Radial Forces within Muscle Fibers in Rigor

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ABSTRACT Considering the widely accepted cross-bridge model of muscle contraction (Huxley. 1969. *Science* [*Wash. D. C.*]. **164:**1356–1366), one would expect that attachment of angled cross-bridges would give rise to radial as well as longitudinal forces in the muscle fiber. These forces would tend, in most instances, to draw the myofilaments together and to cause the fiber to decrease in width. Using optical techniques, we have observed significant changes in the width of mechanically skinned frog muscle fibers when the fibers are put into rigor by deleting ATP from the bathing medium. Using a high molecular weight polymer polyvinylpyrrolidone (PVP-40; number average mol. wt. ($\bar{M}_n$) = 40,000) in the bathing solution, we were able to estimate the magnitude of the radial forces by shrinking the relaxed fiber to the width observed with rigor induction. With rigor, fiber widths decreased up to ~10%, with shrinking being greater at shorter sarcomere spacing and at lower PVP concentrations. At higher PVP concentrations, some fibers actually swelled slightly. Radial pressures seen with rigor in 2 and 4% PVP ranged up to 8.9×10^3 N/m^2. Upon rigor induction, fibers exerted a longitudinal force of $\sim 1 \times 10^5$ N/m^2 that was inhibited by high PVP concentrations (≥13%). In very high PVP concentrations (≥20%), fibers exerted an anomalous force, independent of ATP, which ranged up to 6×10^4 N/m^2 at 60% PVP. Assuming that all the radial force is the result of cross-bridge attachment, we calculated that rigor cross-bridges exert a radial force of 0.2–1.2 $\times 10^{-9}$ N per thick filament in sarcomeres near rest length. This force is of roughly the same order of magnitude as the longitudinal force per thick filament in rigor contraction or in maximal (calcium-activated) contraction of skinned fibers in ATP-containing solutions. Inasmuch as widths of fibers stretched well beyond overlap of thick and thin filaments decreased with rigor, other radially directed forces may be operating in parallel with cross-bridge forces.

INTRODUCTION

Contraction of striated muscle is conventionally thought to involve a cyclic attachment of cross-bridges between thick and thin myofilaments. In this view, changes in interfilament spacing that normally occur with shortening are accomodated by changes in orientation of the linear heavy meromyosin (HMM) S-2 segment of the cross-bridge relative to the thick filament backbone. Upon attachment of the globular HMM S-1 cross-bridge head to the thin filament, tension is generated by a rotation of the head and transmitted

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to the thick filament through the HMM S-2 (Huxley, 1969). Because the HMM S-2 forms an acute angle with the thick filament, tension is directed radially as well as longitudinally. Geometrical calculations based upon surface-to-surface distances between the myofilaments and estimates of the sizes of S-1 and S-2 segments indicate that these radial forces are not inconsiderable, being, for instance, some 10–20% of the longitudinal force at a sarcomere spacing of 1.9 μm in an intact frog muscle fiber (Julian, et al., 1978; Schoenberg, 1980 *a* and 1980 *b*).

Radial forces would tend to draw the myofilaments together. In intact fibers, however, there is little or no change seen in interfilament spacing with contraction (Elliott et al., 1967; Haselgrove and Huxley, 1973). One might not expect to observe significant changes in interfilament spacing in intact fibers, because decreases in fiber volume are counteracted by large osmotic forces across the sarcolemma, which tends to maintain constant cell volume (Dick, 1966). Mechanical removal of the sarcolemma ("skinning") eliminates this constraint and should permit observation of any volume changes associated with cross-bridge attachment.

We describe here an optical method for detecting and quantifying radial forces in mechanically skinned muscle fibers, using the fiber itself as a "strain gauge." The radial stiffness of relaxed skinned fibers was determined by osmotically compressing the fiber in solutions containing a long-chain polymer (polyvinylpyrrolidone, PVP-40) that is largely excluded from the fiber interior (Godt and Maughan, 1977; Maughan and Godt, 1979). The relation between fiber width and external compressive pressure permits us to estimate the magnitude of any radial cross-bridge forces simply by observing changes in fiber width.

To maximize the number of attached cross-bridges and, thereby, the radial forces, we chose to observe fibers in rigor induced by deletion of ATP from the bathing medium. An additional advantage of this preparation is the excellent structural integrity of the fibers compared with the fragility of fibers in ATP-containing medium contracted with high concentrations of calcium. We presume that cross-bridges in the rigor state are structurally similar to those comparably orientated during normal calcium-activated contraction.

METHODS

Skinned fibers were prepared from the distal third of the semitendinosus muscle of the frog *Rana pipiens* (J.M. Hazen & Co., Alburg, Vt.). Details of the skinning and mounting procedures, as well as the experimental apparatus and techniques for observing fiber width and sarcomere spacing, are described elsewhere (Maughan and Godt, 1979). The standard relaxing solution contained (mM): 62 KCl; 5 EGTA, 20 imidazole, 15 creatine phosphate, 8.06 $MgCl_2$, 3.04 Na_2ATP (yielding 3 Mg^{2+} and 3 MgATP using the MgATP and MgHATP binding constants given by Phillips et al. [1966] and Khan and Martell [1966]), pH 7.00; ionic strength was 0.15 M. Rigor solutions were similar except Na_2ATP was omitted, $MgCl_2$ was 5.05 mM, and KCl was increased to 72 mM. Some rigor experiments were conducted in a solution that was similar but contained 68 mM KCl, 5 mM EDTA, and no $MgCl_2$. Further details concerning the calculation of the ionic composition of the solutions are given by Godt (1974). The osmotic pressure, π, of solutions containing PVP-40 ($\bar{M}_n$ = 40,000; Wood,

1970) was estimated from the osmometric data of Vink (1971), although the distribution of molecular weights in our sample may be somewhat different from that used by Vink. The polymer concentrations (grams per liter) referred to in the text denote grams of polymer per liter of final solution (= 10 × %wt polymer/vol final solution). Reagent grade KCl and KOH were obtained from Fisher Scientific Co., Fair Lawn, N. J. Other chemicals were obtained from Sigma Chemical Co., St. Louis, Mo. Experiments were conducted at room temperature (21 ± 1°C).

