SWELLING OF SKINNED MUSCLE FIBERS OF THE FROG

EXPERIMENTAL OBSERVATIONS

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ABSTRACT Frog skeletal muscle fibers, mechanically skinned under water-saturated silicone oil, swell upon transfer to aqueous relaxing medium (60 mM KCl; 3 mM $MgCl_2$; 3 mM ATP; 4 mM EGTA; 20 mM Tris maleate; pH = 7.0; ionic strength 0.15 M). Their cross-sectional areas, estimated with an elliptical approximation, increase 2.32-fold (±0.54 SD). Sarcomere spacing is unaffected by this swelling. Addition of 200 mM sucrose to relaxing medium had no effect on fiber dimensions, whereas decreasing pH to 5.0 caused fibers to shrink nearly to their original (oil) size. Decreasing $MgCl_2$ to 0.3 mM caused fibers to swell 10%, and increasing $MgCl_2$ to 9 mM led to an 8% shrinkage. Increasing ionic strength to 0.29 M with KCl caused a 26% increase in cross-sectional area; decreasing ionic strength to 0.09 M had no effect. Swelling pressure was estimated with long-chain polymers, which are probably excluded from the myofilament lattice. Shrinkage in dextran T10 (number average mol wt 6,200) was transient, indicating that this polymer may penetrate into the fibers. Shrinkage in dextran T40 (number average mol wt 28,000), polyvinylpyrrolidone (PVP) K30 (number average mol wt 40,000) and dextran T70 (number average mol wt 40,300) was not transient, indicating exclusion. Maximal calcium-activated tension is decreased by 21% in PVP solutions and by 31% in dextran T40 solutions. Fibers were shrunk to their original size with 8×10^{-2} g/cm^3 PVP K30, a concentration which, from osmometric data, corresponds to an osmotic pressure (Π/RT) of 10.5 mM. As discussed in the text, we consider this our best estimate of the swelling pressure. We find that increasing ionic strength to 0.39 M with KCl decreases swelling pressure slightly, whereas decreasing ionic strength to 0.09 M has no effect. We feel these data are consistent with the idea that swelling arises from the negatively charged nature of the myofilaments, from either mutual filamentary repulsion or a Donnan-osmotic mechanism.

INTRODUCTION

Skinned skeletal muscle fibers have proven to be a useful model system for studying the mechanochemistry of contraction, since, with the sarcolemma mechanically removed, the solution bathing the contractile apparatus is under experimental control (Natori, 1954).

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A number of workers have noted that, after being skinned, the fibers tend to swell in aqueous relaxing medium (April et al., 1971, 1972; A. M. Gordon in Godt, 1971; Hatchett and Podolsky in Ford and Podolsky, 1972; Matsubara and Elliott, 1972; April, 1975). Since the previous reports indicated that the skinned fiber volumes increased some 40–50%, this is by no means a trivial change, especially since filament lattice geometry is an important aspect of the commonly accepted cross-bridge model of contraction.

The present report describes our experimental attempt to understand and to quantify this swelling phenomenon. It seemed most likely to us that swelling was due to one or both of the following: (*a*) swelling of the sarcoplasmic reticulum in the relaxing medium (observed in electron micrographs of skinned fibers), or (*b*) forces arising from the charged nature of the myofilaments, either electrostatic repulsion between the similarly charged filaments or to a Donnan-osmotic mechanism. We designed our experiments to distinguish between these.

Brief accounts of these results have already appeared (Godt and Maughan, 1973; Maughan and Godt, 1974).

METHODS

Single skinned fibers were prepared from semitendinosus muscles of the frog *Rana temporaria*. After dissection, the intact semitendinosus was kept in a Ringer solution of 111 mM $NaCl_2$, 2.7 mM KCl, 1.8 mM $CaCl_2$, and 1.2 mM $NaHCO_3$, pH = 7. Single fiber segments, isolated from small bundles of fibers, were "skinned" with needles under water-saturated silicone oil (Rhodorsil SI 200, 10 cSt viscosity; Rhône-Poulenc, Paris). The H_2O-saturated oil was prepared by adding a few milliliters of distilled water to about 500 ml of silicone oil and shaking vigorously. A skinned fiber (3–5 mm long) was mounted between fiber clamps, one stationary and one connected to a photo-electric transducer similar to that used by Hellam and Podolsky (1969). The fiber was then transferred into a 6-ml bath of H_2O-saturated oil in a solution changer and the length adjusted to the desired overall sarcomere spacing, as determined from the laser diffraction pattern produced with a 0.5 mW helium-neon laser (155, Spectra-Physics, Inc., Laser Products Div., Mountain View, Calif.). The fiber was subsequently transferred into different solutions contained in other 6-ml baths on the solution changer by depressing the changer until the fiber was free, moving the changer horizontally, and releasing the changer to immerse the fiber in another bath. The procedures for dissection, solution change, and tension measurement have been described previously (Godt, 1974).

