough. The phototransistor force-transducer (Chiu et al., 1982*a*) had a resonant frequency of ~2.5 kHz when loaded with the fiber.

The motor could be servo controlled either with a signal from its own internal position sensor or with a signal from the force-transducer. Switching between the two types of control was accomplished by a diode switching network (Ford et al., 1977; Chiu et al., 1982*b*). The gain of the servo system was adjusted to make a critically damped length step in 0.5 ms. Force steps were usually completed within 5–8 ms. The speed of the tension steps was slowed because the nonlinear elasticity of the muscle is included within the feedback loop when the system is in tension control. Because of this nonlinearity the loop gain of the system is increased when steps are made to higher force, and therefore greater stiffness. The tension feedback loop included an amplifier in which gain was automatically reduced as steps to higher isotonic loads were made. This system provided approximately the correct gain for each size of step, and in the interest of speeding data collection, no adjustment of the servo loop was made for individual sizes of steps. Faster steps could have been made if such adjustments had been done, but individual attention to each step would have slowed data collection enormously.

The tension control portion of the servo loop contained a sample-and-hold circuit that held the isometric force level immediately before the step. This isometric force signal was divided into 16 steps, and one of these steps was used as a command signal for each isotonic step.

The tension and length signals were amplified and then digitized with a Labmaster interface board (Tekmar Co., Cincinnati, OH) in an IBM PC computer. The same computer and interface board were used to time and control experimental events. The timing and interfacing procedures were controlled by the SALT software (Fenster and Ford, 1985; Wirth and Ford, 1986).

Solutions

All experimental solutions contained 150 mM K-propionate, 5 mM Na_2ATP, 6 mM $MgCl_2$, and 10 mM imidazole. The pH was adjusted to 7.0 at room temperature so that the pH at the experiment temperature of 5°C was ~7.3. In addition, all solutions contained one of the following: 5 mM K_2EGTA, in "relaxing" solutions, 0.1 mM K_2EGTA in "rinse" solutions, or 5 mM EGTA plus varying amounts of calcium in "activating" solutions. Full activation solution had a CaEGTA/EGTA ratio of 99:1. Partial activation solutions had a much lower ratio adjusted separately for each condition. Dextran T-70 solutions were made by mixing dry dextran powder with various amounts of solution. In general, dextran in integer number of grams was added to convenient volumes of solution. Because the dextran caused the fluid volume to expand, the resulting concentrations were not described by a round number (e.g., 12 g of dextran added to 100 ml of solution resulted in a concentration of 111 g/liter). This fluid expansion accounts for the results being obtained with irregularly spaced dextran concentrations. The addition of 4, 6, 8, 10, 12, 14, and 16 g of dextran to 100 ml of activating solution led, respectively, to final concentrations of 39, 58, 76, 94, 111, 129, and 145 g/liter.

The T-70 size of dextran was used because (*a*) it does not contain an acid impurity, as does polyvinylpyrrolidone (Godt and Maughan, 1977); (*b*) it is sufficiently large that it does not appear to penetrate the filament lattice (Godt and Maughan [1977] found that dextrans of sizes T-40 and above did not penetrate the lattice); and (*c*) larger sizes of dextran (e.g., T-500) greatly increase solution viscosity and thereby impede solution changes.

Experimental Procedure

Fibers were activated initially by injecting full activating solution into the trough, and usually some degree of activation was maintained until all the data from that fiber was obtained, i.e.,

the fibers were not relaxed between periods of data collection. To avoid sarcomere dispersion the fibers were continuously subjected to a shortening ramp and rapid restretch every 2.5 s (Brenner, 1983; Sweeney et al., 1987). The speed of the ramp was adjusted to bring tension near zero in ~100 ms and the ramps lasted 300 ms. The steps to isotonic loads were imposed on the same schedule. Immediately before a set of isotonic steps was to be made, the computer sensed the onset of the shortening ramp. It then imposed the first isotonic step in a set 2.5 s later. Isotonic steps were imposed in groups of nine. The first five steps proceeded from the lowest to the highest load; the last four proceeded back to the lowest load. In separate control experiments the data obtained during the first five steps were compared with the data in the last five steps. There was no difference between the two sets, suggesting that there was no progressive deterioration of the preparation during the group of nine steps.

At least two sets of nine isotonic steps each were used to define the force–velocity properties under a given condition. After data for control conditions were obtained, the test activating solution was injected into the trough and at least two sets of nine isotonic steps were made. The control conditions were then reestablished by injecting the control activating solution into the trough and another two or more sets of nine isotonic steps were imposed. Usually, but not always, a second group of test and control measurements were made. The force–velocity curves measured under test conditions were always compared with those measured under control conditions that bracketed the test situation. All of the force–velocity data from one fiber were gathered together into two groups, test and control, that were each fitted with the hyperbolic Hill (1938) equation.

