THE MYOFILAMENT LATTICE: STUDIES ON ISOLATED FIBERS

I. The Constancy of the Unit-Cell Volume with Variation in Sarcomere Length in a Lattice in which the Thin-to-Thick Myofilament Ratio is 6:1

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ABSTRACT

The spacing between the thick myofilaments of muscle fibers from the walking legs of crayfish (*Orconectes*) was determined by optical transform analysis of electron micrograph plates of fixed single fibers and by X-ray diffraction of living single fibers. Sarcomere lengths were determined by light diffraction prior to fixation and prior to the in vivo experiments. From these combined measurements, it is demonstrated that the unit-cell volume of the myofilament lattice is constant during muscle shortening, indicating that the myofilament lattice works in a constant-volume manner. It is further demonstrated with X-ray diffraction measurements of living single fibers that the myofilament lattice continues to work at constant volume after the sarcolemma is removed from the fiber. This indicates that the constant-volume behavior of muscle is inherent to the myofilament lattice.

INTRODUCTION

There is general agreement that the structural basis for striated muscle shortening involves an interdigitation of the thick and thin myofilaments as first proposed by A. Huxley and Niedergerke (20) and H. E. Huxley and Hanson (26). Concomitantly, there is a change in the distance separating the myofilaments. H. E. Huxley (22, 24) showed from X-ray diffraction diagrams that the in vivo lattice spacing of relaxed vertebrate muscle is inversely proportional to the square root of the sarcomere length. These results were confirmed and extended to include active muscle by Elliott et al. (8, 10–12). Carlson et al. (5) observed that the myofibrils of fibers studied by electron microscopy are larger in cross-section at shorter sarcomere lengths, but not according to a strict constant-volume manner for they reported a fluid loss of up to 20%. More recently, Brandt et al. (3) found that the lattice volume is constant in frog semitendinosus single fibers fixed at a

variety of sarcomere lengths for electron microscopic study.

This report demonstrates with data obtained by electron microscopy and low-angle X-ray diffraction that a constant-lattice-volume regimen exists in crayfish muscle fibers which have a lattice configuration markedly different from that of vertebrate muscle (April and Brandt, manuscript in preparation). This constant-volume (isovolumic) behavior is maintained after the equilibrium volume is changed osmotically and even after the sarcolemma is removed from the fiber. This precise geometrical variation in lattice spacing with sarcomere length is of interest because the underlying mechanism is not understood and the relation between the interfilament distance and force development in muscular contraction has yet to be determined. Brief accounts of these results have already appeared (1, 2, 2 *a*).

MATERIALS AND METHODS

Dissection of Single Muscle Fibers

Single fibers were dissected from the carpopodite flexor which lies in the meropodite of the walking leg of crayfish (*Orconectes*). The dissections were accomplished according to the method described by Girardier et al. (18) while the muscles were bathed in a crayfish physiological saline solution in a plexiglass chamber fitted with a glass back. The physiological saline solution was a modification of the van Harreveld crustacean saline solution (36), containing 200 mM/liter NaCl, 13.5 mM/liter $CaCl_2$, 5 mM/liter KCl, and 1 mM/liter $NaHCO_3$, or 2 mM/liter Tris.

The proximal end of the muscle fiber was left attached to the chitin which was held securely to the back of the chamber with a stainless steel pressure clip. The distal tendon was cut free of the exoskeleton and attached to a fine stainless steel wire. After the chitin bridge connecting the ischiopodite to the carpopodite was removed, the glass front of the chamber was fitted into place and the chamber was mounted vertically in the optical diffractometer. The wire on the muscle tendon was attached to the strain transducer through an opening in the top of the chamber.

The preparation of the skinned single fibers was similar to that described by Reuben et al. (31). After the dissection in control saline solution, the fibers were equilibrated for 30 min in a solution containing 200 mM/liter potassium propionate, 5 mM/liter $MgCl_2$, 9 mM/liter K_2EGTA, 1 mM/liter calcium propionate, 1 mM/liter ATP and buffered to pH 7 with 20 mM Tris. The sarcolemma was then stripped back along most of the fiber length.

