# CRITICAL DEPENDENCE OF CALCIUM-ACTIVATED FORCE ON WIDTH IN HIGHLY COMPRESSED SKINNED FIBERS OF THE FROG

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ABSTRACT Force development by skinned frog semitendinosus fibers was studied at various levels of lateral compression to compare the results with intact fibers and to evaluate the limits on cross-bridge movements during isometric contraction. The skinned fibers were compressed osmotically using a high molecular weight polymer, dextran T500. Ca-activated force remained constant down to 58% of the fiber width ($w_0$) after skinning, corresponding to a nearly twofold change in separation between the thin and thick filaments in the myofilament lattice. This agrees with the earlier result on intact fibers, and gives additional evidence that the cross-bridge mechanism for force generation is relatively insensitive to large changes in interfilament separation. Further compression, below 0.58 $w_0$, produced a sharp drop in force, and the force was practically zero at a fiber width of 50%. The effect at high compression was the same at all pCa's, which indicates that the Ca sensitivity of the myofilaments is unaffected by radial compression. The stiffness of the fiber remained high in rigor in the presence of dextran, which indicates that the rigor cross-bridge attachment is not inhibited, and actually may be improved, with decreases in the interfilament space. Also, the drop in active force with the highest compression was similar when the compressed fibers were put in rigor before contraction, which suggests that the force drop also was not due to a hindrance to cross-bridge attachment. The results appear to exclude large motions such as tilting and rocking of the bridge as a rigid molecule, but suggest that at least some molecular movement is essential for force development; they also raise the possibility that there is a critical interfilament separation in the fiber, below which the cross-bridge cannot function.

## INTRODUCTION

There is now general agreement that the overall mechanism of muscle contraction involves cyclic interactions of the cross-bridges between the thick myosin filaments and the thin actin filaments, with cross-bridges acting as the chemomechanical generators (H. E. Huxley, 1969; A. F. Huxley, 1974; Kushmerick, 1983; Eisenberg and Hill, 1985). A current question toward the understanding of the contraction mechanism concerns the nature of molecular movements within the cross-bridge during force generation (H. E. Huxley et al., 1983; Matsuda and Podolsky, 1984; Cooke et al., 1984). A useful early insight into this question was obtained by studying the mechanical properties of compressed intact fibers of the frog (Gulati and Babu, 1982), where the osmotic pressure of the bathing solution was varied with permeant and impermeant solutes (KCl and sucrose). The results indicated that force per cross-bridge at 0°C was constant despite radial compression of the interfilament separation in the fiber to the level close to the cross-bridge dimension, which suggested that no major cross-bridge movements were necessary when making force. This idea received immediate support from the studies using electron paramagnetic resonance with skinned fiber bundles modified with external probes (Cooke et al., 1982).

However, the results on intact fibers were in contrast to repeated studies on frog skinned fibers at 22°C (Godt and Maughan, 1977; Maughan and Godt, 1981). The skinned fiber could also be reversibly compressed osmotically by using the high molecular weight polymers dextran and polyvinyl-pyrrolidone (PVP), but studies showed that Ca-activated force in this case decreased steeply with compression. An additional feature was found in a more recent study on a mammalian (rabbit soleus) muscle (Krasner and Maughan, 1984), where the force of skinned fibers first increased a little with moderate amounts of compression with dextran, although once the fiber diameter reached its presumed value in vivo (as judged from the relation between intact and skinned fiber diameters of the frog muscle), additional dextran again caused a progressive decrease in force. Thus, it would appear on the basis of skinned fiber studies that the force-generating mechanism is highly sensitive to interfilament space and this is consistent neither with the results on compression of the frog intact fiber (Gulati and Babu, 1982) nor with the

standard interpretation of the length-tension relationship (Gordon et al., 1966). The present effort was undertaken to re-examine skinned fiber behavior under conditions similar to those used in the frog intact fiber study.