Isotonic Steps

Typical records for a set of nine isotonic steps are shown in Fig 1. The slow tension record in Fig. 1 *A* shows that tension recovered to near its full isometric level before each step. Nine periods of rapid force recording that are not present in the slow record are shown in Fig. 1 *B*. Isometric tension was recorded at a rapid rate for 20 ms before the step. During the next 150 ms the servo system was put into tension control and force was stepped to one of nine relative loads. During the final 30 ms the servo system was returned to length control and a large shortening step was imposed to make the muscle go slack. The zero force level of the tranducer was measured by averaging the last 20 ms of the recording. The order in which the nine force steps were made is shown by the order of the force records in Fig. 1 *B*. In Fig. 1 *C* the length responses for the nine steps are superimposed.

Data Selection

The data set below consists of 210 force–velocity curves derived from 10,329 separate data points. An additional 408 points were excluded from the analysis. In general, the force–velocity records were not scrutinized individually, but two criteria for excluding data points were used. The first was to exclude those points where isotonic force fell below 2.5% of the isometric level (P_o). We noted a great deal of scatter in data obtained at these low loads. A 1–2% P_o error in determining the isotonic force level caused a large error in the proper placement of a data point. A total of 403 points were excluded on the basis of this criterion. The second criterion was a sufficient deviation of a point to cause the fitted curve not to cross the positive force or velocity axis. If omission of no more than one deviant point from a data set would cause such an aberrant curve to cross the axes in the first quadrant, then the point was omitted. A total of five points were omitted on the basis of this criterion. No points were omitted for any other reason.

Statistical Analysis

All data points for a given condition in a single fiber were grouped together and fitted to the hyperbolic Hill (1938) equation using a nonlinear, least-squares (Newton-Raphson) technique. The values of maximum velocity and relative maximum power determined by the fitting procedure, as well as the isometric force measured before the step, were each treated as a single observation for statistical purposes. The values measured under test conditions were divided by the values measured under the control conditions in the same fiber, and the resulting ratios were used as a single observation in determining average values.

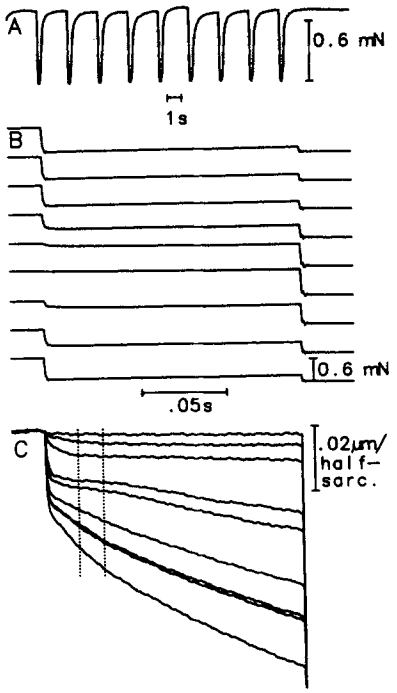

FIGURE 1. Typical force and length records during nine consecutive isotonic steps. (*A*) A slow force record showing the time course of force recovery after each step. The fiber was maximally activated throughout. Recording began immediately after a ramp release and restretch. A 200-ms period of rapid recording associated with each isotonic step is absent in these records and shown in *B*, where the recording speed was increased 125 times. These records show 20 ms of isometric force before the isotonic step is applied, followed by 150 ms of isotonic shortening, and then 30 ms of recording after a rapid step is applied to make the muscle go slack. The zero force baseline was recorded during the last 20 ms of this slack period. The step size progresses in the order shown from top to bottom. (*C*) Superimposed length records. The release to slack length is off scale in these length records. The vertical dotted lines indicate the periods of measurement of force and velocity (graphed in Fig. 2 *A*). Muscle length 1.5 mm, full activation, no dextran. J0489.081.

The relative maximum power is the value of maximum power divided by the isometric force. It has the dimensions of velocity and is used to indicate changes of velocity in the mid-range of isotonic forces (Podolin and Ford, 1986).

