STRETCH AND RADIAL COMPRESSION STUDIES ON RELAXED SKINNED MUSCLE FIBERS OF THE FROG

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ABSTRACT The influence of stretch and radial compression on the width of mechanically skinned fibers from the semitendinosus muscle of the frog (*R. pipiens*) was examined in relaxing solutions with high-power light microscopy. Fibers were skinned under mineral oil. We find that, after correcting for water uptake in the oil, fiber width increased by an average of 28% upon transfer from oil to relaxing medium, with some tendency for greater swelling at longer sarcomere lengths. Subsequently, fibers were compressed by addition of the long-chain polymer polyvinylpyrrolidone (PVP-40, number average molecular weight 40,000) to relaxing solutions. Sarcomere length does not appear to be affected by addition of PVP. At any PVP concentration, the inverse square of the fiber width increased smoothly and linearly with increasing stretch for sacromere lengths between 2.10 and 4.60 μm. At any fixed sarcomere length, fiber width decreased linearly with the logarithm of the osmotic compressive pressure exerted by PVP (2–10% concentration). From this logarithmic relation we estimate that the swelling pressure of the intact fiber is 3.40×10^3 N/m^2, between that of a 2 and a 3% PVP solution. The pressure giving rise to fiber swelling is not due to dilation of the sarcoplasmic reticulum (SR), since the experimental results above were not significantly different after treatment with 0.5% BRIJ-58, a nonionic detergent that disrupts the SR. Swelling may be due simply to elastic structures within the fiber that are constrained in the intact cell. Values of bulk moduli of fibers, calculated from the compression experiments, and preliminary measurements of Young's modulus from stretch experiments, are quantitatively consistent with the idea that skinned fibers behave as nonisotropic elastic bodies.

INTRODUCTION

Single skeletal muscle fibers swell markedly in an aqueous relaxing medium after removal of the sarcolemma. A number of investigators have attempted to come to grips with this swelling phenomenon (April et al., 1972; Matsubara and Elliott, 1972; Elliott, 1973; April and Wong, 1976) because it might yield insight into forces within the muscle cell and indicate a limitation of the skinned fiber preparation as a model muscle system. In previous work (Godt and Maughan, 1977) we examined this phenomenon and came to some tentative conclusions concerning the nature of the swelling force.

In our previous study, fibers were skinned in silicone oil. Recently, however, we discovered that there is uptake of water by silicone oil and that fibers left in silicone oil become progressively dessicated. This makes our previous estimates of swelling artifactually high. In the present study we use mineral oil, which we find imbibes an order of magnitude less water from the fibers; together with measurements of the time-course of fiber dehydration, the use

of mineral oil permits more accurate estimates of the magnitude of swelling and of the swelling force.

In the present work, we set out to examine the swelling phenomenon in more quantitative detail, using high-power microscopy to examine the influence of changes in sarcomere length and externally applied osmotic pressure on fiber width.

METHODS

Preparation and Solutions

Fibers from the semitendinosus muscle of the frog (*R. pipens*) were mechanically skinned under water-saturated mineral oil (Aldrich Chemical Co., Milwaukee, Wis.) and then transferred to relaxing solution containing (in mM): 2.80 $MgCl_2$, 2.14 Na_2ATP, 7 EGTA, 20 imidazole, 15 creatine phosphate, 54 KCl, with pH 7.0, temperature 22°C, ionic strength 0.15 M, and varying concentrations of polyvinylpyrrolidone (PVP-40, number average molecular weight [$\overline{M}_n$] 40,000) or dextran (T500, weight average molecular weight [$\overline{M}_w$] = 478,000; $\overline{M}_n$ = 192,000). These solutions contained 2 mM MgATP and 0.5 mM Mg^{2+} as determined from the appropriate binding constants (Godt, 1974). Details of the skinning procedure can be found in Godt and Maughan (1977). Care was taken to choose fibers that appeared to be round in cross-section, rather than ribbonlike as they sometimes are after skinning. In most cases fibers were pretreated for 10–20 min in relaxing solutions containing 0.5% BRIJ-58 (polyoxyethylene 20 cetyl ether), a nonionic detergent, which extensively disrupts the sarcoplasmic reticulum (Orentlicher et al., 1974). This treatment had no effect upon fiber width or striation spacing, thus strengthening our previous conclusion (Godt and Maughan, 1977) that the sarcoplasmic reticulum plays no role in the swelling phenomenon. Reagent grade KCl and KOH were obtained from Fisher Scientific Co. (Pittsburgh, Pa.); other chemicals, including elastase (type III), were obtained from Sigma Chemical Co. (St. Louis, Mo). Dextran T500 was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.).