We found that the force response of the frog skinned fibers is independent of radial compression over a nearly twofold range of widths, which agrees with the results on intact fibers. Only in very highly compressed skinned fibers, at widths far below the in vivo value at the resting sarcomere length, the force dropped and we found that the drop was very sharp. These results suggest that there is a critical distance between the myofilaments, below which a cross-bridge is unable to function. The possibility of the myofilament lattice collapsing in highly compressed fibers is pointed out as well. A brief report of these findings has been given earlier (Gulati and Babu, 1984*a*).

## METHODS

Most of the studies employed mechanically skinned fiber preparations from semitendinosus muscles of *Rana pipiens pipiens,* made as described earlier (Gulati and Podolsky, 1981; Gulati, 1983). Fibers were selected from the regions opposite to the point of nerve entry to use the fast-twitch fibers. The relaxing solution was made with KCl, 10–20 mM imidazole, 20 mM creatine phosphate (CP), 6.06 mM $MgCl_2$, 5 mM ATP, and 5 mM EGTA. For the activating solutions, part of the EGTA was replaced by Ca-EGTA to obtain the desired pCa. The pCa of the standard activating solution was 4 unless indicated otherwise. Creatine phosphokinase (Sigma Chemical Co., St. Louis, MO) was added to each solution at a level of 250 U/ml (usually ~2 mg/ml), just before the experiment. The rigor solution contained 167 mM KCl, 20 mM imidazole, 2.5 mM EGTA, and 2.5 mM EDTA. The pH of each solution was adjusted to 7.00 ± 0.01 at room temperature. The ionic strength of each solution was kept between 180 and 200 by varying the KCl. Dextran T500 (461,000 mol wt; Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) was added as indicated.

For practical reasons, solutions with dextran were consistently made by adding the desired amount of dextran to the prepared solutions. The volume change of the mixture was significant and was evaluated by measuring the densities of the solutions (Table I). The apparent molal volume ($\bar{v}_2$) for dextran was found to be 0.41 ± 0.04 × $10^6$ ml/mol; this could be used to compute the dilution factor for each solution (Table I) by the equation: dilution factor = $\bar{v}_2 \cdot n_2 + 1$, where $n_2$ = moles of dextran per milliliter.

## Fiber Attachment and Optical Measurements

All skinned fibers were initially checked visually, while in oil, for the overall quantitative sarcomere uniformity. This was done at a magnification of 270 with a compound microscope (model ACM, Carl Zeiss, Inc., New York) before attachment of the ends: one end of the fiber to the force transducer (model AE 801, Aksjeselskapet Mikro-Elektronikk) and the other to a servo-motor. The sarcomere length of the attached fiber was adjusted to ~2.4 μm and monitored throughout the experiment with laser diffraction using a He-Ne laser (4 mW, model 140, Spectra-Physics Inc., Mountain View, CA).

The fiber width was measured at five approximately equidistant points along the length with a stereomicroscope (M3, Wild Heerbrugg Instruments Inc., Farmingdale, NY) at × 80 enlargement, viewing from above. Similar measurements were made in various dextran solutions after a 5–30-min equilibrations.

For the analysis and discussion of the data, the relative changes in fiber widths (in the horizontal plane), by the inclusion of various amounts of dextran, were converted to the related changes in myofilament lattice dimensions. This conversion is appropriate if the measured changes in widths during the experiments truly reflect the changes in fiber cross-sectional area at fixed lengths (i.e., the unit volume). To check this, an additional set of width measurements was made on a number (four) of fibers in the orthogonal plane by using a slanted mirror. The major and minor axes for fibers in the present study differed by no more than 25%, but the means of the two sets of values collected on all the fibers were similar. The relative changes in widths in the horizontal plane were plotted against the fiber volume found by using both sets of values. The data points are shown in Fig. 1. The curve in Fig. 1 is the expected change in volume, estimated assuming the fiber to be cylindrical, with a diameter equal to its width in the horizontal plane. A good correlation was found between the measured and the expected values, which indicates that the compression of the fiber is uniform in the entire cross-section and that relative changes in fiber widths measured in the horizontal plane in the present study may adequately represent the relative changes in fiber volume.