Measurement of Sarcomere Length by Light Diffraction

The diffraction of light by the regular sarcomere repeat of muscle was utilized to determine accurately the sarcomere length (see, for example, 35). A 0.3-mw He-Ne laser (wavelength 6328 A; Optics Technology Inc., Los Angeles, Calif., Model 170) was mounted on an optical bench with a leaf shutter, the muscle fiber, and a Polaroid packet holder which was approximately 7 cm beyond the fiber. The exact specimen-to-film distance was determined from an optical transmission grating placed in the saline solution in the specimen plane. The muscle chamber and hence the fiber could be positioned in the laser beam by rack and pinion accessories. The beam intensity was modulated with neutral density filters so that the diffraction pattern could be recorded in $\frac{1}{50}$th–$\frac{1}{25}$th sec on Polaroid type 57 film. The muscle fiber could be exposed, however, to the unfiltered beam for several hours without apparent injury.

The diffraction patterns (Fig. 1) were measured on the Polaroid print with a 7× magnifying micrometer. Because the sarcomere length in these muscle fibers is of the order of 10 μ, the angle of diffraction is very small (about 4°) and the sarcomere length (L_s) is inversely related to the measured distance between the selected order of diffraction and the incident spot according to the grating equation:

$$n\lambda = d \sin\theta, \text{ approximated as } n\lambda = L_s \tan\theta$$

The strain transducer (Grass Instrument Co., Quincy, Mass., Model FT. 03C) was mounted with a micrometer mechanism to the platform carrying the muscle chamber. The muscle fiber length and hence the sarcomere length was adjusted by moving the strain transducer vertically. The position of the muscle chamber was always readjusted so that the same area of the fiber was measured at the various degrees of stretch. Tension changes resulting from stretch or fixation were recorded on an ink-pen polygraph (Grass Instrument Co., Model 7).

Preparation for Electron Microscopy

After single fibers had been isolated, the sarcomere lengths were adjusted to a definite length (9.6 μ, about 1.18 × L_o) and the diffraction patterns were recorded. The control saline solution was replaced by a similar solution which contained 1 mg/ml procaine hydrochloride as a means of preventing contraction of the fiber when cold fixing medium (1% OsO_4 substituted for an equal osmolar amount of NaCl in the control saline, pH 7.4) was perfused through the chamber for 3 min. A final record was made of the diffraction pattern before the fiber was removed from the chamber and placed in a cold solution of 1%