Mounting and Observation

Segments of the skinned fibers (40–100 μm width, 1–3 mm long) were mounted between clamps, transferred to a 1-ml solution chamber, and observed with an inverted compound microscope (Invertoscope D. Zeiss, Oberkochen, West Germany). Photographs were taken with a 35-mm camera (PM-6, Olympus Corporation of America, New Hyde Park, N.Y.). Laser diffraction patterns from the fibers were produced with a 0.5-mW He-Ne laser (model 155; Spectra-Physics Inc., Laser Products Div., Mountain View, Calif.). Zero- and first-order diffraction lines were focused on the rear focal plane of the microscope objective lens (40×, N.A. 0.60, 1.3 mm working distance) and projected for measurement onto a grid with a focusing telescope (10×). The system was calibrated with diffraction gratings of known spacing.

Striation Spacing and Fiber Width Measurements

As a check on the condition of each fiber, we observed the laser diffraction pattern to monitor striation spacing during the course of the experiments. However, measurements of both striation spacing and fiber width used for quantitative analysis were taken from light micrographs (image enlargement, 699×). All direct observations and photography of striations and fiber width were taken in the center of the observed region (~0.3 mm long), at the same spot on the fiber under all experimental conditions.

Prints were made from negatives using a microfilm printer-copier (model 400; 3M Co., St. Paul, Minn.). Width measurement was made at a specified place, using landmarks on the fiber. Striation spacing was estimated by averaging 10 individual sarcomeres at five places, from one edge of the fiber to the other, along the line used for the width measurement. Sarcomere length was taken to be the average of the five determinations (representative standard deviations given in Fig. 2). As monitored by the

landmarks, fibers did not change orientation (i.e., roll or twist) in the field of view when they were stretched or when shrunk with PVP.

To rule out optical artifacts arising from the different refractive indices of the PVP solutions, a platinum wire (diameter 87.2 μm), roughly the size of a fiber, was photographed in all of the PVP solutions used, as well as in mineral oil. The wire was the same size in all solutions, ruling out optical artifacts in the width measurements.

Correction for Fiber Dehydration in Oil

Muscle fibers appear to lose water to the oil in which they are skinned, thus reducing the fiber width (Hatchett and Podolsky in Ford and Podolsky, 1972). To test whether oil takes up water, we measured the uptake of water by oil, using tritium-labeled water (New England Nuclear, Boston, Mass.) diluted to an activity of 10 μCi/ml. Labeled water (5 ml) was vigorously mixed with an equal volume of either silicone oil (Dow Corning 200, Dow Corning Corp., Midland, Mich., 10 cs) or mineral oil in 20 ml plastic scintillation vials on a shaker (New Brunswick Scientific Co., Inc., Talmadge, N.J.). After various periods of shaking, the phases were separated by centrifugation at 500 *g* for 5 min. Samples (40 μl) from the oil phase were dissolved in a toluene-based fluor and radioactivity was measured in a liquid scintillation counter. Because of the low radioactivity, each sample was counted for 50 min. Subsequently, internal standards of ^{3}H-toluene were used to determine individual counting efficiencies for each sample. Equilibration of water into oil was essentially complete with 10 min. The actual amount of water in the oil phase was extremely small, 64–122 nl H_2O/ml of silcone oil and 10–18 nl H_2O/ml of mineral oil, thus demonstrating that although oil and water are practically immiscible, oil does nevertheless take up water. Inasmuch as the water capacity of mineral oil is some tenfold less than silicone oil, mineral oil is preferred.

Consider now the actual amount of water associated with a typical skinned fiber. For a fiber 50 μm wide, each millimeter of length has a volume of ~2 nl, most of which is water. It is easy to see how such a fiber might be dessicated if even 10 nl H_2O are taken up per milliliter of oil. In previous studies we have attempted to minimize this problem by working with water-saturated oil. However, the oil may not remain saturated during the course of an experiment since our experimental chamber is open to the atmosphere. To examine the pertinent case of water extraction from a skinned fiber under our conditions, we observed the time-course of the width changes of fibers in mineral oil over a 60-min period (fibers are normally in oil for between 10 and 40 min). The fractional decrease of width of 11 unskinned and 4 skinned fibers was approximately linear with time over the 60-min period. The rates of width decrease of skinned and unskinned fibers were not significantly different ($P < 0.05$). Expressed as a fraction of the initial extrapolated width the rate of decrease was 0.11 (±0.14)% per minute. In further tests of dehydration rate, three fibers were skinned and mounted under oil and transferred to aqueous relaxing medium to rehydrate the fiber and to establish a standard initial condition. They were then returned to oil for observation and photographed at various times. The rate of width decrease in these fibers was not significantly different ($P < 0.05$) from that obtained on the fibers that had not been rehydrated. To assess whether width decrease was uniform along the length of a fiber, we estimated the mean width of each of these same three fibers from low-power photographs (175×). The mean width of the fiber was determined from the length of the fiber and the area of the fiber image on the print. The rate of decrease of the mean width was not significantly different ($P < 0.05$) than that determined previously for these fibers.