TABLE I
CORRECTION FACTORS FOR FORCE

| Added dextran T500 | Measured density, 20°C | Dilution factor* | Apparent molal volume for dextran, $\bar{v}_2$ | Estimated ionic strength‡ | Correction factor for force at 0–2°C§ |
|---|---|---|---|---|---|
| *g/ml* | *g/ml* | | *ml/mol* | *mM* | |
| 0 | — | — | — | 190 | 1.00 |
| 0.03 | 0.9994 | 1.031 | 0.48 × $10^6$ | 184 | 1.10 |
| 0.07 | 1.0067 | 1.063 | 0.41 × $10^6$ | 179 | 1.15 |
| 0.13 | 1.0283 | 1.099 | 0.41 × $10^6$ | 173 | 1.21 |
| 0.26 | 1.0469 | 1.236 | 0.36 × $10^6$ | 154 | 1.35 |
| | | | 0.41 ± 0.04 × $10^6$ | | |

*The dilution factor is estimated as total mass per density. Thus, for 0.03 g/ml dextran, the dilution factor = 1.03/0.9994. For solutions where density was not measured, this factor was found from: dilution factor = $\bar{v}_2 n_2 + 1$, where $n_2$ = moles of dextran per milliliter, and $\bar{v}_2$ = 0.41 × $10^6$, the mean value for apparent molal volume.

‡The ionic strength of the standard solution was 190 mM. For other solutions, the ionic strength was (190/dilution factor).

§The correction factor is the ratio of force at the estimated ionic strength to that in 190 mM. This ratio is found from the linear force-KCl relationship on frog fibers in Fig. 3 of Gulati and Podolsky (1981).

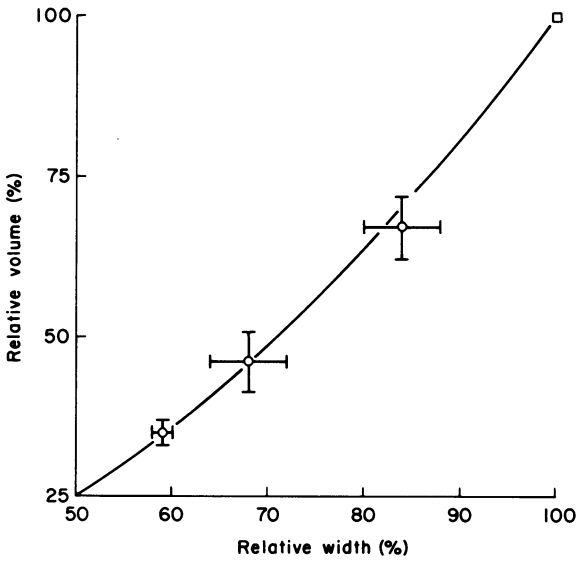


FIGURE 1 The relation between the changes in fiber width and fiber volume (at a fixed sarcomere length of 2.4 $\mu$m) on compression with dextran T500. Measurements of widths on the abscissa were made in the horizontal plane, with the 100% point being the skinned fiber width in relaxing solution without dextran. For the ordinate, an additional measurement was made in a plane perpendicular to the first by using a 45° mirror, and assuming the fiber to be of elliptic cross-section. The data points are means ± SEM of measurements on four fibers. The solid line is found theoretically by assuming a circular fiber. The results show that for relative changes brought about by compression, fiber width was an adequate measure of fiber volume in this study.

## Fiber Activation

The first activation on each fiber was made in pCa 4 solution with no dextran. When the fiber was activated in dextran solutions and with other levels of pCa, the reversibility of force was checked in the above solution as an indicator of fiber stability, and the experiment was discarded if the force was not reversible to within 10%.

The solutions were contained in thermoelectrically cooled chambers (Gulati and Podolsky, 1978; Gulati and Babu, 1984*b*) and the experiments were carried out at 0–2°C. There was one exception at 20°C.