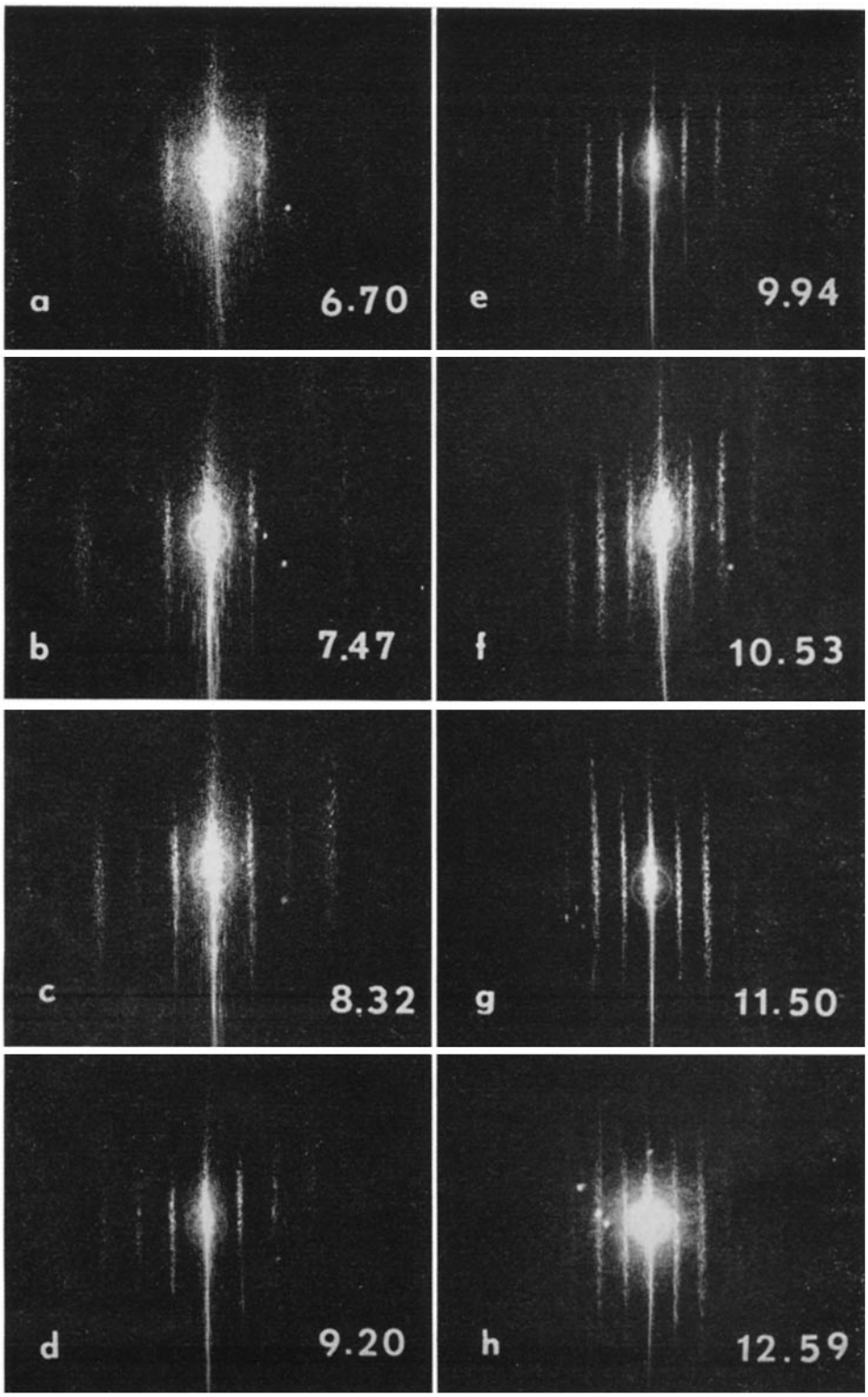

FIGURE 1

FIGURE 1 Light-diffraction patterns from a single muscle fiber stretched to various sarcomere lengths (indicated in microns). Four orders of diffraction are visible on either side of the incident spot. The sarcomere length is inversely proportional to the distance between any order and the incident spot. The specimen-to-film distance is approximately 7.1 cm and the photographs are reproduced at approximately × 75.