Control Observations

Force–velocity measurements. Velocity measurements were made by fitting a linear, least-squares regression to the length record over a brief interval. Isotonic force was averaged over the same interval. Timing of the interval was varied because the length records obtained with isotonic releases frequently showed prominent velocity transients (Fig. 1 *C*). These transients made it impossible to measure the force–velocity values at a fixed time after the release to an

isotonic load. A force–velocity curve obtained by measurements at such a fixed, short interval after the release is shown in Fig. 2 *A*. Velocities in the mid-ranges of force are very low, and frequently below those obtained at higher loads. Inspection of the length records shows the reason for this. At intermediate loads the measurements were made at a time in the transients when shortening had slowed to its minimum value. With the more rapid shortening at lower loads, no velocity transients were evident in the length record and the time of the measurements seemed appropriate. With higher loads the measurements were sometimes made during the early, high velocity phase of the transient.

Several methods of determining the appropriate time for making the force–velocity measurements were assessed. In developing a method, we believed that it was important to find a procedure that could be applied arbitrarily by the computer. There were two reasons for this belief: (*a*) it was more valid statistically, and (*b*) it was far more convenient. The results presented here are based on >10,000 separate force–velocity points. Assessing this many records individually would have been extremely time consuming.

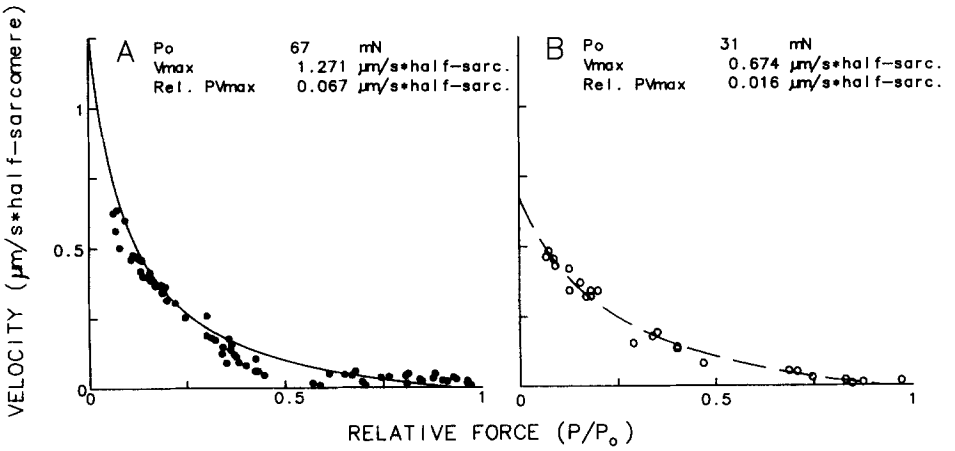

FIGURE 2. Typical force velocity curves. (*A*) Force and velocity measured from 15 to 30 ms after the onset of step. Note the very low velocities at intermediate loads (35–60% P_o). Data were taken from seven groups of nine isotonic steps, including the set shown in Fig. 1. J0489C.003. (*B*) Force–velocity data obtained at later periods, as described in the text. Data from three groups of nine isotonic steps, including the set shown in Fig. 3. O2589C.001.

Ford et al. (1985) have shown that quasi-isotonic velocity transients in intact frog fibers are limited to the time taken to shorten ~16 nm/half-sarcomere, ~1.6% of muscle length. This observation suggests that velocities should be measured at later times with slower shortening. To obtain a better estimate of the correct timing of the measurements, at the conclusion of the experiments we built a device for tracking the position of the first order diffraction pattern of a laser beam passed through the fiber. The incident angle of the laser beam was set to one-half the expected diffracted angle to maximize Bragg angle effect (Goldman and Simmons, 1984). Cylindrical lenses were used below the fiber to extend the beam along the fiber axis so as to include all sarcomeres and to narrow the beam onto the axis of the fiber. An additional cylindrical lens was used to narrow the diffracted first order beam onto a linear position sensing device (LSC/5D United Detector technology, Hawthorne, CA) placed 8 cm above the fiber. A resistor shaping network was used to convert the position of the first order to a linear function

of sarcomere length over the region of 2.27–2.5 μm (Goldman et al., 1984). The system was calibrated using the separately masked 10th and 11th diffraction orders (corresponding to spacings of 2.5 and 2.27 μm) of an eyepiece reticle having 40 divisions/mm.

This system does not give a reliable measure of absolute sarcomere length because the signal must be adjusted for light intensity, which undergoes unpredictable fluctuation. It can, however, give a good estimate of the relative changes in sarcomere length as illustrated in Fig. 3.