Determination of Young's Modulus

Young's modulus was estimated for resting skinned fibers near slack length. Fibers were stretched by ~10% from a resting sarcomere spacing near 2.2 μm. The associated passive tension was measured after stress relaxation, when tension was very nearly steady (1–2 min after the stretch). The cross-sectional area of the fiber was approximated by $\pi D^2/4$, were D is the fiber width. Young's modulus was calculated as stress (tension per cross-sectional area of fiber before stretch) divided by strain (sarcomere length change per initial sarcomere length).

RESULTS

Amount of Swelling of Skinned Fibers

When a skinned fiber is transferred from mineral oil to relaxing solution it swells rapidly (Godt and Maughan, 1977). To quantitate the swelling, we must have a value for the *in situ* size that the particular collection of myofibrils had in the intact cell, which should correspond closely to that size seen in mineral oil. However, the observed width must be corrected for the dehydration of each fiber in mineral oil, which, from our control experiments, amounts to a width decrease of 0.11% per minute. After correction, the average amount of swelling of the fiber width (for sarcomere lengths between 1.98 and 2.54 μm) in mineral oil is 28 ($\pm$13)%, for 10 fibers. Fibers at longer sarcomere lengths tended to swell more, but this tendency was not statistically significant using a population correlation coefficient t-test (Ott et al., 1978, p. 416).

Variation of Fiber Width with Osmotic Compressive Pressure

Fig. 1 demonstrates the ability of increasing PVP concentrations to shrink a skinned fiber. Complete data from this same fiber is shown in Fig. 2. In general, increasing PVP concentration does not appear to change sarcomere spacings. In converting PVP concentrations to osmotic pressures we assume that the exclusivity of the fiber to this rather large random-coil polymer is similar to that of the membrane of a membrane osmometer. That is, the relation between polymer concentration and osmotic pressure determined by Vink (1971) holds for our fibers. For a detailed discussion of this point, see Godt and Maughan (1977).

The data in Fig. 2 can be fitted between 2 and 10% PVP with an empirical expression of the form

$$D/D_0 = b \ln (\pi/\pi_0) + 1; \quad \pi > 0, \tag{1}$$

where D is the fiber width, D_0 is the width in 4% PVP solution at any given sarcomere length, π is the osmotic pressure exerted by PVP, π_0 is that pressure exerted by a 4% PVP solution

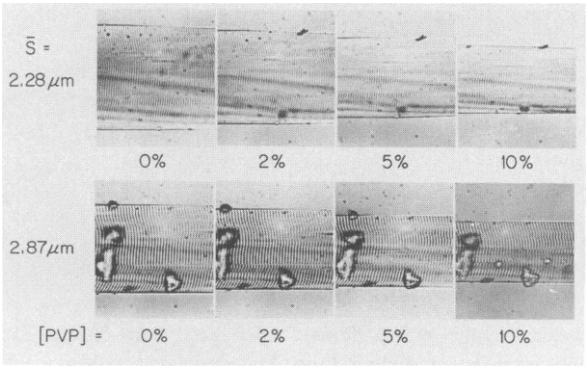

FIGURE 1 Variation of fiber width with increasing PVP concentrations at two sarcomere lengths. Percent concentration of PVP is in grams per milliliter (e.g., 2% is 0.02 g PVP per cm^3 total solution). Width and sarcomere length measured in the center of the frame. Sarcomere lengths differ by no more than 1.8% when PVP concentration is changed. Note from the position of dust particles and oil droplets adhering to the fiber that stretching and shrinking do not cause the fiber to roll or twist. Fiber 11-17-77.