RESULTS

Calibration of Fiber as Radial Strain Gauge

Skinned fibers behave as elastic bodies, resisting radial compressive forces

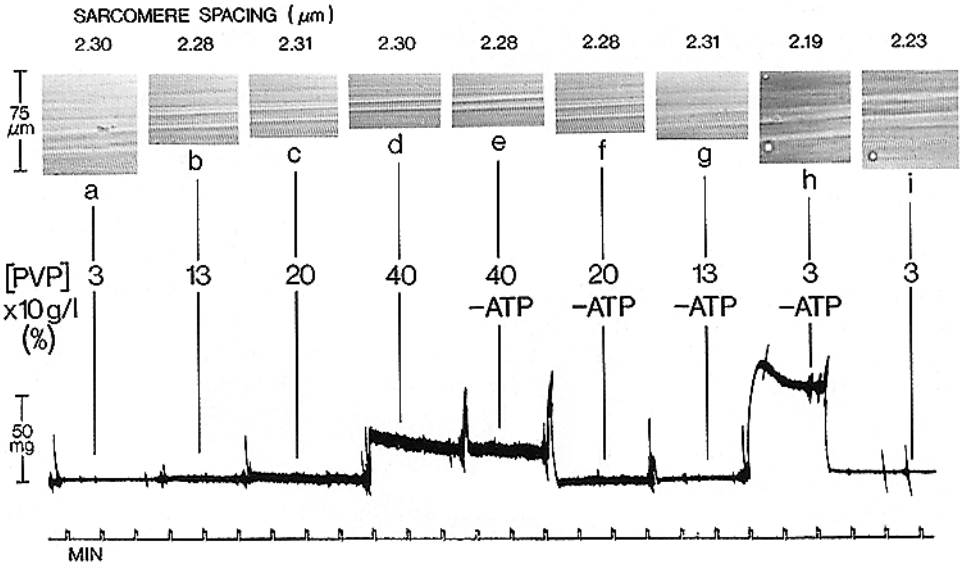

FIGURE 1. Fiber compression and force development in the presence and absence of ATP. *Top panel*: photographs of the central segment of a mechanically skinned frog muscle fiber immersed in solutions containing various concentrations of PVP-40, with (*a–d*, *i*) and without (*e–h*) ATP. Sarcomere spacing is indicated above the photographs. *Bottom panel*: corresponding force records from the strain gauge at the end of the muscle fiber. Note, at 3% PVP (30 g/1), the appreciable (7%) increase in fiber width when going from rigor (*h*) to relaxation (*i*). At higher PVP concentrations, differences in width between rigor and relaxation are less noticeable.

(Maughan and Godt, 1979). The relation between fiber width and osmotic compressive pressure can be utilized to estimate the magnitude of radially directed forces (arising, for instance, from cross-bridge attachment) in the same manner as a conventional strain gauge is used to measure longitudinal tension. The upper panel of Fig. 1 demonstrates the relation between fiber width and PVP concentration at a constant sarcomere spacing in both ATP-containing and ATP-free rigor solutions.

The protocol was as follows. Each fiber was immersed in a series of relaxing solutions with increasing polymer concentrations. This established a width-pressure relationship for each fiber. The fiber was then transferred through a series of rigor solutions containing decreasing concentrations of polymer. Some fibers were taken into rigor at a low concentration of polymer and transferred through a series of rigor solutions with increasing PVP concentrations. Near slack lengths, fibers tended to be damaged when rigor was initially induced at low PVP concentration. This was not the case when rigor was induced in high PVP concentrations. As a control, the fiber was reimmersed in the initial series of relaxing solutions. There usually was no significant change in sarcomere spacing in the relaxing solutions or in the rigor solutions containing high concentrations of polymer. Fibers whose sarcomere spacing changed by >2% over this series of solution changes were rejected. Upon transfer into rigor solutions with a lower concentration of polymer (Fig. 1, *g* to *h*), fibers usually generated tension and tended to shorten somewhat (e.g., 4% for the fiber in Fig. 1); fibers shortening more than 7% were rejected.

Fiber Shrinkage with Rigor Induction

In low concentrations of PVP, induction of rigor caused skinned fibers to shrink markedly. As can be seen in Fig. 1, the transfer from rigor to relaxing solution with 3% PVP leads to a noticeable increase in fiber width. Fig. 2 shows the complete width-pressure relation at several sarcomere spacings for the fiber of Fig. 1, as well as that of another typical fiber in a more extensive experiment. Note that fibers tend to shrink in rigor solutions, with the amount of shrinkage less at higher PVP concentrations. (In fact, at intermediate and high polymer concentrations [7–20% PVP], some fibers swelled slightly in rigor solutions.) Note also that rigor shrinkage was generally greater at shorter sarcomere spacing, as illustrated in Fig. 3, where pooled data on rigor shrinkage in seven fibers is plotted against sarcomere spacing for 2, 4, and 20% PVP.