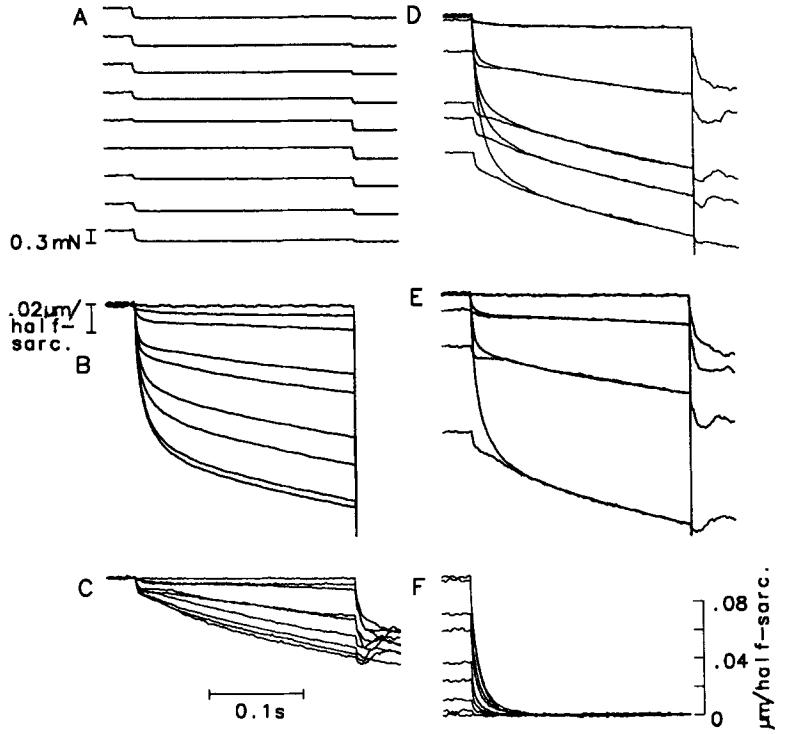

FIGURE 3. Set of nine isotonic steps as in Fig. 1, including records of sarcomere shortening determined by laser diffraction. (*A*) Force records showing time course of force steps. (*B*) Overall fiber length records. (*C*) The nine sarcomere length records superimposed. (*D* and *E*) Superimposed sarcomere length and overall length records. The records for the first five steps are shown in *D;* records for the last four steps are shown in *E*. The gain and offset of the sarcomere length records were adjusted so that they superimpose on the muscle length records at 50 and 142 ms after the onset of shortening. (*F*) The difference records of overall length minus sarcomere length. The difference declines to zero by 30 ms after the onset of shortening. Muscle length 1.5 mm, full activation, no dextran. O2589.014.

The records of sarcomere length (Fig. 3, *C–E*) have been scaled so that they match the overall length records at 50 and 142 ms of shortening. As shown by the superimposed traces (Fig. 3, *D* and *E*), the records match very closely over this period of steady isotonic shortening. The traces are very different at the onset of shortening and converge over the first 30 ms of shortening. This is better shown in Fig. 3 *F*, where the difference between the overall muscle

length and sarcomere length are plotted. As shown, the difference is reduced to zero by 30–32 ms.

The difference between the overall muscle shortening and sarcomere shortening is easily explained by damped recoil of the elastic elements in the crushed tissue at the ends of the preparation. The magnitude of this recoil, measured as the initial difference between the records, is plotted in Fig. 4. As shown, between the lowest and highest loads the series elastic elements are stretched by an amount equivalent to ~100 μm/half sarcomere, or ~8% of muscle length when sarcomere length is 2.4 μm. It should be emphasized that the relatively large series elastic element extension is due in part to the method of gripping the fibers, but mainly to the relatively short length of the segments (~1.5 mm).

The main observation to be made from these records is that velocity transients that are very prominent in the sarcomere length records are obscured by the damped series elastic recoil in the length record. This is especially true at low forces. Even at the lowest loads the transients can sometimes last for up to 20 ms. In addition, the damped series elastic recoil causes measurements of overall muscle velocity to overestimate sarcomere shortening up to 30 ms after the onset of the step. Because of these observations the force–velocity measurements were made at the following times, based on the relative force in the muscle: (*a*) for isotonic loads up

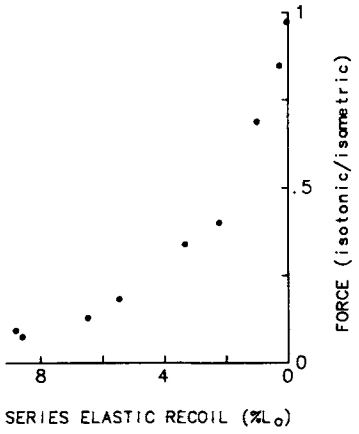

FIGURE 4. Series elastic force–extension data. Series elastic recoil was measured as the initial difference (before the isotonic step) between the sarcomere length and overall muscle length traces in Fig. 3 *F*.