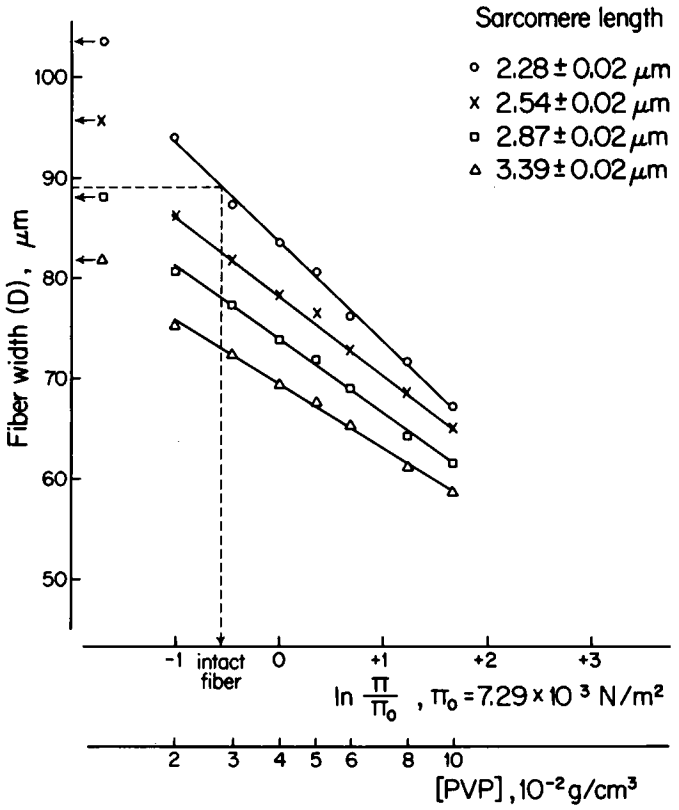

FIGURE 2 Variation of fiber width with osmotic compressive pressure at four sarcomere lengths (fiber of Fig. 1). Pressure (π) calculated from pressure-concentration relation for PVP determined by Vink (1971): $\pi = k_1c + k_2c^2 + k_3c^3$, where $k_1 = 0.878$ atm · cm^6/g^2, $K_2 = 17.25$ atm · cm^6/g^2, and $k_3 = 144.1$ atm · cm^9/g^3, osmotic pressure (π) and PVP concentration (c) are given in atmospheres and grams per cm^3, respectively. Pressure normalized at 4% PVP concentration, where $\pi(=\pi_0)$ is 0.0719 atm, or 7.29×10^3 N/m^2. Symbols and arrows on abscissa indicate width of fiber at indicated sarcomere spacings in PVP-free solution. Horizontal dashed line indicates corrected width in mineral oil for fiber at 2.28 μm.

(7.29×10^3 N/m^2), and b is a constant for any given sarcomere length. A least-squares fit of pooled data for 10 fibers (2.2–3.7 μm) yielded $b = -0.097$ ($r = 0.99$).

By using relations such as those illustrated in Fig. 2, the swelling pressure of the intact fiber can be determined from the concentration of PVP necessary to compress the fiber back to its width in oil, as corrected for dehydration. For example, the fiber in Fig. 2 spent 43 min in mineral oil during which the fiber was skinned, mounted in the muscle chamber, and photographed. At the end of this time the fiber width was 84.9 μm and sarcomere length was 2.31 μm. As mentioned in Methods, we observe an average decrease of 0.11%/min in fiber width. Thus the initial fiber width is 84.9 μm/[1 − (0.0011) (43)] = 89.1 μm. This corresponds to a pressure of 4.2×10^3 N/m^2, somewhat less than that exerted by the 3% PVP solution (Fig. 2). Stated another way, this skinned fiber could be returned to its physiological, *in situ* width by including between 2 and 3% PVP in the bathing medium. A similar treatment for five skinned fibers with sarcomere spacing between 1.98 and 2.51 μm gave a swelling pressure of $3.2 (\pm 1.3) \times 10^3$ N/m^2, also midway between a 2 and 3% PVP solution. In these

fibers we saw no consistent relationship between swelling pressure and either sarcomere length or fiber width.

Variation of Fiber Width with Sarcomere Length

Returning once again to Fig. 1, note that, in a given solution, the fiber grows thinner as it is stretched. Fibers stretched over a broad range of sarcomere lengths (2.0–4.6 μm) indicate that width decreases as a hyperbolic function of sarcomere length (Fig. 3), obeying the empirical expression

$$D_0'^2/D^2 = a_1 S + a_0 \tag{2}$$

where S is the sarcomere length, D_0' is the width at $S = 2.2$ μm at each PVP concentration, and a_0 and a_1 are constants for each PVP concentration. This expression also fits data obtained from fibers stretched in mineral oil. This equation fits the data quite well, with high correlation coefficients. However, a linear equation of the form $D/D_0' = a_1' S + a_0'$ fits the data almost as well, although the correlation coefficients were somewhat lower that for the hyperbolic relation.

Table I presents mean values for a_0 and a_1 of Eq. 2 for seven fibers stretched between 2.0 and 4.6 μm. Note that in all cases, the widths do not decrease as rapidly with stretch at higher PVP concentration; i.e., the parameter a_1 decreases with increasing PVP concentration. The 95% confidence limit for the true mean intercept of each linear regression curve indicates, with reasonable certainty, that $a_0 > 0$ for 10% PVP and $a_0 < 0$ for mineral oil. There is, however, a distinct tendency to approach constant volume behavior (i.e., $a_0 = 0$) in solutions containing between 2 and 4% PVP.