Quantitation of Radial Pressures in Rigor

Radial pressures can be quantified by using relations between fiber width and pressure such as those shown in Fig. 2. That is, one asks, how much additional osmotic compressive pressure is required to cause a change in width equivalent to that seen in rigor? In Fig. 2 *b* this increment in pressure is indicated by the horizontal lines. Fig. 4 shows the radial pressure as a function of sarcomere spacing for two concentrations of PVP. Though there is considerable scatter in the data, there seems to be a tendency for radial pressure to be smaller at longer sarcomere spacings. At higher concentrations of PVP, scatter was too great to permit further analysis.

Shrinkage with Applied Longitudinal Force in Rigor Solutions

If fiber shrinkage with rigor induction is in some part due to attachment of cross-bridges one might expect that longitudinal force applied to the ends of a rigor fiber would be transmitted from one filament to the other by the

angled cross-bridges, resulting in a radially directed component of force. This radial force would tend to cause further shrinkage of the fiber. This is, in fact, what is observed. In rigor solutions with no Mg or ATP, application of force up to 1 × 10^5 N/m^2 caused fiber width to decrease by 4% (relative to the average of the widths before and after the stretch) as indicated in the example given in Table I. In view of the irreversible changes in fiber width after this procedure, an overall 4% change in width may be an overestimate. Although

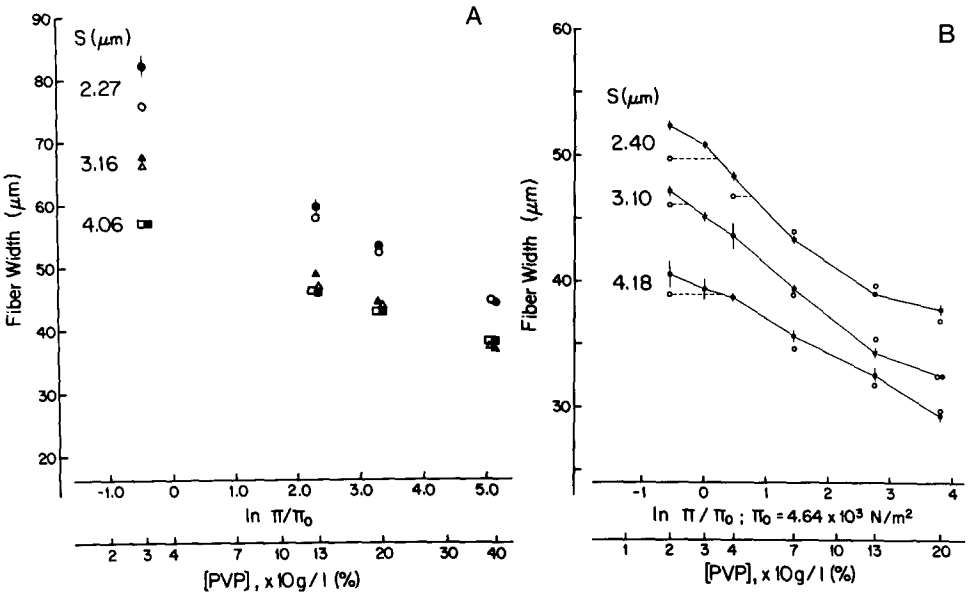

FIGURE 2. Width-pressure relations for two fibers at several different sarcomere spacings. Fiber width D versus ln (π/π_0) plotted, where π is the osmotic compressive pressure exerted by the concentration of PVP indicated (π_0 = 4.64 × 10^3 N/m^2; i.e., the pressure exerted by a 3% PVP solution). Experiments in ATP-containing (relaxing) solutions are indicated by filled symbols; ATP-free (rigor) solutions, by open symbols. Vertical bars indicate range of width measurements in relaxing solutions (one set of measurements in relaxing solution taken before, and one set after, the set taken in rigor solutions). (*A*) Fiber of Fig. 1 at sarcomere spacings of 2.27, 3.16, and 4.06 μm. (*B*) another fiber at sarcomere spacings of 2.40, 3.10, and 4.18 μm. Curves were drawn by eye. Horizontal lines refer to the additional pressure required to cause a change in width in the relaxed fiber equivalent to that seen in rigor.

the applied longitudinal forces were substantial (up to 100% of the maximum calcium-activated tension expected from these fibers, 1–3 × 10^5 N/m^2; Godt [1974]), sarcomere spacing remained more or less constant.

Longitudinal Forces in Skinned Fibers

In the experiments such as those illustrated in Fig. 1, we observed two different types of longitudinal force: one associated with the withdrawal of ATP (a

"rigor" force) and the other associated with high concentrations of polymer (an "anomalous" force). An example of the rigor force is shown in the lower panel of Fig. 1. The force developed in rigor solution with 3% PVP is abolished