to 25% of isometric force the velocity was measured over an 18-ms interval beginning 32 ms after the onset of the step; (*b*) for loads between 25 and 40% isometric, the values were obtained over a 20-ms period beginning 70 ms after the step; (*c*) for loads between 40 and 60% isometric, the values were obtained over a 20-ms period beginning 110 ms after the step; and (*d*) for loads >60% isometric, the values were obtained over a 33-ms period beginning 117 ms after the step (i.e., at the end of the record). The greater interval in the steps to higher loads helped to reduce noise in the measurements. Velocity was determined as the slope of a least-squares linear regression fitted to the overall length record over the specified interval. Isotonic force was averaged over the same interval.

It should be mentioned that before the sarcomere length sensor was developed, the same records were analyzed with several different protocols, and the relative relationships between the values obtained in different dextran concentrations and different levels of activation were always similar to those described below. The absolute values of maximum velocity and maximum power varied inversely with the time of the measurement, but the relative values were always similar.

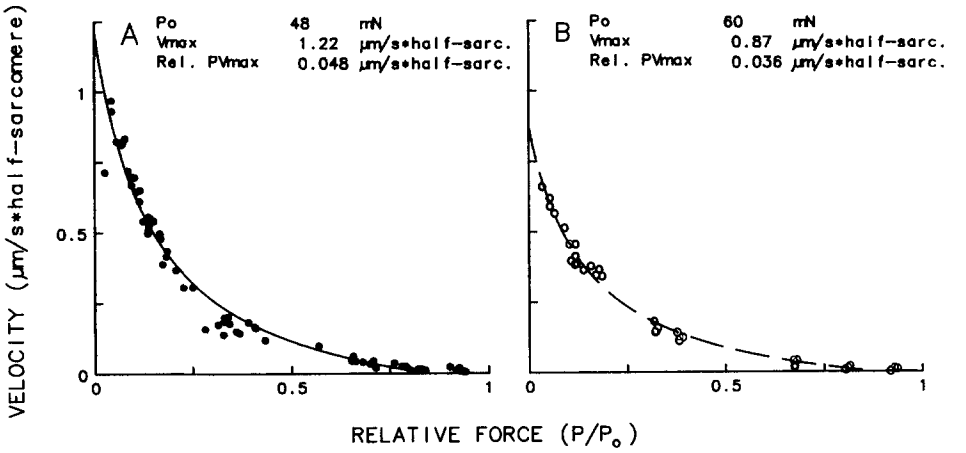

FIGURE 5. Force–velocity data with no dextran (*A*) and 94 g/l dextran (*B*). D2288.008.

RESULTS

The presence of dextran T-70 in the activating solution tended to diminish velocities at low loads without having much influence on velocity at intermediate and high loads (Fig. 5). To quantify the effects of osmotic compression, the force–velocity curves were fitted with the hyperbolic Hill equation to determine maximum velocity and relative maximum power. Changes in relative maximum power were used to assess the effects of dextran T-70 on intermediate velocities, while changes in maximum velocity were used to assess the effect on unloaded shortening velocity. In addition, the changes in isometric force were measured directly. The reference values of isometric force, maximum velocity, and maximum power obtained in the absence of dextran are listed in Table I.

In the presence of 39–111 g/liter dextran T-70, isometric force was increased by ~6–36%. At 145 g/liter the isometric force declined below control levels (Fig. 6 *A*). Relative maximum power remained constant to concentrations of 76 g/liter and then decreased gradually as dextran was increased (Fig. 6 *B*). Maximum velocity fell substantially (~35%) when the dextran concentration was raised to 58 g/liter. It remained nearly constant at concentrations >58 g/liter (Fig. 6 *C*). The results show that osmotic compression reduces the high velocities at low force levels with less depression of velocities at higher forces.