## Data Analysis

In the experiments described here, we measured the changes induced in fiber widths on incubation with varying amounts of dextran. To relate these changes to the accompanying alterations in the lattice constant $d_{10}$, the relationship in the inset to Fig. 7 was used for skinned fibers. The two points for drawing the line are taken from the results of Matsubara et al. (1984, their Fig. 4), using the data for relaxed fibers at 2.4 $\mu$m sarcomere length in 0 liter and 75 g/liter PVP. (The assumption is made that the shrinkage in various high molecular weight solutes occurs similarly.) The arrows in the inset to Fig. 7 mark the positions for the relaxed, noncompressed intact fiber. The position of the arrow on the abscissa was found from the mean of two sets of data on intact and skinned fibers. In one, 11 intact fibers (7 tibialis and 4 lumbricalis; *R. temporaria* frogs; range of fiber widths: 88–183 $\mu$m) were chemically skinned (with glycerol, as described by Julian and Moss [1981]) and the diameters were found to be expanded to 1.32 ± 0.02. (The isolation and treatment of intact fibers was the same as described earlier [Gulati and Babu, 1984*b*]). The second set of data was found by comparing the diameters of 37 mechanically skinned fiber segments in oil and relaxing solution; the expansion in solution was 1.44 ± 0.03 (width range in oil: 43–110 $\mu$m).

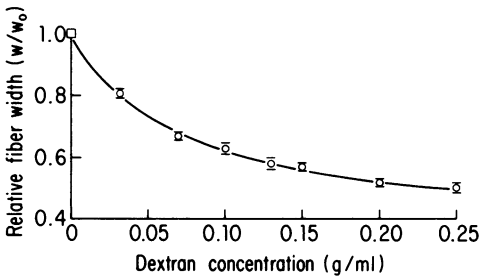


FIGURE 2 Effect of dextran concentration on fiber width. The fiber width ($w$) in each dextran concentration is normalized to the width ($w_0$) in dextran-free relaxing solution. The data points are the means ± SEM measurements on 16 fibers.

The inverse of the mean of all values (1.41) is 0.71. To ascertain the position for the arrow on the ordinate of the inset to Fig. 7, the lattice spacing for intact fibers was taken from the data of Elliott et al. (1963) on whole muscles.

The center-to-center distance between the actin and myosin filaments in the lattice is two-thirds the lattice constant, $d_{10}$. To estimate the free space between the two sets of filaments, we took 14 nm as thickness for the myosin filament and 7 nm for the actin filament (Squire, 1975).

## RESULTS

The addition of dextran T500 in the bathing medium decreased the skinned fiber width, as shown in Fig. 2. Fig. 3 shows the biphasic force response of these fibers with varied dextran. The force in each case is normalized to the

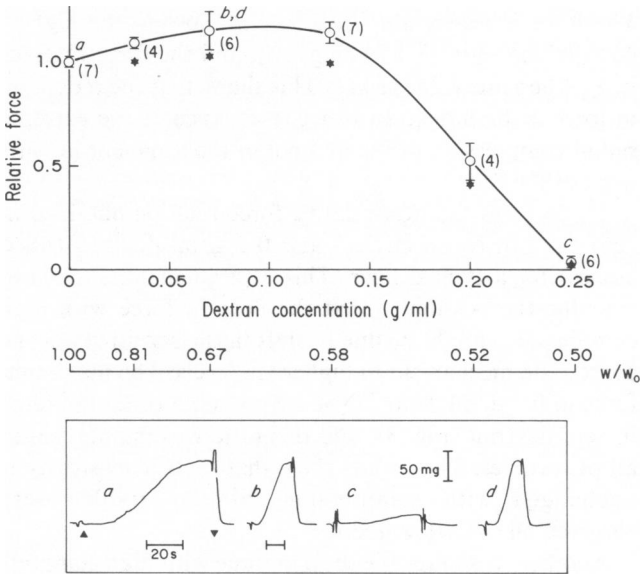


FIGURE 3 Effect of dextran concentration on skinned fiber force development. Force of the skinned fiber in each activating solution (pCa 4) is normalized to that in dextran-free solution (open circles). Stars indicate the corrected values according to Table I. The numerals in parentheses indicate the number of fibers. The scale below the abscissa indicates the relative width wherever force measurement was made. Note the rise in measured force at moderate values of dextran. *Inset:* reversibility of force after the highest compression (pCa 5). *a*, *b*, *c*, and *d* correspond to the dextran values indicated above. Note that the time scale is different for the first and the remaining three traces.

force of the fiber in zero dextran. The addition of dextran up to 0.13 g/ml is associated with an increase in the force, and the fiber width reached a value ~60% of its initial value, $w_0$. Increases in dextran above 0.13 g/ml produced only a small additional change in width but caused progressive drops in force. The force was nearly zero (0.03 ± 0.01) in 0.26 g/ml dextran. The fiber width in this solution was 0.50 $w_0$.