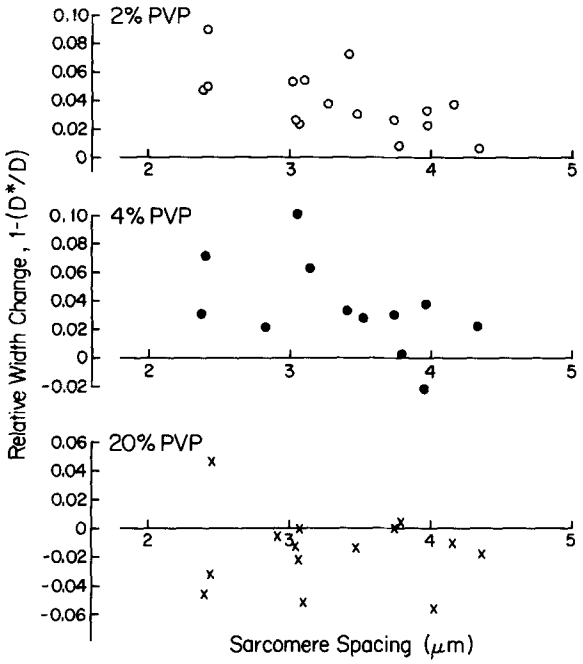

FIGURE 3. Rigor-induced shrinkage as a function of sarcomere spacing in fibers bathed in 2, 4, and 20% PVP-containing solutions. Data pooled from seven fibers. The width of each fiber in rigor (D^*) is expressed as a fractional change from the average width of the relaxed fiber (D); i.e., $1 - D^*/D$. Note that positive values indicate a net shrinkage, negative values a net swelling.

TABLE I

FIBER SHRINKAGE WITH APPLIED LONGITUDINAL FORCE IN RIGOR SOLUTION CONTAINING 2% PVP

Force	Width, D^*	Relative width	Sarcomere spacing
$\times 10^4$ N/m^2	μm		μm
0	79.3	0.98	2.48
0.71	79.7	0.99	2.46
4.97	77.2	0.96	2.40
8.54	74.5	0.92	2.43
9.96	73.7	0.91	2.43
0	74.8	0.93	2.41

Relative width = D^*/D, where D = 80.6 μm, the width of the fiber in 2% PVP-containing relaxing solution. Fiber 10/12/79. Rigor solution contained no Mg or ATP (cf. Methods).

by transfer to relaxing solution with 3% PVP (*h* to *i*). Note that high concentrations of polymer tend to antagonize the development of rigor force (*g* to *h*). Fig. 5 illustrates the influence of polymer concentration on rigor force.

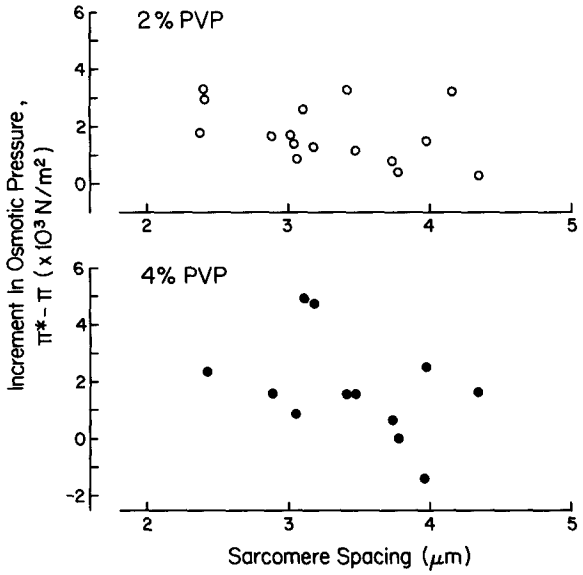

FIGURE 4. Rigor-induced radial pressure as a function of sarcomere spacing in fibers bathed in 2 and 4% PVP-containing solutions. Data taken from fibers of Fig. 3. Radial pressure is determined by measuring the increment in osmotic pressure ($\pi^* - \pi$) required to cause a change in width in the relaxed fiber equivalent to that seen in rigor (see horizontal lines of Fig. 2 *B* for examples).

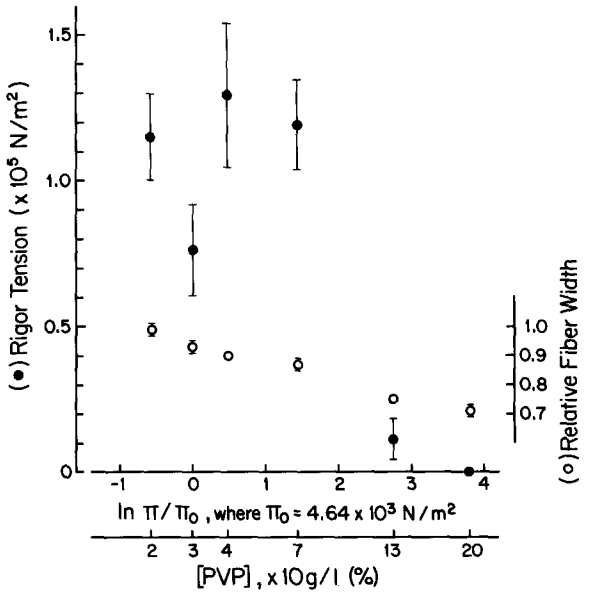

FIGURE 5. Influence of PVP concentration on muscle rigor force in eight fibers. Mean force (±1 SEM) referred to the cross sectional area (circular approximation, $\pi D^2/4$, where D is the observed width of the fiber in 3% PVP-containing relaxing solution; width is given relative to that of each fiber in 3% PVP-containing relaxing solution). Sarcomere spacing ranged from 2.07 to 2.70 μm.

Rigor tension from each fiber was expressed relative to the width of the fiber in 3% PVP relaxing solution at the same sarcomere spacing. Pooled data for Fig. 5 came from fibers with sarcomere spacings between 2.07 and 2.70 μm (average, 2.4 ± 0.2 μm). The magnitude of rigor tension in 2–7% PVP is large, comparable to maximal tension observed in calcium-activated skinned fibers in ATP-containing medium. Although rigor tension was not significantly different in 2, 3, 4, and 7% PVP, there was a tendency in some fibers for rigor tension to rise as polymer concentration was increased in this range. In some cases, rigor tension was abolished in 13% PVP and, in all cases, in 20% PVP. As can be seen in Fig. 5, fiber width decreased with increasing polymer concentrations, with no abrupt change between 7 and 13%.