TABLE I

Average Reference Values at Full Activation without Dextran

Parameter	Value	Standard deviation	Dimensions
Isometric force	0.351	±0.121	mN
Maximum velocity	0.857	±0.401	μm/half sarcomere
Relative maximum power	0.044	±0.016	μm/half sarcomere
n = 59 fibers			

Partial Activation

To test whether the effects of dextran on velocity were due to an internal load, the force–velocity curves were measured at full and half activation and at different concentrations of dextran. To compare the curves, isotonic force was normalized to its isometric values immediately before the isotonic steps. As shown in Fig. 7, the relative force–velocity curves at full and partial activation superimpose almost exactly. These results are summarized in Fig. 8, which shows that at half-maximum activation neither the relative maximum power or the maximum velocity were

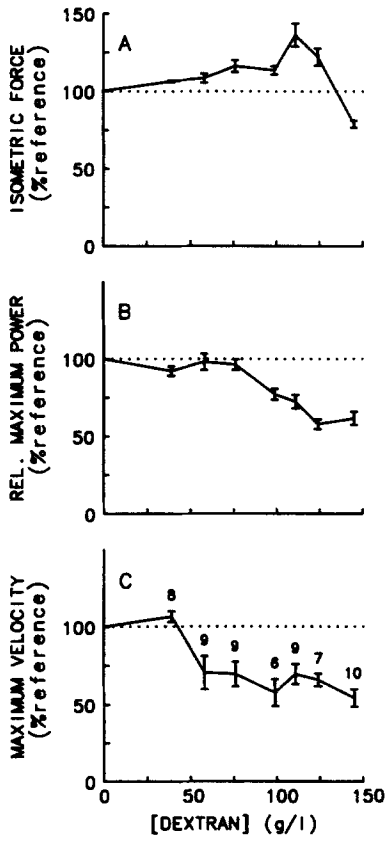

FIGURE 6. Effect of dextran concentration on isometric force (*A*), relative maximum power (*B*), and maximum velocity at full activation (*C*). Values are expressed as a fraction of the value in the absence of dextran. Error bars indicate SE. Numbers in *C* indicate the number of fibers studied at each dextran concentration.

significantly different from the values at full activation. Even at the highest concentration of dextran (145 g/liter) there was no significant decline of maximum velocity at partial activation. This finding argues strongly against dextran causing an internal load.

Further Observations of Isometric Force

As discussed below, the increase of isometric force measured in dextran here was substantially larger than that described in the past. In addition, the increase in force

extended to dextran concentrations that have been described as producing a force decrease. This latter observation raised the question of whether the dextran solution changes had been adequate. Earlier investigation of the solution change using dyes indicated that the technique was more than adequate when dextran was not present (Chiu et al., 1985). The additional observation that the usual solution change promptly activated and relaxed fibers in dextran solutions further suggests that the methods used were adequate. As a final test, we made additional measurements of isometric force in the manner described above, except that the fibers were relaxed after each group of nine steps, and the dextran concentration was changed using large volumes of rinse solution before the fibers were activated. The isometric force was averaged for two sets of nine steps each for bracketing control conditions without dextran and test conditions with dextran. The force measured in the dextran test solutions was divided by the average force in the dextran-free control solution. Six

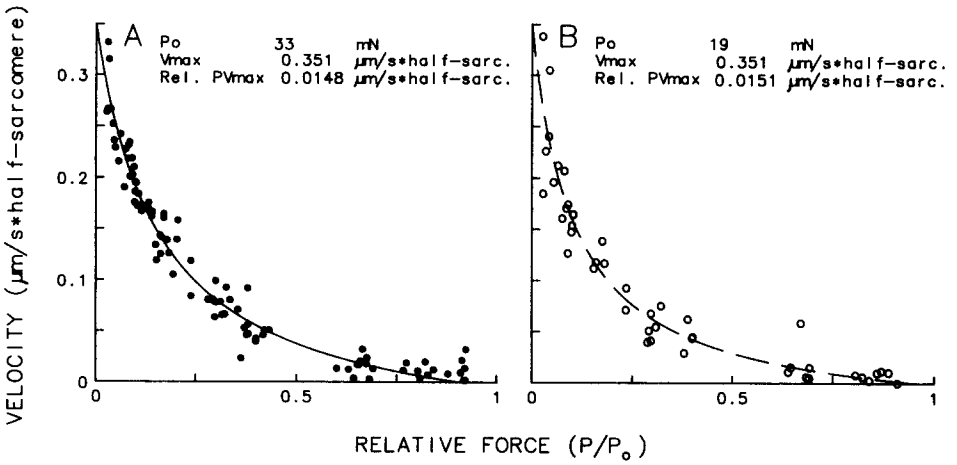

FIGURE 7. Force–velocity data at full (*A*) and 57% (*B*) activation in 58 g/liter dextran. A2888.102.

fibers were studied in each of two dextran concentrations. Isometric force was increased by 8 ±0.5% (SD) in the 76 g/liter dextran and by 2 ±0.3% in the 111 g/liter dextran.