Correction was needed in the force data for the increase in solution volume because of the large "apparent molal volume" of dextran in all solutions (Table I). The dilution factor owing to the dextran "volume" was used to calculate the actual ionic strength, and the correction factors for force were obtained from the linear ionic strength-force relation of Gulati and Podolsky (1981, their Fig. 3) on frog fibers. These correction factors are listed in Table I for each of the dextran solutions and the mean relative force values are indicated by stars in Fig. 3. After the correction, the force development by skinned fibers was found to be nearly constant up to 0.13 g/ml dextran, while the fiber was radially compressed down to 0.58 $w_0$.

## Highly Compressed Fibers

The compression of skinned fibers below 0.58 $w_0$ was associated with a decrease in active force in pCa 4, and the force was fully reversible, as shown in the lower panel of Fig. 3. Traces *a–d* were made in sequence and correspond to the dextran concentrations shown in the main graph. The force is nearly abolished in *c* at close to the highest dextran concentration (0.25 g/ml), and the force reverses in *d* to the same value as in *b*. This shows that the reduction in force in high dextran is a consequence of the extreme radial compression per se and not of a permanent impairment of the fiber.[1]

Fig. 4 gives the pCa-relative force relation obtained in zero dextran (open circles) and 0.2 g/ml dextran (filled circles) for a typical fiber. This experiment was made to examine the possibility that the drop in force with high compressions might be due to shift in the sensitivity of the contractile mechanism to higher Ca levels. The maximum force in 0.2 g/ml dextran (pCa 3) was 0.5 times the force in zero dextran (Fig. 3), and this ratio was maintained at all pCa values. The results show that the Ca sensitivity is unchanged with compression. Similar results were observed on two other fibers.

Another reason for the drop in force with high compressions might be the inability of the detached cross-bridge in the relaxed fiber to reach the attachment site because of steric hindrance. To check this possibility, a set of experiments was performed in which the fiber was placed in rigor before contraction, so that the majority of the bridges were already attached (see below). Typical results of this study are shown in Fig. 5. In force trace *a*, the fiber in rigor (relative width = 0.91 $w_0$) was compressed with 0.24 g/ml dextran (width = 0.53 $w_0$; "rigor 1," see below) and then transferred to activating solution (pCa 3) containing the same amount of dextran. This force response was compared with the response in trace *b*, where the fiber was compressed in the relaxing solution (0.52 $w_0$) and then activated. The force was identical in the two modes, which shows that the force response to Ca activation is independent of whether the cross-bridges are attached or detached at the time of activation. This argues against a hindrance to the cross-bridge for reaching the attachment site as a likely explanation for the drop in force in the compressed fiber.

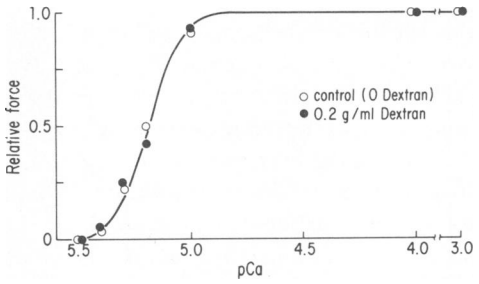


FIGURE 4 pCa-relative force relationship with and without dextran. 0.2 g/ml dextran was selected because the fibers produce about half-maximal force. Force at each pCa is normalized to the force in pCa 3 in the same dextran. Data points are from one fiber and the line is a fit with Hill's equation: relative force = $[Ca]^n/(K^n + [Ca]^n)$, with $n$ = 5.8. A similar lack of effect of this dextran on the pCa-force relationship was seen on two other fibers.