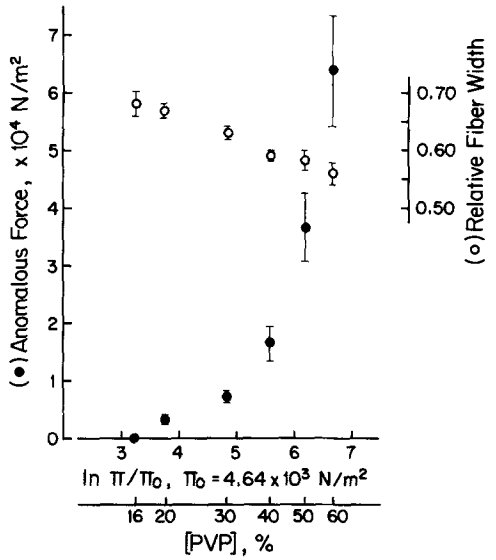

FIGURE 6. Relation between anomalous force and PVP concentration for four fibers. Mean force (±1 SEM) referred to the cross sectional area (circular approximation) as in Fig. 5. Sarcomere spacing ranged from 2.24 to 3.67 μm.

Rigor tension can also be abolished by decreasing the separation between the clamps holding the fiber. Rigor tension drops precipitously but redevelops slowly, along with a detectable, slow shortening of the sarcomeres. Force redevelopment and shortening can be prevented by deleting magnesium as well as ATP from the bathing medium. This suggests that in the rigor state induced by ATP deletion with Mg present, cross-bridges are probably cycling slowly rather than statically attaching, as they appear to be in Mg- and ATP-free rigor solutions.

At very high concentrations of PVP, fibers generate an anomalous force that appears to be unaffected by ATP (cf. Fig. 1). The relation between anomalous force and polymer concentration is shown in Fig. 6. Note in Fig. 1 *b* and *c* that the stiffness of the preparation, as reflected by the width of the force trace (resulting from small perturbations of fiber length from building

vibrations) also increases with increasing polymer concentrations, even at polymer concentrations at which there is no detectable anomolous force. Anomolous tension drops after a short decrease in fiber length but, unlike rigor tension in the presence of Mg, does not redevelop.

DISCUSSION

To quantify any possible radial force component of cross-bridge attachment, we examined the relation between overall fiber width and osmotic compressive pressure during rest and induction of rigor. The elastic behavior of the fiber served as a strain gauge, monitoring changes in radial pressure between the two states. This method rests on a number of assumptions.

First, we assume that the polymer used to exert osmotic compressive forces is excluded from the fiber to the same extent as from the membrane of the osmometer used to determine the osmotic pressure. We have dealt with this problem elsewhere (Godt and Maughan, 1977; Maughan and Godt, 1979). We have observed (Maughan and Godt [1979] Fig. 3) that changes in fiber width in solutions with PVP-40 ($\bar{M}_n$ = 40,000) were similar to those in solutions of the same calculated osmotic pressure made up with a much larger polymer (Dextran T500, $\bar{M}_n$ = 192,600). Thus, we felt that PVP-40 was, for all practical purposes, excluded from the fiber, although some low molecular weight fractions of the PVP undoubtedly enter (see below).

Second, we assume that the elastic structures that determine the width-pressure relation are unaffected by deletion of ATP from the bathing medium. We know of no evidence indicating that a significant amount of ATP binds to the major elastic structures in frog striated muscle (M lines, Z bands and "connectin," cf. Maughan and Godt [1979]). Thus, it seems unlikely that ATP would affect their elastic behavior.

Third, we assume that forces arising from changes internal to the fiber (i.e., attachment of cross-bridges) affect fiber width similarly to forces arising external to the fiber (i.e., osmotic compressive forces). That is, the structure of the fiber is tightly linked mechanically under our experimental conditions. This means that changes in myofilament lattice spacing should be proportional to changes in overall fiber width. There is evidence that this is the case for externally applied forces. Magid and Reedy (1980) and Magid[1] observed changes in interfilament spacing of chemically skinned frog sartorius muscle in relaxing solutions similar to ours, containing various PVP-40 concentrations. Over the range 2–10% PVP there is a strong correlation (r = 0.97) between changes seen in fiber width with PVP concentration (Maughan and Godt, 1979, Fig. 2) and changes in interfilament spacing with PVP concentration. We have no direct evidence, however, that changes in myofilament lattice due, for instance, to cross-bridge attachment would change fiber width proportionately, although the data in Fig. 3, which relate fiber width to sarcomere spacing in 4% PVP, in conjunction with preliminary data on interfilament spacing changes with sarcomere spacing in 4% PVP (Goldman et al., 1979) support this assumption.

[1] Personal communication.