The width of the central region of a fiber was measured with a Zeiss dissecting microscope at 80x magnification (Carl Zeiss, Inc., New York). The thickness of the same region was measured from the fiber's reflection in a tiny front-surfaced mirror mounted at a 45° angle on a small Plexiglas platform placed very close to the fiber. With practice, these dimensions could be determined with a precision of 5 μm. The fiber, after sarcomere length adjustment and size measurement in the oil, was transferred into a bath containing standard relaxing medium: 60 mM KCl, 3 mM $MgCl_2$, 3 mM ATP; 4 mM EGTA (so that $Ca^{2+} < 10^{-8}$ M), and 20 mM Tris maleate; pH = 7.0; ionic strength 0.15 M. The width and thickness were measured in standard relaxing medium and in other modified relaxing solutions approximately 1 min after transfer to the solution to allow for complete equilibration. Since skinned fibers are seldom round, cross-sectional areas of the fiber in various media were calculated as: $A = (\pi/4)(w \times t)$ where A is cross-sectional area, w is width, and t is thickness.

Analytical grades of KCl, $MgCl_2$, $CaCl_2$, NaCl, $NaHCO_3$, Tris-Cl, maleic acid, and sucrose were obtained from Merck (Darmstadt, W. Germany); Na_2ATP was obtained from Sigma Chemical Co. (St. Louis, Mo.); EGTA and polyvinylpyrrolidone (PVP) K30 ("Plasdone") from Fluka AG (Buchs, Switzerland) and dextran T fractions from Pharmacia Fine Chemicals Inc. (Uppsala, Sweden).

RESULTS

Amount of Swelling

Transfer from oil to standard relaxing medium took about 10 s. Some swelling was observed as soon as this change was accomplished and swelling was complete within 30 s. We define the amount of swelling (S) as the ratio of fiber cross-sectional area in relaxing medium to area in H_2O-saturated silicone oil. The average ratio ($\bar{S}$) for 49 fibers was 2.32 ($\pm$0.54 SD) for sarcomere lengths between 2.02 and 3.16 μm.

The initial sarcomere spacing in oil did not differ from that of the swollen fiber in relaxing medium by more than a few percent; the amount of swelling was not dependent in any consistent way upon sarcomere length in the range investigated.

Added Sucrose

The sarcoplasmic reticulum (SR) of skinned fibers, especially the lateral sac region, tends to swell in relaxing medium (cf. plate I, Taylor and Godt, 1976) and can be partially reversed by addition of sucrose (200 mM) to the relaxing medium (Godt, unpublished observations). This is to be expected owing to osmotic effects, if, as seems likely, SR membranes are as impermeable as plasma membrane to sucrose. If swelling of the entire fiber arises from swelling of the SR, the fiber should shrink in solutions containing sucrose. Addition of sucrose in concentrations of 100 or 200 mM to the normal relaxing medium, however, had no significant effect on fiber cross-sectional areas at either short (2.12–2.28 μm) or long (2.70–3.16 μm) sarcomere spacings. (Only 200 mM sucrose was used at long spacing.) Thus it seems unlikely that the SR plays a role in swelling of skinned fibers.

Changes in Solution pH

Mutual filamentary repulsion and Donnan-osmotic forces arise from the net charge of the myofilaments. The charge of the myofilaments, like that of any charged protein, can be neutralized by titration to its isoelectric point. If swelling forces depend upon filamentary charge, then changes in solution pH should affect fiber size.

The effect of pH upon swelling was tested by transferring fibers from normal relaxing solution (pH 7.0) to relaxing solutions with altered pH. Fig. 1 shows that fiber cross-sectional areas at pH 6.0 were not significantly different from controls in pH 7.0. But fibers shrank as pH dropped below 6.0 so that at pH 5.0 they were only slightly larger than their initial (H_2O-oil) size. These effects were entirely reversible and fibers gave good laser diffraction bands in these low pH solutions. On the other hand, when placed into "relaxing" solutions of pH 8.0, fibers contracted strongly (even though EGTA was present), their laser diffraction pattern disappeared, and the fibers took on