## Fiber Stiffness in Rigor

The above conclusion rested on the assumption that the rigor bridges remained attached with fiber compression. To check this, the stiffness of the rigor fiber was measured

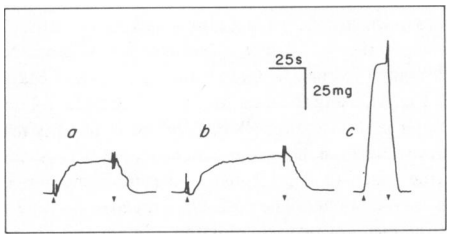


FIGURE 5 Comparison of force development on compressions in rigor and relaxing solutions. In *a*, the fiber was first put in rigor and then compressed, still in the rigor solution with 0.24 g/ml dextran. The rigor tension was relieved by shortening the fiber by ~1% of its length. The arrowheads indicate when the fiber was transferred to pCa 3 solution with the same dextran. In *b*, the fiber was compressed in the relaxing solution and then activated. Force levels are identical in both cases. *c* is the control activation in the absence of dextran.

[1]The intact fibers survived less well or not at all upon compression with sucrose above 200 mM (i.e., above 1.8 times the normal osmolarity of 249 mosmol) compared with frog skinned fibers compressed with dextran to the same level or below. Therefore, contraction experiments at such high compressions were not carried out with intact fibers in a systematic manner.

by studying the force response to stretching (Table II). The compressed rigor state was achieved in two ways: in one ("rigor 1"), the fiber was put in rigor in dextran-free solution and then transferred to dextran (0.25 g/ml); in the second ("rigor 2"), the fiber was compressed while relaxed and then transferred to the rigor solution containing the same dextran. The uncompressed rigor stiffness was found to be 0.12 $P_0$/nm · half-sarcomere, and the value nearly doubled (0.2 $P_0$/nm) with high compression. The results show that the rigor bridges were attached in the compressed fiber, which supports the conclusion that radial compression does not inhibit the attachment step in the cross-bridge cycle. Incidently, the increase in rigor stiffness with radial compression implies that the stiffness per cross-bridge may rise with decreasing interfilamentary space (Goldman and Simmons, 1978) or that the number of attachments is increased but this point was not followed up further in the present study.

## Activation at 20°C

As mentioned earlier (see Introduction), there was no flat region in the force-width relationship in the studies at 22°C (Maughan and Godt, 1981). Since the flat region in the present study (near 0°C) has a wide span and has significance for the cross-bridge mechanism, it seemed worthwhile to make a check at the higher temperature.

Fig. 6 gives the typical results. A set of traces was made in zero dextran (first trace) and 0.13 g/ml dextran (second trace) at 20°C. This amount of dextran was selected because we expected the force to be constant (from the 0°C data), whereas if high temperature were responsible for the drop in force in the previous studies, force at 20°C would be close to zero. In Fig. 7, we find that force in the dextran solution is 1.1 times control (fiber width: 0.6 $w_0$), within the range in Fig. 4 at 0°C. In the course of these experiments, we observed that frog fibers were much less stable (i.e., had incomplete reversibility) after contraction at 20°C in high

TABLE II
FIBER STIFFNESS IN RIGOR IN THE NORMAL AND HIGH COMPRESSED STATES*

| Rigor‡ | Compressed rigor 1§ | Compressed rigor 2‖ |
|---|---|---|
| 0.12 ± 0.01 | 0.21 ± 0.02 | 0.20 ± 0.02 |

*Stiffness was measured from the change in force to stretching with a ramp (0.5% $L_0$ in 500–600 μs). Stiffness values are given as $P_0$ per nanometer per half-sarcomere. The values are means ± SEM of four measurements.

‡Rigor state was achieved by direct transfer of the fiber from the relaxing solution to the rigor solution. Stiffness 0.12 $P_0$/nm half-sarcomere is similar to the value in Goldman and Simmons (1977).

§For rigor 1, the fiber in rigor state was transferred to a rigor solution containing 0.25 g/ml dextran.

‖For rigor 2, the fiber was first compressed in the relaxing solution with 0.25 g/ml dextran and then transferred to the rigor solution with the same amount of dextran.