Fig. 3 also illustrates that the fiber width in 4% PVP solution is similar to that in a solution of the same osmotic pressure containing a much larger polymer (dextran T500, $\overline{M}_w$ = 478,000, $\overline{M}_n$ = 192,600). This supports our previous suggestion (Godt and Maughan, 1977) that PVP-40 ($\overline{M}_n$ = 40,000) is for all practical purposes excluded from the fiber. Low molecular weight fractions of both polymers will, of course, enter the fiber, but the amount of polymer that enters is probably insignificant.

Fibers stretched in solutions containing more than 10% PVP do not shorten when the muscle ends are brought closer together again; they simply go slack. This occasionally occurs as well in solutions containing as little as 8% PVP. Only when the fibers are returned to solutions containing lower concentrations of PVP do fibers shorten rather than slacken. Fibers also get progressively stiffer and more resistant to passive stretches at higher PVP concentrations. In 20% PVP, in fact, we couldn't change the striation spacing without breaking the fiber.

Elastic Properties

In this section we examine some consequences of treating the fiber as if it were an elastic body. The modulus of elasticity for compression of an elastic body is called the bulk modulus, which is defined by the equation

$$dP = -K\frac{dV}{V}, \tag{3}$$

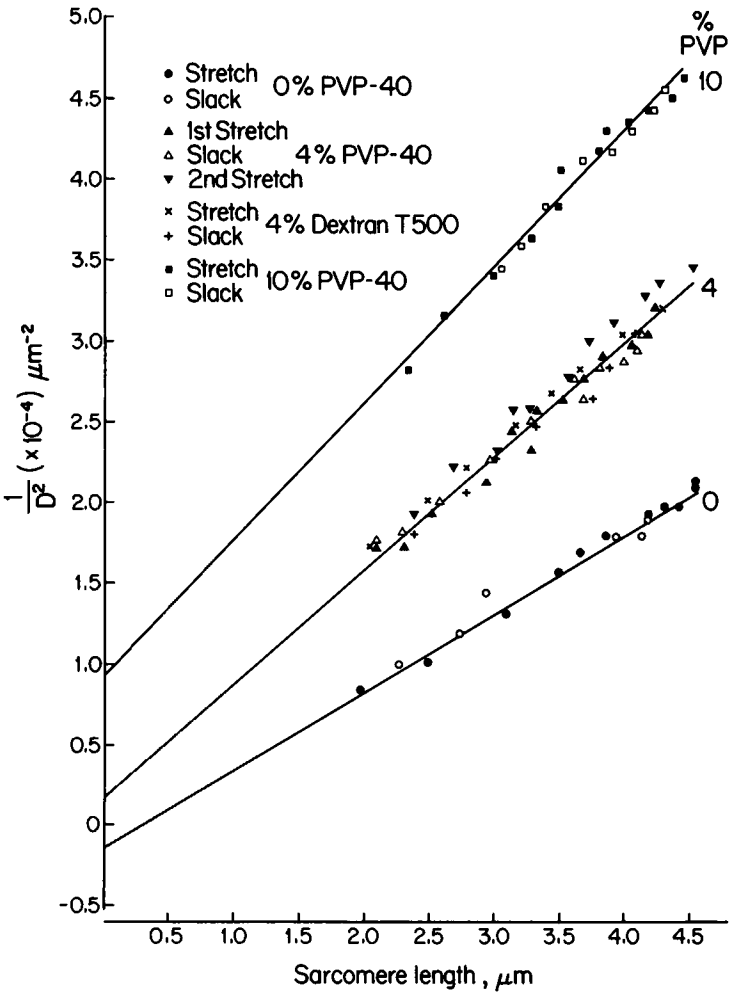

FIGURE 3 Variation of the inverse square of the fiber width with sarcomere length (2.1–4.6 μm) at 0, 4, and 10% PVP-40 (circles, triangles, and squares, respectively) and 4% dextran T500 (crosses). Closed symbols (and x) refer to data obtained after stretching the fiber, open circles (and +) after slackening the fiber. Second stretch in 4% PVP performed after run with dextran. Solid lines, least-squares fit of Eq. 2 to data points, where the values of the parameters at 0% PVP: a_0 = 0.139, a_1 = 0.518, r (correlation coefficient) = 0.99; 4% PVP (mean of first and second stretch): a_0 = 0.097, a_1 = 0.411, r = 0.98; 10% PVP: a_0 = 0.937, a_1 = 0.835, r = 0.99. Note closeness of fit of dextran points to 4% PVP curve; both solutions have an osmotic pressure of 7.29 × 10^3 N/m^2. PVP osmotic pressure determined from Vink's (1971) equation (cf. Fig. 2). Dextran pressure determined from Granath (1958) equation 21: $\pi/RT = A_1c + A_2c^2$, where $A_1 = 1/\overline{M}_n$ and $A_2 = 160\,[\eta]/\overline{M}_w$, where $[\eta]$ is the intrinsic viscosity. For dextran T500 (lot 7863), $\overline{M}_n$ = 192,000, $\overline{M}_w$ = 478,000, $[\eta]$ = 0.51 dc/g. Fiber 8-11-78.