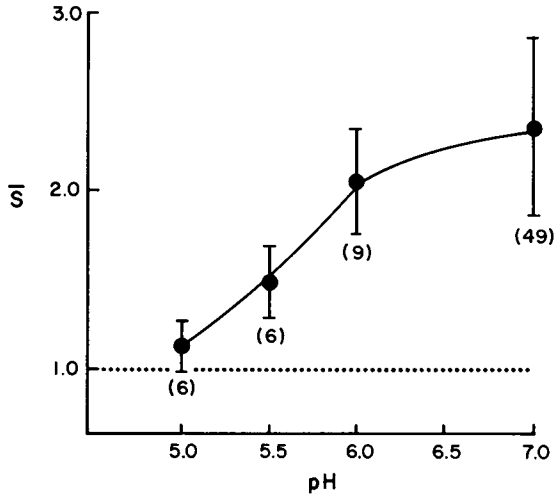

FIGURE 1 Effect of pH upon swelling of skinned fibers. Average swelling ($\bar{S}$) is defined as the ratio of the fiber cross-sectional area in test relaxing medium to the area in H_2O-saturated silicone oil. The number of fibers tested is indicated in parentheses and the bars indicate the standard deviation.

a granular, clotted appearance. In three cases where dimensions were taken there was no difference between the cross-sectional areas in pH 8.0 and those in pH 7.0.

Changes in Magnesium

Myofilament charges can also be altered by specific binding of ions, such as magnesium (Martonosi et al., 1964; Beinfield et al. 1975) to the filaments. With this in mind, the effect of altered $MgCl_2$ in the relaxing solution was tested. In these solutions the amount of added ATP was kept constant at 3 mM and the added $MgCl_2$ was either dropped to 0.3 mM or increased to 9 mM. The concentrations of free Mg in these solutions is given in Table I, calculated with the Mg and H binding constants for ATP given in Godt (1974). Transfer from standard relaxing medium to low-Mg relaxing medium

TABLE I

FREE MG IN RELAXING SOLUTIONS

Solution	Added $MgCl_2$	Calculated free Mg
	mM	*mM*
Standard	3	0.29
Low Mg	0.3	0.003
High Mg	9	6.0

Added ATP, 3 mM; pH = 7.0; other constituents as in standard relaxing solution. Binding constants used: $K_{MgATP} = 6 \times 10^4 M^{-1}$; $K_{MgHATP} = 6 \times 10^2 M^{-1}$; $K_{HATP} = 8.91 \times 10^6 M^{-1}$ (see Godt, 1974).

caused an average swelling of 10% in four fibers; transfer to high-Mg relaxing medium caused an 8% shrinkage in two fibers. These changes were fully reversible.

Altered Ionic Strength

Effects of myofilament charge can be modified by changes in ionic screening, which will be altered by changing the ionic strength of the bathing medium. Forces due to filament charge would be diminished by raising ionic strength and enhanced by decreasing ionic strength. When fibers were transferred from standard relaxing medium containing 60 mM KCl (ionic strength 0.15 M) to a solution with 200 mM KCl (ionic strength 0.29 M), they swelled still further. In the six fibers tested, fiber cross-sectional areas increased an average of 26%. On the other hand, decreasing the ionic strength to 0.09 M by deletion of KCl from normal relaxing solution had no effect on fiber cross-section. There was no correlation between fiber length and cross-sectional area over a range of sarcomere spacings from 2.12 to 3.28 μm.

When the "swelling force" was estimated with the long-chain dextran T40 (see below), we found that increasing ionic strength to 0.39 M with 300 mM KCl decreased swelling force by 8 mM (a 23% decrease), whereas decreasing ionic strength to 0.09 M had no significant effect on swelling force (see Discussion).

Long-Chain Polymers

Swollen fibers can be shrunk by adding long-chain polymers to the normal relaxing medium. If the polymer molecules are too large to penetrate into the tightly organized myofibrillar array, they will provide a counter-osmotic force. The magnitude of the swelling force can be estimated from the amount of polymer necessary to shrink the fiber to its original size and from physical chemical determinations of the osmotic pressure of that concentration of polymer.

Fig. 2 shows the relation between S and concentration for the addition of poly-

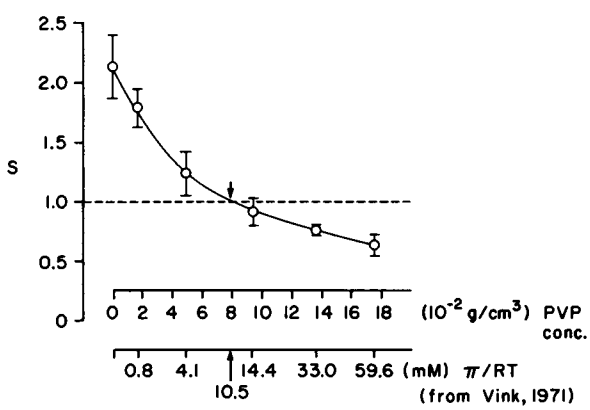

FIGURE 2 Skinned fiber cross-sectional area in relaxing solutions with PVP K30. Each point is the average from eight fibers except for the polymer-free control, which is from 49 fibers; bars give standard deviation. Osmotic pressure was (Π/RT) calculated from concentration by using membrane osmometric data of Vink (1971).