As would be expected from attachment of angled cross-bridges, fibers shrink markedly when rigor is induced at low to moderate PVP concentrations. At higher concentrations, however, some fibers unmistakably swell with rigor induction. Although this swelling might seem paradoxical, Schoenberg (1980 *a* and 1980 *b*) has calculated that under certain circumstances of close myofilament separation, attachment of cross-bridges could lead to an *outwardly* directed radial force. Using accepted dimensions for the S-1 and S-2 portions of HMM, Schoenberg (1980 *a* and 1980 *b*) has examined the biophysical consequences arising from the geometrical constraints of the cross-bridge system. As might be expected, at large interfilament spacing, cross-bridge attachment gives rise to an inwardly directed radial force. However, at small interfilament distances, the S-1 molecule is then long compared with the filament separation, so that the S1-S2 junction actually lies behind the part of the myosin filament from which the cross-bridge projects. Thus, cross-bridge attachment can give rise to an expansive radial force on the myofilament lattice. In accord with Schoenberg's analysis, we find that most fibers swell slightly with rigor at high concentrations of PVP, at which fiber width and, presumably, interfilament spacing are decreased. E. W. April,[2] using x-ray diffraction techniques, has observed similar expansive radial changes in myofilament dimensions and fiber width in skinned crayfish muscle with rigor induction at small interfilament spacing.

Although skinned fiber width changes seem qualitatively consistent with current views of cross-bridge attachment in rigor, accurate quantitation by our method of the magnitude of the radial pressures is hampered by scatter in the experimental data. Certainly, imprecision in measurements of fiber width and uncertainties in the effective osmotic pressure of PVP solutions on the fiber contribute to the variance of the estimate. However, the major cause of the variability in estimates of radial pressure probably arises from uncertainties in the determination of the exact slope of the width-pressure relationship due to slight hysteresis of fiber width as it is altered with rigor and with changes in PVP concentration. We constructed width-pressure curves (Fig. 2) using the average width of the fiber in relaxing solution at each PVP concentration before and after rigor induction (cf. Fig. 2, legend). Generally, fiber width in relaxing solution after rigor was somewhat less than the initial width. Typical ranges are shown in Fig. 2. Because the abscissa is logarithmic, any uncertainties in slope have a large effect on the estimate of radial pressure as reflected in the scatter. This seems unavoidable with the present technique. The most reliable estimates are obtained at low osmotic pressure. Thus, we confined our analysis to data obtained from fibers in 2 and 4% PVP solutions. In 2% PVP rigor solutions, the radial pressure ranged from 0.3 to 3.2×10^3 N/m^2, and in 4% PVP, ranged from -1.3 to 8.9×10^3 N/m^2 (with inwardly directed pressures being positive).

Assuming that all the radial pressure in rigor solution results from cross-bridge attachment, we can utilize these estimates to calculate the radial force per cross-bridge. As outlined in the Appendix, if two-thirds of the available

[2] Personal communication.

cross-bridges are attached in rigor, an upper bound for the radial force per cross-bridge is $\sim 6 \times 10^{-12}$ N and appears to be independent of sarcomere spacing. The radial force per thick filament is 1.2×10^{-9} N at near rest length ($S = 2.2$ μm). A lower bound for the radial force per cross-bridge is $\sim 2.7 \times 10^{-12}$ N, and for the radial force per thick filament, 1.9×10^{-10} N. Using x-ray diffraction to measure interfilament spacing, Goldman et al. (1979) have also examined radial forces in frog skinned fibers in rigor. In accord with our results, they calculate that radial forces per thick filament are 10^{-10} to 10^{-9} N.

The longitudinal rigor force we observed, when normalized to force per thick filament, was somewhat smaller than the radial force per thick filament. From the geometry of the myofilament lattice and x-ray diffraction data (Magid and Reedy, 1980), one can calculate that there are $\sim 7 \times 10^{14}$ thick filaments/m^2 for skinned fibers near rest length in 3% PVP relaxing solution. Thus, for rigor tension of $\sim 1 \times 10^5$ N/m^2, the longitudinal tension per thick filament is $\sim 1.4 \times 10^{-10}$ N.

TABLE II

INCREASE IN RADIAL STIFFNESS MODULUS WITH INDUCTION OF RIGOR (NINE FIBERS, SARCOMERE SPACING 2.27–4.36 μm)

PVP concentration	π	+ATP (rest) K^*	−ATP (rigor) K^*
%	$\times 10^3$ N/m^2	$\times 10^4$ N/m^2	$\times 10^4$ N/m^2
2	2.59	1.50 ± 0.24	1.70 ± 0.26
3	4.64	2.55 ± 0.42	2.91 ± 0.45
4	7.29	3.84 ± 0.66	4.40 ± 0.71
7	19.79	9.42 ± 1.79	10.97 ± 1.92

* ± 1 SD.

Application of longitudinal force to a fiber in rigor causes the fiber width to decrease. The 4% decrease seen with application of 10^5 N/m^2 is similar to that predicted (~2%) by Schoenberg (1980 *b*) from geometrical considerations. Longitudinal stiffness of rigor fibers is high, because sarcomere spacing remains virtually unchanged with applied forces nearly the same as those in maximal contraction. The radial stiffness modulus of rigor fibers can be calculated from the pressure-width relation. Following our previous derivation (Maughan and Godt, 1979), the radial stiffness modulus, K, is determined from the equation $d\pi = -K\,[d(D^2)/D^2]$, which relates changes in fiber width, D, to osmotic compressive pressure, π, at constant sarcomere spacing. Values of radial stiffness moduli for fibers in relaxing and rigor solutions are given in Table II. Although the moduli for relaxed fibers are similar to those previously described (Maughan and Godt [1979], Table III), the moduli for rigor fibers are 13–16% greater.