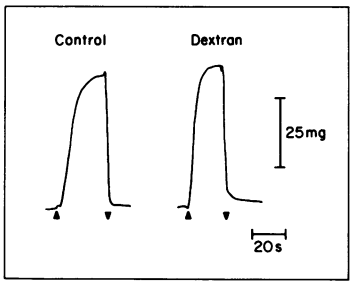


FIGURE 6 Force development of the frog fiber with 0.13 g/ml dextran at 20°C. The upward arrowhead indicates when the fiber was plunged into pCa 4 solution and the downward arrowhead shows when it was returned to the relaxing solution. The fiber was compressed in dextran to ~60% of its width in control. Note that the measured force in dextran solution is slightly greater than control, which is similar to the result at 1°C in Fig. 3.

dextran than at 0°C. However, we were able to record results like those in Fig. 6 on two other fibers, both showing full reversibility after activation in dextran. Thus, high temperature as such cannot account for the lack of a flat region in the force-width relationship in the previous study. The ionic strength was slightly lower in the study of Godt and Maughan (1981; 150 vs. 180–190 mM in this study), but this protocol was not explored further since there are often permanent changes in frog fiber properties with repeated activation at low ionic strengths (Thames et al., 1974; Julian and Moss, 1981; Gulati and Podolsky, 1981).

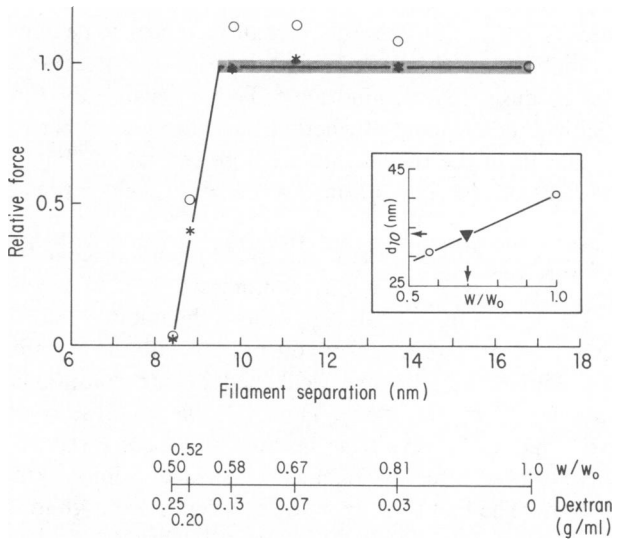


FIGURE 7 Relation between the estimated filament separation and force. The open circles and the stars show the same data as in Fig. 3. The stippled strip indicates the range of separations for intact fibers where the force was constant also (intact fiber data used from Gulati and Babu, 1982). *Inset:* the relation used for converting changes in skinned fiber width to lattice dimensions. The two points (circles) are taken from Matsubara et al. (1984), and a linear relation is assumed for the other widths. Arrows indicate the respective values for the resting intact frog fiber at 2.4 μm sarcomere length as explained in the text (p. 7). The solid triangle is the back intersection point for the arrows.

## DISCUSSION

The principal finding of this study is that osmotically induced radial compression of the frog skinned fiber, from its originally expanded state ($w_0$) down to 58% of the width (0.58 $w_0$), has practically no effect on isometric force development, which supports the results on intact fiber (Gulati and Babu, 1982). Further compression of the skinned fiber by <10% completely abolished the Ca-activated force. To interpret these findings at the cross-bridge level, it is useful to relate the observed width changes to the accompanying changes in the lattice dimensions. Using the relation in the inset to Fig. 7, the dextran (or width)-force relationship in Fig. 3 was converted to that in Fig. 7 relating force to filament separation (i.e., distance from the surface of a thick filament to the nearest thin filament). The flat portion of the relationship in Fig. 7 overlaps with the results on intact fibers described previously (Gulati and Babu, 1982). These findings reinforce the conclusion that force per cross-bridge is constant over a nearly twofold change in filament separation. The abrupt drop in the force-generating capability of the frog skinned fiber around the estimated 8–9-nm separation range[2] suggests that while a relatively small free space between the filaments is sufficient for cross-bridges to make full force under isometric conditions, the cross-bridge mechanism is sharply constrained at a critical value of the interfilament space.