While the increases in force were not as great as seen in the original experiments, they were nonetheless present at dextran concentrations where others have reported a decrease. In the course of making these observations we developed the impression that the isometric force levels either increased or did not decline as rapidly when the fibers were not relaxed between each set of nine steps in higher concentrations of dextran. This raised the question of whether the release and rapid restretch protocol produced some extra component of isometric force that would not contribute to force generation during shortening. Such an extra force component might derive from frictional drag or from the force of cross-bridges stretched to positions where they

detach very slowly. To test for this extra component of force, we examined the isometric force obtained by extrapolating the force–velocity curves to zero velocity. This extrapolated isometric force obtained in the three highest concentrations of dextran were compared with the values obtained in the same fibers contracting in the absence of dextran. They were 100, 94, and 99% of control in 111, 129, and 145 g/liter dextran, respectively. The lack of a difference in extrapolated isometric force suggests that rapid restretch of a fiber shortening in high concentrations of dextran

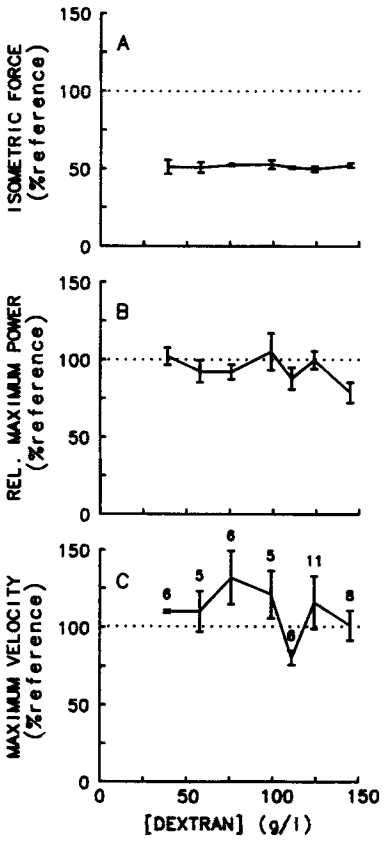

FIGURE 8. Effect of dextran concentration on isometric force (*A*), relative maximum power (*B*), and maximum velocity at half-maximum activation (*C*). Values are expressed as a fraction of the value at full activation in the same concentration of dextran. Numbers in *C* indicate number of fibers studied at each dextran concentration.

does not produce an extra component of force above that which contributes to the force–velocity properties of the fiber.

Fiber Shrinkage in Dextran

To obtain some estimate of the osmotic effect of the dextran, the influence of different concentrations of the polymer on the diameter of a separate group of 14 unstimulated fibers was measured. There was a progressive decline in fiber diameter as the concentration was increased (Fig. 9).

DISCUSSION

The results described here for fully activated fibers are similar to those of Goldman (1987). The absence of a decline in maximum velocity and maximum power at partial activation in the presence of dextran strongly suggests that the decline in maximum velocity caused by dextran is not due to a passive internal load. By recalculating Huxley's (1957) equations for force–velocity curves with and without buckling, Goldman (1987) has shown that osmotic compression will have its greatest effect on high velocity shortening. Since a passive internal load such as a viscosity will have similar effects on the force–velocity curves, it is important to exclude such effects.

Effects of Activation on Maximum Velocity

An ancillary conclusion to be drawn from these experiments is that the maximum velocity is not altered by changes in activation when the interfilament distance is in the physiological range. The lack of effect of activation on maximum velocity has been shown for nonshrunken skinned fibers (Podolsky and Teichholz, 1970; Thames

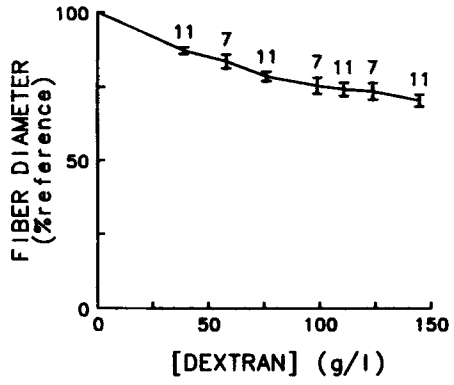

FIGURE 9. Resting fiber diameter relative to control without dextran vs. dextran concentration. The reference was the average of two diameter measurements made without dextran before and after each measurement with dextran. Mean reference diameter of 14 fibers was 66.3 (±13.7 SD) μm.

et al., 1974; Podolin and Ford, 1986) and the present study shows that this result is not peculiar to the swollen state. It should also be emphasized, however, that this finding applies to a relatively limited range of conditions with activation levels down to ~40–50% of maximum, and for velocities measured after a very small amount of shortening. There is a growing consensus that maximum velocity declines when it is measured at levels <30–40% of maximum, particularly after a substantial amount of shortening has occurred (Moss, 1986; Farrow et al., 1988).