By way of comparison, one can ask, how much does the longitudinal stiffness (Young's modulus) increase with rigor? In our experiments, there was no detectable change in sarcomere spacing with applied force within the resolution of our technique, which is ~1%. Thus, a rough estimate of the lower

bound of Young's modulus in our fibers is $\sim 1 \times 10^7$ N/m^2, some two orders of magnitude greater than that for relaxed skinned fibers (Maughan and Godt, 1979). This value seems reasonable since Kawai and Brandt (1976) obtain a value for Young's modulus of crayfish skinned fibers of 1.08×10^7 N/m^2 under comparable conditions ("high rigor").

The observation that, with induction of rigor, longitudinal stiffness increases much more than radial stiffness is consistent with the widely accepted cross-bridge model proposed by Huxley (1969). In this model, the S-1 head firmly attaches to the thin filament, with hinge points between S-1 and the rigid S-2 arm and between the S-2 arm and the light meromyosin in the backbone of the thick filament. Huxley proposes that upon attachment to thin filaments, heads rotate in a preferential direction, thereby giving rise to a longitudinal force in the fiber. When the fiber is in rigor, the head is presumably firmly attached, and attempts to lengthen the fiber are resisted by the stiffness of the S-2 arm and by the reluctance of the head to rotate contrary to its preferred direction. Thus, the longitudinal stiffness will be high. However, radial compression is not so stoutly resisted because the S-2 is hinged at both ends, because the angle that the arm makes with the thick filaments is rather small, and because heads would not be forced to rotate contrary to their preferred direction. However, if heads attach firmly in rigor, one would expect radial stiffness to increase somewhat with rigor, as is observed.

The origin of the anomalous force seen in high concentration of PVP is unclear. Fiber stiffness under these conditions increases, with both the increased stiffness and force being independent of the presence of ATP. Possibly, interfilament spacings are decreased to the point that filaments interfere sterically or interact directly, or that cross-bridges attach even in relaxing solution.

Let us reexamine the notion that skinned fibers shrink with rigor induction as a result of attachment of cross-bridges. If shrinkage is solely the result of cross-bridges, one would expect that shrinkage would decrease with increasing sarcomere spacing and that there would be no shrinkage at sarcomere spacings beyond which myofilaments no longer overlap, i.e., beyond 3.60 μm in frog muscle (Julian et al., 1978). Although Fig. 3 shows that shrinkage does decrease with sarcomere length, there is nevertheless still unmistakable shrinkage at lengths well beyond overlap (e.g., even at 4.34 μm in 2% PVP).

X-ray diffraction work by Goldman et al. (1979) showed that myofilament lattice spacing decreased when skinned fibers were put into rigor in PVP-free solutions containing no ATP or Mg. The shrinkage they observed decreased with increasing sarcomere spacing up to 3.5 μm, the longest spacing they used. From the regression lines they present it would appear that there would be shrinkage of the lattice beyond 3.60 μm, in agreement with our data at 2 and 4% PVP.

One possible explanation is that myofilaments at sarcomere spacings beyond 3.60 μm are no longer in complete registration. This misregistration would allow some filaments to overlap, although the distance between the center of the I bands observed optically would be >3.6 μm.

Another possibility is that changes in other radial forces might occur. In particular, there may well be changes in the radial force due to electrostatic repulsion between similarly charged proteins in the fiber. Pemrick and Edwards (1974) used glass microelectrodes to study potentials in glycerol-extracted rabbit psoas muscle. They proposed that these potentials arise from the fixed charges in the myofilaments and obey a Donnan equilibrium. From observations of potential differences between fibers in relaxation and rigor, they concluded that the concentration of fixed negative charge is decreased markedly (~50%) when fibers go into rigor, possibly because of conformation changes in the proteins. Such a decrease in fixed charge concentration could have a pronounced effect upon fiber width. As we have shown (Godt and Maughan [1977], Fig. 1) fiber width decreased significantly by decreasing solution pH from 7 to 5, a procedure that would be expected to greatly decrease myofilament charge by titrating charged groups on the proteins toward their isoelectric points. If radially directed electrostatic repulsive forces do play a role in the stabilization of the myofilament lattice, as has been proposed (Elliott, 1973; April and Wong, 1976), and if these forces can be altered by deletion of ATP as proposed by Pemrick and Edwards (1974), these forces, acting in parallel with cross-bridge forces, might explain the shrinking of rigor fibers beyond overlap lengths. This would tend to decrease our estimate of the radial cross-bridge force.

APPENDIX

Radial Force per Cross-Bridge

We have obtained an estimate of the extra radial pressure exerted on the bulk of the fiber when fibers go into rigor. If we assume that all the extra radial force is due to attachment of cross-bridges, we can calculate the magnitude of force exerted by each attached bridge.

To transform radial pressure into force, we make use of the static equilibrium between the outwardly and inwardly directed radial force components at the periphery of the skinned fiber: first consider the balance of forces upon a representative thick filament in the region of myofilament overlap at the fiber periphery (Fig. 7). In PVP-containing relaxing solutions, for a given fiber of width D (with a corresponding myosin-to-myosin spacing d_{mm}) and sarcomere length S, the inwardly directed osmotic force F_i is counterbalanced by an outwardly directed force F_o. We assume the force F_o arises from structures whose elastic moduli can be determined for each resting fiber by compression and stretch experiments (Fig. 2 and Maughan and Godt [1979]). Thus, at a specific D and S, $F_i(D, S) + F_o(D, S) = 0$. In the relaxed fiber we estimate the outward forces as $F_o(D, S) = \pi(D, S)d_{mm}L$, where the outward pressure per thick filament of length L on the periphery is applied to the area extending halfway in either circumferential direction from the thick filament to the neighboring thick filaments. As outlined in the text, when the fiber goes into rigor, we estimate the additional inward rigor force as $F^*(D^*, S^*) = \pi(D^*, S^*)d^*_{mm}L - \pi(D, S)d_{mm}L$, where the starred variables refer to those of the rigor state. This is illustrated in Fig. 2 *B*.