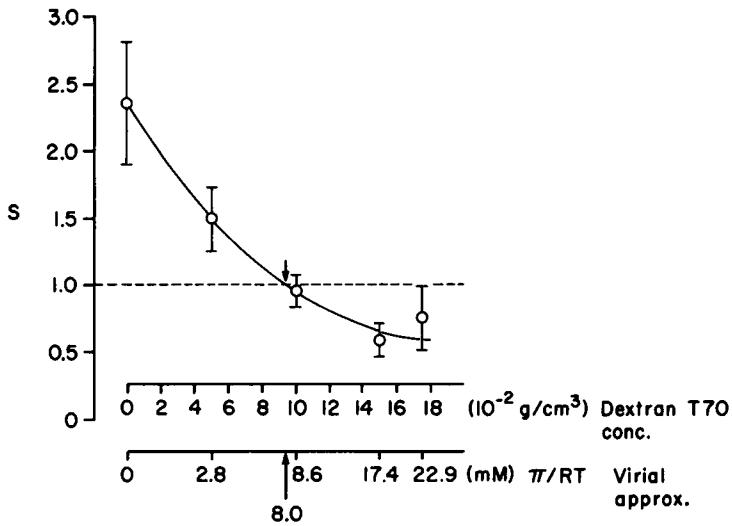

FIGURE 3 Skinned fiber cross-sectional area in relaxing solutions with added Dextran T70. The polymer-free control value is the average of 49 fibers; each other point is the average from 7 fibers; bars give standard deviation. Osmotic pressure (Π/RT) was calculated from the virial approximation as mentioned in the text.

vinylpyrrolidone (PVP) (K30, $\bar{M}_w$ = 76,000, $\bar{M}_n$ = 40,000, private communication, Dr. D. P. Wyman, GAF Corporation, Wayne, N.J.)[1] to normal relaxing medium. The abscissa is plotted both as PVP concentration and as Π/RT, where Π is the osmotic pressure, as calculated from the membrane osmometric measurements of Vink (1971), and R and T have their usual significance. Note that the relation between PVP concentration and osmotic pressure is not linear, a common finding with polymers. The swelling force, interpolated from the curve as the value of the osmotic counter pressure when S is unity, amounts to a Π/RT of 10.5 mM.

The PVP K30 we obtained from Fluka evidently contained acidic impurities since addition of PVP to neutral relaxing solution caused the pH to drop to 6.3–6.8, with higher PVP concentrations causing a greater decrease in pH. In an attempt to purify the polymer, we dialyzed a solution of PVP under pressure in an Amicon filtration cylinder (Amicon Corp., Lexington, Mass.). The resultant concentrated PVP solution was subsequently lyophilized. Addition of cleaned PVP to neutral relaxing solution only decreased the pH to 6.95, indicating that much, though not all, of the acidic impurity was eliminated. There was, however, no difference between the effects of impure and cleaned PVP on skinned fiber size.

Fig. 3 shows the results of analogous experiments with dextran T70 ($\bar{M}_w$ = 68,500; $\bar{M}_n$ = 40,300), a polymer whose average dimensions are similar to those of PVP K30 (see Discussion). Since we have no osmometric data for dextran T70, the relation be-

[1] $\bar{M}_w$, weight-average molecular weight; $\bar{M}_n$, number-average molecular weight.

tween concentration (c) and osmotic pressure for this polymer was estimated by the virial expansion: $\Pi/RT = A_1c + A_2c^2$, where $A_1 = 1/\bar{M}_n$ and $A_2 = 160[\eta]/\bar{M}_w$ (Granath, 1958, Eq. 21), where $[\eta]$ is the intrinsic viscosity. With this approximation the swelling force, Π/RT, was interpolated to be 8 mM. When a smaller dextran, the T40 fraction ($\bar{M}_w$ = 41,000; $\bar{M}_n$ = 28,000), was used, the interpolated "swelling force" appeared to be larger (35 mM), which we take to indicate that dextran T40 is less completely excluded from the myofilament lattice than dextran T70 or PVP K30.