Relationship to the Physiological State

One of the major reasons for undertaking these experiments was to attempt to determine the correct amount of compression for studying skinned fibers. If the force–velocity relations and the stiffness change substantially when the fibers swell, the results obtained with swollen fibers may not adequately reflect the normal physiological condition. It might be expected that there would be a range of interfilament spacings over which physiological results could be obtained, since the

distance between filaments changes with variations of sarcomere length such that a constant lattice volume is maintained (Huxley, 1953; Elliott et al., 1963). The observations that maximum velocity maintained a nearly constant value in dextran concentrations >58 g/liter and that maximum velocity in intact fibers is nearly independent of sarcomere length (Gordon et al., 1966) suggests that a physiological range of filament spacing might have been achieved above concentrations of 58 g/liter. The further observation that relative maximum power remained constant up to 76 g/liter suggests that the physiological range of lattice spacing might have been achieved over the range of 58–76 g/liter dextran.

Relationship to Earlier Work

Amount of fiber shrinkage. The earliest descriptions of fiber swelling and compression by dextran suggest that fiber diameter might increase by 47% when the fibers are skinned and that it required >90 g/liter dextran T-70 to shrink them to the physiological size (Godt and Maughan, 1977). Subsequent studies by the same authors (Maughan and Godt, 1981) reduced these values to ~10% diameter swelling and 20–40 g/liter dextran to reverse it. These lower numbers are in agreement with the x-ray diffraction findings of Matsubara and Elliott (1972) and Matsubara et al. (1985) that the filament lattice swells ~8–13% when fibers are skinned, and that ~30 g/liter dextran is required to return the lattice to its physiological spacing at rest length. This is somewhat less reduction in diameter and less osmotic strength than was required here (16% diameter reduction, 76 g/liter dextran) to bring maximum velocity to a plateau. This observation raises the question of whether the plateau of maximum velocity correlates with the physiological state as we have concluded above. We have no independent measurements of lattice spacing in this study, but there are significant differences that could reconcile the present study with the lesser degree of swelling in previous work. The earlier work was done on freshly skinned fibers, which may have retained more restrictions to swelling, and therefore required a lower polymer concentration to restore the physiological size. In addition, there may be differences in ionic strength that could alter the osmotic strength required to restore physiological spacing.

Isometric force. The earliest studies of the effect of osmotic compression on skinned fibers reported that it decreased isometric force (Godt and Maughan, 1977). More recent studies report a decrease in force at high degrees of compression, but they have also reported a small (<10%) but definite increase of force at intermediate degrees of compression (Godt and Maughan, 1981; Krasner and Maughan, 1984; Metzger and Moss, 1987). The increase of force seen here at intermediate degrees of shrinkage was substantially larger than that reported earlier. A major difference between these other studies and this work is the lower temperature used here. This lower temperature was chosen because the striation pattern was much better preserved. It also caused a reduction in isometric force. It is possible that compression of the filament lattice partially overcomes some of the force reduction caused by low temperature. Another possible explanation of the greater force increase is that we used more closely spaced polymer concentrations and consequently may have been closer to the optimum lattice spacing for force development.

It should be emphasized that an increase of isometric force might be expected from the simplest interpretation of the effects of activation and rigor on skinned muscle fibers. The diameters of swollen skinned fibers diminish when the fibers are activated (Matsubara et al., 1985) or put into rigor (Maughan and Godt, 1981; Matsubara et al., 1984), but do not change or expand slightly if the fibers are osmotically compressed. This finding suggests that some of the cross-bridge force might be directed radially to partially overcome the osmotic force in swollen fibers. To the extent that the total force generated by the cross-bridge is not affected by lattice spacing, the radial direction of cross-bridge force will diminish the axial force. Since this radial force must partially overcome substantial osmotic force (Maughan and Godt, 1981; Matsubara et al., 1984), the fraction of cross-bridge effort must also be substantial. Thus, fiber compression that reduces the radial component of force is expected to increase the axial component measured with a force transducer.

Shortening velocity. Decreases in maximum velocity with fiber compression have been reported both by Metzger and Moss (1987, 1988) and by Goldman (1987). The main reason for extending this work was to determine if any of the decline in velocity caused by polymers could be attributed to an increased viscosity (apparently it cannot) and to determine if there is some range of fiber compression where velocity is not strongly dependent on the polymer concentration. The results suggest that concentrations of dextran T-70 in the range of 60–90 g/liter fulfill this requirement.

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