where P is pressure, V is volume, and K is the bulk modulus. By analogy, for skinned fibers in PVP solutions, we have

$$d\pi = -K\frac{d(D^2S)}{D^2S}, \tag{4}$$

TABLE I

VARIATION OF FIBER WITH (D) WITH SARCOMERE LENGTH ($2.0 < S < 4.6$ μm) ACCORDING TO THE EXPRESSION $D'^2_0/D^2 = a_1S + a_0$, WHERE D'_0 IS THE WIDTH OF THE FIBER AT $S = 2.2$ μm IN EACH PVP SOLUTION

Fiber	Mineral oil			0% PVP			2% PVP			3% PVP			4% PVP			10% PVP		
	a_0	a_1	(r)	a_0	a_1	(r)	a_0	a_1	(r)	a_0	a_1	(r)	a_0	a_1	(r)	a_0	a_1	(r)
8/11/78‡	—	—	—	−0.139	0.518	(0.99)	—	—	—	—	—	—	0.097	0.411	(0.98)	0.338*	0.301	(0.99)
11/17/77	—	—	—	0.290*	0.323	(0.99)	—	—	—	—	—	—	0.316*	0.311	(0.97)	0.509*	0.223	(0.84)
11/30/77	−0.289*	0.586	(0.97)	−0.448*	0.658	(0.98)	—	—	—	—	—	—	0.248*	0.342	(0.99)	0.357*	0.292	(0.95)
12/14/77	−0.387*	0.631	(1.00)	−0.210*	0.359	(1.00)	—	—	—	—	—	—	0.301*	0.318	(1.00)	—	—	—
1/12/78	−0.113*	0.506	(1.00)	−0.423*	0.647	(0.98)	−0.130	0.513	(0.99)	—	—	—	0.070	0.423	(1.00)	—	—	—
10/11/78‡	—	—	—	—	—	—	−0.007	0.420	(0.99)	−0.006	0.457	(1.00)	0.059	0.392	(1.00)	—	—	—
1/18/79‡	—	—	—	−0.108	0.504	(0.98)	−0.179*	0.536	(0.99)	−0.285*	0.584	(0.99)	0.116	0.402	(0.96)	—	—	—

*Indicates that a_0 is significantly different from 0 ($P < 0.05$); i.e., that the 95% confidence limits for the true a_0 intercept lies outside the point $a_0 = 0$. The symbol r refers to the Pearson product-moment correlation coefficient (Ott et. al., 1978, p. 412), which measures the strength of the linear relationship between D'^2_0/D^2 and S.
‡Indicates experiments carried out in solutions containing (in mM): 8.06 $MgCl_2$, 3.04 Na_2 ATP, 5 EGTA, 61.2 KCl, 20 imidazole, 15 creatine phosphate, yielding 3 mM Mg ATP and 3 mM Mg^{2+}; other concentrations as given in the Methods section.

assuming that D^2S is proportional to fiber volume (V) and that the fiber approximates a right circular cylinder, where π is the osmotic squeezing pressure, D is width, and S is sarcomere length. Since we observe that S does not appear to vary with π, this equation simplifies to

$$d\pi = -K\frac{d(D^2)}{D^2}. \tag{5}$$

This expression then allows us to use data such as represented in Fig. 2 to determine the bulk modulus of skinned fibers.

Consider the range of PVP concentration from 2–10%. From Eq. 1,

$$-\frac{1}{K} = \frac{d(D^2)}{D^2 d\pi} = \frac{2b}{1 + b \ln \dfrac{\pi}{\pi_0}}. \tag{6}$$

Thus, with rearrangement,

$$K = -\frac{\pi\left(1 + b \ln \dfrac{\pi}{\pi_0}\right)}{2b}. \tag{7}$$

Table II gives values for K as a function of π for the fiber shown in Fig. 1. In this fiber, there was a tendency for the bulk modulus to increase with stretch, although this was not a consistent finding. Note, however, the marked increase of bulk modulus with PVP concentration, showing that as the fiber is compressed it becomes more resistant to further compression. This was a consistent finding in all six fibers studied (Table III).