The drop in force in the highly compressed fibers is not the effect of altered Ca sensitivity, as the relative pCa-force relations were the same in compressed and normal fibers (Fig. 4). Nor does the force drop appear to be due to the inability of the cross-bridge to reach the attachment sites because of steric hindrance. This is because the force level was independent of whether the compressed fiber was in rigor or in the relaxed state before activation (Fig. 5), and because the rigor stiffness was high in the compressed state.

The possibility may arise that the filament lattice in frog fibers collapses in the highly compressed fiber to account for the drop in force. This is in view of the findings that the equatorial intensities in x-ray diffraction with the mammalian skinned bundles are weakened during compression (Matsubara et al., 1985). However, the collapse of the lattice has to be reversible because the force response is fully restored after the fiber is returned to a low-dextran medium. The fact that rigor stiffness remained high in the compressed state (Table II) is also not consistent with the explanation that the lattice has collapsed, but the possibility still deserves a more direct investigation in the future.

Another possible explanation for the inhibition of force following the flat region in the force-width relationship is that there are severe constraints on the cross-bridge movements, after the bridges are attached, such that the force-generating stroke in the cross-bridge cycle is blocked in highly compressed fibers.

Combining the structural information with the force-width relationship as in Fig. 7 gives additional interesting insights into the cross-bridge mechanism. The current estimates for the cross-bridge (S-1) dimensions range from 16 to 20 nm for lengths and ~6 nm for thickness (Elliott and Offer, 1978; Winkelmann et al., 1985; see also Table III in Wakabayashi and Toyoshima, 1981). In this light, the constancy of force in skinned fibers down to an estimated 8–9-nm filament separation range supports the earlier view that the cross-bridge does not perform large movements during the force step (Gulati and Babu, 1982; Fajer et al., 1985) and challenges the concept (e.g., H. E. Huxley, 1969) that the cross-bridge functions by tilting or rocking as a rigid molecule. This also draws attention to the current interpretation of contraction transients (A. F. Huxley and Simmons, 1971; Ford et al., 1985), as it takes the working stroke to be ~12 nm, only slightly lower than the maximal deformation of the rigid cross-bridge with rocking (to change the orientation from 90° to 45°). The sharp drop in force in the highly compressed fibers suggests, however, that at least some molecular movement is essential for the cross-bridge function. This could be achieved, for instance, if the S-1 moiety of the cross-bridge is internally flexible and only a small domain of the bridge deforms during the force-generating part of the cycle, which is also consistent with the assumptions made in a number of current schemes (e.g., A. F. Huxley, 1974; Eisenberg and Hill, 1985). Recent electron microscopic studies (Knight and Trinick, 1984) have shown that the globular part of myosin in the isolated thick filament is variably curved, which also suggests a flexible cross-bridge. Similarly, Craig et al. (1985) have found that the S-1 moiety of the cross-linked acto-S-1 is fatter and shorter during ATP hydrolysis, and this could be indicative of an additional flexibility in the cross-bridge during the actual turnover cycle. Future studies of isotonic contraction as well as those further combining the structural and mechanical measurements under the present conditions should be helpful in gaining additional insights into the molecular movements of the cross-bridge in the fiber.

[2]The explanations for the biphasic width-force relationship of skinned fibers of rabbit soleus muscle are presumably the same as for frog fibers. However, in addition, since the drop in force with compression of rabbit fiber started at ~0.8 $w_0$ (close to the in vivo width; see Introduction), compared with 0.58 $w_0$ in the frog fiber, the possibility may be considered that the relation between lattice spacing and cell width is different in mammalian soleus and frog semitendinosus and/or that the skinned (zero dextran) lattice spacing was different in the two preparations.

*Note added in proof:* H.E. Huxley and Kress (1985) have recently introduced a new model in which the cross-bridge is also internally flexible and has a force stroke of merely 4 nm. *J. Muscle Res. Cell Motil.* 6:153–161.

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