If we assume provisionally that all the extra inward force in rigor is due to cross-bridge attachment, from simple geometry, the sum of the inwardly directed forces due to cross-bridges between a peripheral thick filament and the underlying three

thin filaments is $F_{xb} = [2 (\sin 30°) + 1] f_{xb} yn/6 = f_{xb} ny/3$, where f_{xb} is the radial force of the individual cross-bridge, $n/2$ is the number of inwardly directed, attached cross-bridges in the overlap region that project from a peripheral thick filament in each sarcomere, and y is the fraction of overlap of thick and thin filaments at sarcomere length S. Given the values for thick and thin filament lengths in frog muscle from Julian et al. (1978), $y = 1/1.4\ (3.6 - S)$ for S between 2.2 μm (full overlap) and 3.6 μm (no overlap). Thus, if $F^*(D^*, S^*) = F_{xb}$ (i.e., all the extra force is due to cross-bridges), the radial force per cross-bridge is given by $f_{xb} = (3L/ny)\ [\pi(D^*, S^*)\ d^*_{mm} - \pi(D, S)\ d_{mm}]$. If we assume, as mentioned in the text, that under our experimental conditions changes in interfilament spacing are reflected proportionately by changes in fiber width, $D^*/D = d^*_{mm}/d_{mm}$. Then, for $2.2 < S < 3.6$, $f_{xb} = (3d_{mm}L/ny)\ [\pi(D^*, S^*)\ (D^*/D) - \pi(D, S)]$.

To calculate f_{xb}, values of d_{mm} (i.e., $(2\sqrt{3}/3)d_{1,0}$) were taken from x-ray diffraction experiments conducted at S in 2% PVP-containing relaxing solutions by A. Magid and M. K. Reedy[3] and in 4% PVP-containing relaxing solutions by Goldman et al. (1979). The length of the thick filament was taken to be 1.6 μm (Julian et al., 1978); with an H-zone distance of 0.15 μm (Craig, 1977), we calculate an n of 304 (Squire,

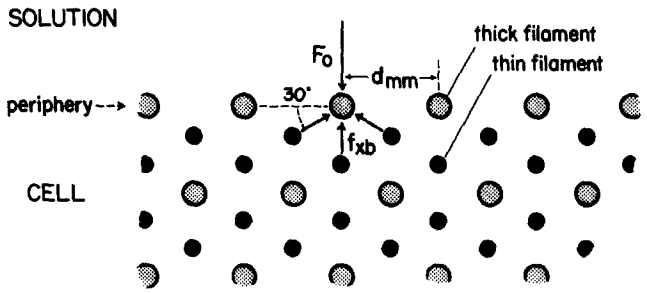

FIGURE 7. Schematic illustration of myofilaments at fiber periphery, showing the balance of forces between outwardly and inwardly directed radial force components during rigor. See Appendix for details.

1974). Assuming that two-thirds of the available cross-bridges (ny) are attached (Squire, 1974), we find that f_{xb} is $\sim 5 \times 10^{-12}$ N for sarcomere spacings between 2.2 and 3.2 μm in seven fibers bathed in 2 and 4% PVP-containing rigor solutions (values for $\pi\ (D^*, S^*)$, $\pi\ (D, S)$, D^*, D, S^*, and S from 14 individual experimental runs, including those at S^* (mean) = 2.40 and 3.10 μm in the experiment illustrated in Fig. 2 *B*). The data suggest that there are no large differences between the radial force f_{xb} at 2% PVP and that at 4% PVP ($P < 0.05$). Furthermore, f_{xb} was relatively constant over the range of striation spacing of 2.21–3.54 μm ($P < 0.05$). This is contrary to what one would expect on the basis of geometrical calculations (Schoenberg, 1980 *b*), although the calculations also suggest that these effects would be relatively small. The radial force per thick filament (nyf_{xb}) was $\sim 1.2 \times 10^{-9}$ N at $S^* = 2.2$ μm (complete myofilament overlap).

We were surprised that this calculated mean radial force per thick filament was an order of magnitude larger than the mean longitudinal force per thick filament (1.4×10^{-10} N). As mentioned in the text, the estimate of radial force depends upon the assumption that PVP-40 is effectively excluded from the fiber. If appreciable amounts of the low molecular weight fractions do enter the fiber, use of PVP-40 would tend to

[3] Personal communication.

overestimate the value of radial force. Previously (Maughan and Godt, 1979) we observed that 4% PVP-40 shrank fibers to the same extent as a 4% solution of Dextran T500, a much larger polymer. If we use data from Granath (1958), a 4% Dextran T500 solution has the same osmotic pressure as a 4% PVP-40 solution. If, however, we use data from Vink (1971), a 4% Dextran T500 solution has an osmotic pressure some threefold lower than a 4% PVP-40 solution. Similarily, the osmotic pressure of a 2% Dextran T500 solution is but a fourth of that of a 2% PVP-40 solution. Thus, on the basis of Vink's (1971) data, our estimate of the *effective* osmotic pressure of 2–4% PVP-40 solutions may be too high by a factor of 3 to 4. This would lower our estimate of radial rigor force per cross-bridge to 2.7×10^{-12} N and radial force per thick filament to 1.9×10^{-10} N at $S^* = 2.2$ μm.

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