For an elastic body, the one-dimensional elasticity (Young's modulus, E) is related to the bulk modulus by the equation

$$K = \frac{E}{3(1 - 2\nu)} \tag{8}$$

TABLE II
VARIATION OF BULK MODULUS (K) WITH COMPRESSION AND STRETCH
(FIBER 11-17-77)

		Sarcomere length (micrometers)				
PVP	π	2.32 ± 0.05*	2.28 ± 0.02	2.54 ± 0.03	2.87 ± 0.02	3.39 ± 0.02
%	$(N/m^2)(\times 10^3)$	K (From Eq. 7) in N/m^2 $(\times 10^4)$				
2	2.59	1.26	1.26	1.46	1.47	1.61
3	4.64	2.12	2.12	2.48	2.51	2.75
4	7.29	3.17	3.16	3.74	3.74	4.15
5	10.64	4.41	4.41	5.25	5.30	5.85
6	14.78	5.91	5.88	7.06	7.12	7.89
8	25.77	9.59	9.54	11.59	11.70	13.04
10	40.97	14.30	14.22	17.47	17.66	19.78

*The bulk modulus data in column 1 (S = 2.32 μm) was obtained from the fiber before it was treated with BRIJ. After subsequent treatment with BRIJ, the data in column 2 (S = 2.28 μm) was obtained and were not different. Since the bulk modulus is unchanged by BRIJ treatment, this strengthens our conclusion that the sarcoplasmic reticulum plays no significant role in fiber swelling.

where ν is Poisson's ratio, a measure of the relative ability of material to resist changes in shape and volume, which must, for physical reasons, lie between 0–0.5 (Wainwright et al., 1977). Young's modulus, obtained by stretching fibers, and the bulk modulus, obtained by compressing fibers, will therefore determine a Poisson's ratio.

We know of no experiments where the Young's modulus of mechanically skinned frog fibers has been carefully determined. However, from preliminary experiments, we find that E is 3.6×10^4 N/m^2 (S.D. 1.6×10^4, $n = 4$) for fibers stretched in the range 2.22–2.60 μm in 4% PVP solutions, assuming that the fiber shape is that of a right circular cylinder. For this value of E and the average value of K in 4% solutions, Poisson's ratio is 0.35, a value between that of polyethylene (ν = 0.33) and hard rubber (ν = 0.39) (cf. Wainwright et al., 1976). The characteristic of an isotropic elastic body is that its Poisson's ratio is 0.25 (Timoshenko, 1958); thus our data indicate that skinned fibers are not isotropic, which the available morphologic data support.

DISCUSSION

Upon transferring the fiber from mineral oil to relaxing solution, we observe a 28% increase of fiber width, on the average, after correction for fiber dehydration in mineral oil. In previous experiments (Godt and Maughan, 1977) we measured a swelling of cross-sectional area of 2.32-fold which, assuming that the fiber shape is approximately that of a right circular cylinder, corresponds to an 52% increase of fiber width. Part of this increase was most certainly an artifact due to the uptake of fiber water by silicone oil. Our new corrected value for the increase in width is more in accord with others in the literature for frog muscle: 20% (Ford and Podolsky, 1972) and 25% (Gordon et al., 1973).

As mentioned in Methods, we deliberately chose to study only those fibers which, after skinning, appeared to be more or less cylindrical upon inspection under the dissecting microscope. Furthermore, the assumption that these skinned fibers can be approximated as a right circular cylinder seems justified for the following reason: in previous experiments (Godt

TABLE III
VARIATION OF AVERAGE BULK MODULUS WITH COMPRESSION FOR SIX FIBERS (S = 2.26–2.42 μm)

PVP concentration	π	K	SD
%	$(N/m^2)(\times 10^3)$	$(N/m^2)(\times 10^4)$	$(\times 10^4)$
2	2.59	1.56	0.319
3	4.64	2.66	0.575
4	7.29	4.01	0.900
5	10.64	6.31	1.707
6	14.78	7.60	1.828
8	25.77	12.54	3.184
10	40.97	18.98	5.062

and Maughan, 1977, p. 110) we measured both the width and thickness of the fiber at the same point and found that, in 49 fibers similar to the kind studied here, the area calculated on the basis of an elliptical approximation was not significantly different ($P > 0.1$) than that calculated on the basis of a cylindrical approximation using only the width measurement.

The data relating fiber width, D, to osmotic compressive pressure, π, are nicely fit between 2 and 10% PVP (~2.6 to 40 kN/m^2) by the expression: $D/D_0 = 1 + b \ln(\pi/\pi_0)$, ($\pi > 0$, Eq. 1), where D_0 is the width in 4% PVP at each sarcomere length, and π_0 is the osmotic pressure in 4% PVP (7.29×10^3 N/m^2). It is of interest to note here that LeNeveu et al. (1977), using a similar technique of polymer-induced osmotic pressure to determine forces between lecithin bilayers, observed that the distance between bilayers is also proportional to the logarithm of the external osmotic pressure.