With PVP K30 and dextran T40 and T70 the fibers shrank promptly ($\sim$10 s) and remained at constant size for up to 15 h. With solutions containing a smaller dextran, fraction T10 ($\bar{M}_w$ = 10,400; $\bar{M}_n$ = 6,200), however, fibers shrank and then swelled again to an intermediate size over the course of 1–2 min. Our interpretation is that the larger polymers are more completely excluded from the myofilament lattice than the smaller dextran T10, which seems able to penetrate the lattice to some extent, albeit more slowly than would be expected from free diffusion.

Preliminary X-ray diffraction data from Alan Magid (personal communication) indicate that the myofilament lattice of chemically skinned frog sartorius muscle in relaxing medium is swollen relative to the lattice of intact muscle. Furthermore he finds that both PVP ($\bar{M}_n \sim$ 40,000) and dextran ($\bar{M}_n \sim$ 28,000) shrink the lattice dimensions and that higher concentrations of the dextran than of PVP are required to return the lattice to near its original (intact fiber) size. Similarly, April et al. (1977) have reported that addition of PVP-10 (approximate mol wt 10,000) to relaxing solution can reverse the swelling of the filament lattice of skinned crayfish muscle fibers observed by means of low-angle X-ray diffraction.

Fiber Size and Calcium-Activated Tension

We wished to determine whether skinned fiber swelling had any influence upon the ability of the fiber to contract in high calcium solutions. Contractions were elicited in solutions with added $CaCl_2$ ($Ca^{2+} = 10^{-4}$ M); the other constituents of the solution were identical to the standard relaxing medium. If free Ca^{2+} was raised from 10^{-4} M to 5×10^{-4} M, there was no further increase in tension, ensuring that the control contractions were fully maximal. The effect on Ca-activated tension of shrinking a skinned fiber back to near $S = 1$, i.e., its original volume, was tested by transferring fibers into a contracting medium containing 10^{-4} M or 10^{-3} M free Ca^{2+}, and 9.4×10^{-2} g/cm^3 PVP. Before each contraction, fibers were equilibrated in standard relaxing medium with 9.4×10^{-2} g/cm^3 PVP. In PVP-containing solutions maximal tension occurred at the same free Ca^{2+} (i.e., 10^{-4} M), but was appreciably reduced. The effect was completely reversible; upon return to polymer-free solution maximal contraction increased to the control value. In the four fibers tested, the maximal calcium-activated tension in the PVP-containing solutions was 21% less than control contractions of the same fiber in PVP-free solutions. Similarly maximal tension was 31% less in three fibers with 9.55×10^{-2} g/cm^3 Dextran T40, indicating that shrinkage per se, rather than some specific effect of PVP, in some way diminishes the maximum calcium-activated tension that skinned fibers can generate.

DISCUSSION

Magnitude of Swelling

A number of workers have reported that, after skinning, fibers tend to swell dramatically in relaxing media containing Mg, ATP, KCl, and EGTA. Hatchett and Podolsky (reported in Ford and Podolsky, 1972) found that frog fibers skinned under silicone oil increase their diameters by 20% upon transfer to their relaxing medium; this increase corresponds to an $S(A/A_{oil})$ of 1.44. They attributed this phenomenon to an uptake of water from the fibers by the silicone oil, so that the swelling is only relative, being merely a restoration of the original volume by rehydration. However, this seems unlikely since we also observed swelling when frog fibers are skinned under water-saturated silicone oil. Additionally, intact single fibers in relaxing medium swell upon skinning. As the sarcolemma is rolled down the fiber, the freshly skinned region immediately balloons out (Godt and Maughan, unpublished observations). Gordon (unpublished, reported in Godt, 1971) observed a diameter increase of 25% ($S = 1.56$) on transfer of skinned fibers from water-saturated silicone oil to relaxing medium. Similarly, Matsubara and Elliott (1972), using low-angle X-ray diffraction patterns, observed a swelling of the myofilament lattice when a single frog muscle cell was skinned in relaxing medium. They observed that the myofilament lattice swelled by a factor of 1.40 at a sarcomere spacing of 2.2 μm, similar to previous observations in oil. This phenomenon is not confined to amphibian muscle, because April et al. (1971, 1972) have observed a similar increase (1.6-fold) of lattice volume in crayfish muscle fibers mechanically skinned in relaxing medium, and Rome (1972) found that the lattice of glycerol-extracted rabbit psoas fibers in relaxing medium was swelled about 1.25-fold relative to the lattice of intact fibers at a sarcomere spacing of 2.25 μm.

In our experiments, we observed a swelling (S) of 2.32 ($\pm$0.54 SD) when frog fibers, skinned under water-saturated silicone oil, were transferred to our standard relaxing medium. To reduce possible misestimates of swelling due to preferential fiber orientation during skinning or mounting, we measured both the width and the thickness of a fiber and assumed its cross-sectional shape was elliptical. But if we assumed the shape was a right circular cylinder and used only the width measurement to calculate swelling, the estimates were not significantly different ($P > 0.1$).