From data such as presented in Fig. 2, one can determine that to shrink a fiber back to that size which the particular collection of myofibrils had in the intact muscle (its *in situ* size), a PVP concentration between 2 and 3% is required. Assuming that, in PVP-containing solutions, fiber width varies in proportion to the myofilament lattice spacing, this value is in accord with recent observations in chemically-skinned frog muscle.[1] From x-ray diffraction studies,[1] the myofilament lattice was observed to return to its original dimensions in a relaxing solution containing approximately 3% PVP-40. Our previous estimate of 8% (Godt and Maughan, 1977) was too high because our estimate of *in situ* size was low, due to dehydration of fibers in the silicone oil used in those experiments.

We also determined the relationship between fiber width and sarcomere length, S: $D_0'^2/D^2 = a_0 + a_1 S$ (Eq. 2), where D_0' is the width at 2.2 μm, and a_0 and a_1 depend on PVP concentration according to Table I. In general, skinned fibers move toward a constant volume relation ($a_0 = 0$) at a PVP concentration below 4%; i.e., toward the range of concentration required to shrink swollen fibers back to their *in situ* size (see above). Above this range of PVP concentrations, in PVP-free relaxing solution, and in mineral oil, skinned fibers do not obey a constant volume relation. Note that these data are not in accord with what one would expect on the basis of Matsubara and Elliott's (1972) observations relating myofilament lattice

[1]Magid, A. 1979. Personal communication.

spacing to sarcomere spacing of fibers bathed in PVP-free solution. Assuming that lattice spacing and fiber width change proportionately, one would expect that, with stretch, the width of the fiber would change considerably less than we observe, and that the intercept a_0 would be substantially greater than zero rather than somewhat less. We have no explanation for these differences except to say that, in PVP-free solutions, the assumption of proportionality between changes in lattice spacing and fiber width does not hold. Unfortunately, Matsubara and Elliott (1972) did not report measurements of fiber width concurrent with their measurements of lattice spacing.

Since fibers do not simply fall to pieces when their sarcolemma is removed, and since skinned fibers are mechanically robust and can be reversibly deformed by stretch or compression, there clearly must be some continuous elastic structure permeating the fiber. With this in mind, we treated the skinned fiber as an elastic body and, using the relationship between fiber width and osmotic pressure (with the assumption that the volume is proportional to D^2S), were able to determine bulk modulus under different conditions of stretch and compression. Focusing on the elastic behavior of skinned fibers near slack length and *in situ* diameter, we determined Young's modulus in 4% PVP solutions, assuming that the fiber cross-sectional area was proportional to D^2. From Young's modulus and the corresponding bulk modulus in 4% PVP solutions we found that skinned fibers behave as a nonisotropic elastic body with a Poisson's ratio near that for polyethylene, a semicrystalline polymer with crosslinked molecular chains that tend to be oriented (Flory, 1953). We suggest that fibers swell upon being skinned because the elastic structures are not at equilibrium, i.e., are constrained, in the intact fiber and that removal of the sarcolemma removes the constraint. This concept is similar to that of Matsubara and Elliott (1972), Elliott (1973), and April (1975), who suggest that the myofilament lattice is constrained in the intact fiber. We have used PVP to estimate the constraining pressure, (i.e., the pressure necessary to shrink the skinned fiber back to its *in situ* size) and found it to be 3.4×10^3 N/m^2.

What are the possible elastic structures in skinned fibers from frog muscle? Available morphological data on the myofilament lattice point to M-line (Matsubara and Elliott, 1972; Luther and Squire, 1978) and Z-band (Kelly, 1967) structures. In addition, there is some suggestive evidence for the presence of an elastic matrix in frog muscle apart from the thick and thin filament array (Maruyama and Natori, 1978). Maruyama and colleagues (Maruyama et al., 1977 *a, b*) have isolated a protein, connectin, which they propose functions as the parallel elastic component of muscle. This protein composes ~5% of the total myofibrillar protein in rabbit psoas muscle and forms fine networks, as observed in electron micrographs. Maruyama and his colleagues were able to prepare ghost skinned fibers by extracting both actin and myosin from conventional skinned fibers (a procedure that presumably left mostly connectin), which retained both the shape and much of the elasticity of the original preparation. Furthermore, they found that the protease elastase was able to digest purified connectin and also to disrupt and weaken skinned fibers to the point of rupture. We have confirmed this observation and find that less than 1 mg/ml elastase added to BRIJ-containing relaxing solution completely disintegrates skinned fibers.

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