We have no explanation for the fact that we observed much more swelling than did other workers. It seems likely that the structures that preserve the integrity of the fibers after skinning would provide forces counter to the swelling force and so limit the amount of swelling. Insofar as these structures are unknown at present, comparison of our data and that of others may not be meaningful.

Mechanism of Swelling

Fundamentally the amount of swelling is less important physiologically than the mechanism underlying the swelling. One possibility is that swelling arises from the enlargement of the SR when skinned fibers are in relaxing medium (cf. plate I, Taylor and Godt, 1976). This is discounted, however, since addition of up to 200 mM sucrose to

our standard relaxing medium had no effect on skinned fiber cross-sectional area, whereas sucrose did shrink dilated SR as observed in electron micrographs of skinned fibers (Godt, unpublished observations).

The experiments with alterations of solution pH and $MgCl_2$ suggest that the charge on the myofilaments plays a role in the swelling. It is well known that the myofilaments are net negatively charged at pH = 7 (Collins and Edwards, 1971; Elliott, 1973) and that a decrease of solution pH will diminish their net charge. The isoelectric point (i.e., the pH at which a protein has no net charge) of myosin is about 5.4 (Erdös and Snellman, 1948), that of actin is 5.2 (Szent-Györgyi, 1947), and that of tropomyosin is 5.1 (Young, 1963). Fig. 1 shows that as relaxing solution pH is decreased below 6.0, skinned fibers shrink and at pH 5.0 their cross-sectional areas are nearly back to normal, in good accord with the isoelectric point of the major myofilament proteins. April et al. (1972) report a similar shrinkage of the myofilament lattice of skinned crayfish fibers as relaxing solution pH is decreased below 7.0. Contrary to our experience and that of Matsubara and Elliott (1972) with frog skinned fibers, April et al. found that the change is irreversible if pH is less than 6.4.

As seen in Table I, the concentration of free Mg^{2+} in our standard relaxing solution was about 0.3 mM, in the low-Mg solution about 0.003 mM, and in high-Mg solution about 6 mM. It is known that there is a class of Mg^{2+} binding sites on "actin" (probably actin and tropomyosin) with a binding constant around $10^3 M^{-1}$ (Martonosi et al., 1964). Beinfeld, et al. (1975) report that myosin also has Mg^{2+} binding sites with an affinity constant of about $10^3 M^{-1}$. Thus decreasing free Mg^{2+} from 0.3 to 0.003 mM will likely increase the net negative charge on the myofilaments. Conversely, increasing free Mg^{2+} to 6.0 mM will likely decrease the net negative myofilament charge. These changes in Mg^{2+} (and presumably in net filament charge) are reflected in a slight swelling of skinned fibers in the low-Mg solution and a slight shrinkage in the high-Mg solution.

The ionic strength experiments, on the other hand, are more difficult to interpret in terms of filamentary charge mechanisms. Decreasing ionic strength would presumably enhance either interfilament repulsive or Donnan-osmotic forces by less effective screening of the charged filaments and thus should lead to a swelling. Conversely, increasing ionic strength would decrease repulsive or Donnan-osmotic forces due to more effective screening and should lead to a shrinking (Brenner and McQuarrie, 1973; Overbeek, 1956). In fact, no change was seen in fiber size with a decrease of ionic strength and the fibers actually tend to swell slightly in higher ionic strength solutions. We do not understand these effects of ionic strength. However, we do know that since fibers do not swell indefinitely upon skinning, some sort of restraining forces must exist. (These could be structural forces arising from M-line structures, for instance.) The direction of size change with ionic strength in the fully swollen fibers will depend upon the nature of these countervailing forces and one can speculate that increased ionic strength might weaken these restraining forces more than it weakens the swelling forces, with the result that the fibers would tend to swell further in high ionic strength solutions.

To test the hypothesis more directly, we examined the influence of ionic strength on the swelling pressure as measured by shrinking fibers back to their original size with dextran T40. We observed that decreasing ionic strength from 0.15 M to 0.09 M had no significant effect on swelling pressure, while increasing ionic strength to 0.39 M caused swelling pressure (Π/RT) to decrease by about 8 mM relative to that in normal relaxing medium. Subsequent calculations of expected changes in swelling pressure, using equations we developed for a Donnan-osmotic swelling mechanism, are in good accord with our experimental observations. Using the values of 3.15×10^{17} e^-/m^2 and 2.55×10^{17} e^-/m^2 for the effective surface charge densities on the thick and thin filaments, respectively, we calculate that swelling pressure should decrease by 8 mM in the high ionic strength solution and increase by 2 mM in the low ionic strength solution relative to the value in normal relaxing medium as determined with dextran T40.

Swelling, Shrinking, and Calcium-Activated Tension

The experiments on maximal calcium-activated tension of skinned fibers shrunken to near their original volumes with PVP or dextran are a bit puzzling. The absolute tension was most definitely diminished from that obtainable in the same fiber without polymer present, was not due to submaximal activation, since increasing free Ca^{2+} did not increase the tension, and was completely reversible. This phenomenon bears some resemblance to the potentiation of tetanic tension of intact muscle by hypotonic solutions (Edman and Andersson, 1968; Okada and Gordon, 1972; April and Brandt, 1973), whereby a fiber yields more tetanic tension when swollen in the hypotonic solution than it gives in control solutions. Although hypotonic potentiation in intact fibers can be explained to some extent by the potentiating effect of decreased internal ionic strength (down to $\Gamma/2 = 0.10$ M) (Gordon et al., 1973), in our case ionic strength remained constant. One can speculate that under our conditions, when fibers are highly swollen, there may be some influence of myofilament lattice volume on tension production. Although April and Brandt (1973) report that swelling of the lattice of crayfish muscle fibers up to 130% in hypotonic but isosmotic KCl solutions has little influence on caffeine-induced tension, our observations indicate that frog skinned fibers swollen to more than 200% of their initial volume are able to generate more calcium-activated tension, although the reasons for this remain obscure.

Magnitude of Swelling Force

In an attempt to quantify the swelling force, we applied an osmotic counter-force by adding the long-chain polymers PVP and dextran to standard relaxing solution. As interpolated from data in Fig. 2, the fibers were shrunk back to their original size at a PVP concentration of 8×10^{-2} g/cm^3. This corresponds to an osmotic pressure, Π/RT, of 10.5 mM, by assuming that PVP is completely excluded from the fiber interior (Vink, 1971). In similar experiments with dextran T70, fibers were shrunk to their original size at a polymer concentration of 9.5×10^{-2} g/cm^3, which corresponds to an osmotic pressure of approximately 8 mM. The value of swelling pressure inter-

polated from experiments with dextran T40 is, however, 35 mM. We take this to indicate that dextran T40 is less completely excluded than PVP K30 or dextran T70. The "size" of PVP K30 and of dextran T70 is, however, very similar to that of dextran T40. The radius of gyration of dextran T70 and PVP K30 is about 7.8 nm and that of dextran T40 is about 7.3 nm (but see below), and one would expect rather complete exclusion of polymers of this size from the myofilament lattice due to steric hindrance. Synthetic polymers are, however, seldom of a single molecular weight, more often being a heterogeneous collection of fractions with a range of chain lengths. For example, data on the dextran T40 molecular weight distribution obtained by gel filtration (Pharmacia data sheet, lot 2771) indicates that molecular weights of fractions of this chemical range up to 130,000 or so but with 90% below 70,000. Dextran T70 fractions range up to 250,000 with 90% below 140,000. We have no similar data on the range of molecular weights for PVP K30, but it is clear from the weight- and number-average mol wt that PVP K30 has a distribution similar to that of dextran T70. With a greater preponderance of high molecular weight fractions, dextran T70 and PVP K30 would be better excluded from the myofilament lattice than would dextran T40 or T10.

The values for osmotic pressure of PVP for Fig. 2 were calculated from data obtained with a membrane osmometer. It is very likely that the selectivity of the membrane of this device, which would exclude virtually all polymer molecules above about 10,000 mol wt, would be different from that of the myofilament lattice, which would probably only exclude those above a somewhat larger molecular weight. On the other hand, since we have no information on the exclusivity of the lattice, we shall use the osmometric data as a good approximation of the effective osmotic pressure of PVP solutions on the lattice. In our subsequent work we have therefore used the PVP-derived value of 10.5 mM as the true swelling pressure in our relaxing solution.

One measure of the effective "size" of random-coil polymers is their radius of gyration (R_G), defined as the root-mean-square weight-average distance of each mass element of the polymer from the center of mass of the entire polymer. For any particular polymer, R_G can be derived from measurements of intrinsic viscosity $[\eta]$, molecular weight, and from relations between $[\eta]$ and R_G in polymer theory. For two random-coil polymers, *a* and *b*, of the same molecular weight, Tanford (1961) shows that: $[\eta]^a/[\eta]^b = [R_G^a/R_G^b]^3$. (Note, however, this equation strictly applies only to polymers homogeneous with respect to molecular weight. We use it here only to give an approximation for the "average" size of these heterogeneous polymers and as a basis for comparison between them). Vink (1971) gives values of $[\eta] = 0.26$ dl/g for K30 PVP and $[\eta] = 0.21$ dl/g for T40 dextran, which yield: $R_G^{PVP}/R_G^{dextran\ T40} = 1.07$. For linear dextran, $M_W = 41{,}000$, $R_G = 7.3$ nm (Granath, 1958); thus for K30 PVP, $R_G = 7.8$ nm. Similarly for dextran T10 $[\eta] = 0.102$ dl/g, $R_G = 5.8$ nm, and for dextran T70 $[\eta] = 0.26$ dl/g and $R_G = 7.8$ nm.

Physiological Implications of Swelling

Skinned fibers in relaxing medium differ in three respects from intact fibers in Ringer's solution: (*a*) the sarcoplasmic reticulum of skinned fibers is swollen, (*b*) interfilament spacings are greater than in intact fibers, and (*c*) the constant volume behavior of the myofilament lattice is lost (Matsubara and Elliott, 1972). Swelling may well affect the ability of the SR to sequester or release calcium and could be a complication in the use

of the skinned fiber preparation for study of SR function (cf. Endo, 1977). For instance, Thorens and Endo (1975) report that "depolarization"-induced calcium release from skinned fibers of *Xenopus laevis* is completely inhibited by addition of 40 mM or higher concentrations of sucrose or other sugars to relaxing medium. Since higher concentrations (200 mM) of sucrose can partially reverse SR swelling, this raises the possibility that depolarization-induced release may only occur in abnormally swollen SR. In this regard, however, shrinking skinned fibers (and presumably their SR as well) to near their oil volume with either PVP or dextran T40 has no effect on either calcium-induced or depolarization-induced release of calcium from skinned fibers of *Rana temporaria* (Godt, unpublished observations).

When skinned fibers swell, the distance between myofilaments increases; if swelling of the whole fiber accurately reflects the swelling of the lattice, our observations would indicate that interfilament distances increase 1.52-fold ($\sqrt{2.32}$) on the average. In spite of this, frog skinned fibers produce maximal calcium-activated tensions in the range 2–3 kg/cm^2, comparable to maximal tetanic tension in intact single fibers. Contrary to our expectation, however, shrinking skinned fibers to their original size decreased maximal tension somewhat. Thus it would appear that interfilament spacing may well influence generation of tension by skinned fibers. This is not consistent with Edman and Andersson (1968) or April and Brandt (1973), who concluded, from studies with intact fibers, that myofilament spacing has no effect on tension generation.

The loss of constant volume behavior in skinned fibers may have an effect upon dynamic properties of the contractile apparatus, although very little is known about the physiological importance of this constancy of lattice volume. In intact muscle, interfilament spacings, and thereby cross-bridge working distances increase as shortening progresses. In mechanically skinned fibers, Matsubara and Elliott (1972) showed that the increase in interfilament spacing with shortening is very much smaller. Similarly, Magid (unpublished observations) has found that chemical skinning of frog sartorius muscle with the detergent Triton X-100 abolishes the constant lattice volume behavior. On the other hand, Rome (1972) reported that the myofilament lattice of rabbit psoas fibers chemically skinned by glycerol-extraction retains its constant volume behavior in relaxing medium. If constant lattice volume is critical to normal cross-bridge function, these observations indicate that not all skinned fiber preparations have similar dynamic properties and that some skinning procedures may be preferable to others for studies of contractile behavior.

We have no clear reason to recommend the routine use of polymers in bathing solutions for skinned fibers. On the one hand, fiber swelling can be minimized by including a long-chain polymer in all solutions. On the other, shrunken fibers generate less tension and inclusion of the polymer does not restore the constant volume behavior of the lattice (A. Magid, personal communication). Further complications are possible binding of ionic constituents by the polymer and the question of impurities in commercially available polymers, especially PVP. (In this regard, dextran T70 is probably somewhat better). Currently we are not including polymers in our experimental solutions.

Nature of Swelling Force

Having discounted SR swelling and having implicated myofilament net charge as factors in the swelling of skinned fibers, we believe that there are then basically two mechanisms that could be responsible for the swelling force. The first is the mutual repulsive force arising between the similarly charged filaments in the lattice. The second, and to us more likely, is the Donnan-osmotic force due to water entering the filament lattice to dilute the excess concentration of counter-ions surrounding the charged filaments. The nature of these forces and our reasons for believing that swelling is due to a Donnan-osmotic mechanism will be described in a subsequent publication.

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