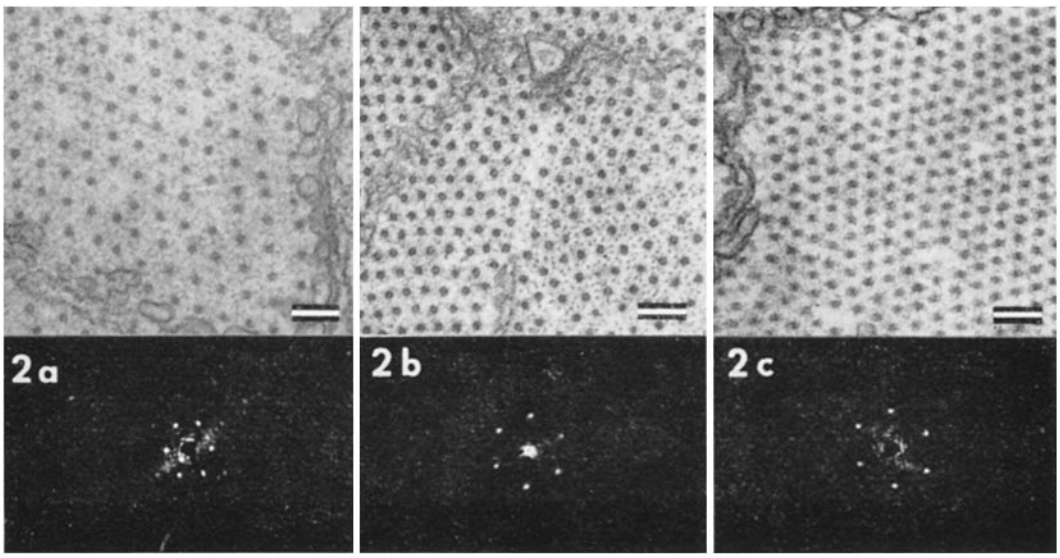

FIGURE 2 Electron micrographs and corresponding optical transforms of the cross-sectional lattice of single fibers fixed at three different sarcomere lengths: Fig. 2 *a*, 6.8 μ; Fig. 2 *b*, 8.9 μ; and Fig. 2 *c*, 12.3 μ. The fixation is with 1% OsO_4 in the control saline solution. The electron micrographs are printed here at a magnification of × 59,000; however, the optical transforms were obtained from similar plates of the same fibers at about × 3000 with a specimen-to-film distance of 1 m. The distance between the first order diffraction points and the incident spot is an inverse function of the thick myofilament spacing.

OsO_4 and Veronal acetate at pH 7.4 (30) and fixed for an additional 15 min. The Epon embedding and electron microscopic procedures were those described by Brandt et al. (4). The thin cross-sections were obtained from the same portion of the fiber used previously in the determination of the sarcomere length by light diffraction, and the specimens were carefully oriented so as to ensure that the sections were normal to the fiber axis.

Measurement of the Lattice Volumes of Fixed Fibers

The area of the unit cell which is the cross-sectional area of the myofilament lattice exclusive to one thick filament (see, for example, 23) was determined by optical transform analysis with the use of a modification of the method described by Klug and Berger (27). Low-magnification (× 3,000) electron micrograph plates of single fibers in cross-section were placed in the laser beam. With the use of appropriate lenses, the beam was diverged to cover the approximate area of one myofibril and then refocused in the film plane. The resulting diffraction pattern (optical transform of the myofilament lattice) was photographed on Polaroid type 57 film (Fig. 2). Once again, since the diffraction angles are small, the distance between the first orders of diffraction and the incident spot is inversely proportional to the distance between the $\bar{1}$, $\bar{0}$ lattice planes ($d_{\bar{1},\bar{0}}$) from which the myosin interfilament distance (d_{m-m}) can be determined according to the following relation:

$$d_{\bar{1},\bar{0}}/\sin 60^\circ = d_{m-m}$$

The accuracy of the determination of the myosin-to-myosin spacing is greatly improved by this method since the myofilament distances within one myofibril (about 1 μ in diameter) are averaged together. Compression effects incurred during sectioning are minimized by averaging the first order diffractions in all three lattice planes. From the interfilament distance the cross-sectional area and volume of the unit cell were obtained according to the following relation:

$$d_{m-m}^2 \cdot \sin 60^\circ \cdot L_s = K_v$$

where d_{m-m} is the center-to-center distance between the thick myofilaments, ($d_{m-m}^2 \cdot \sin 60^\circ$) is the cross-sectional area of the unit cell, L_s is the sarcomere length, and K_v is the volume constant of the unit cell.

Measurement of the Lattice Volumes of Living Fibers

Single muscle fibers were dissected in a plexiglass chamber fitted with a miniature micrometer mech-

anism to permit sarcomere length adjustment and mylar windows to minimize X-ray absorption. The chamber was mounted over openings in a copper block which was kept at 4°C with a cold stage cooling module (De La Rue Frigister, Ltd.). After the sarcomere length was adjusted in the optical diffractometer the muscle was perfused with the experimental solution for 1 hr. The chamber was then mounted in an optically focusing low-angle X-ray camera of the basic Franks design (17) as modified by Elliott and Worthington (7, 15). This type of camera has a small beam size (about 200 μ wide) at the specimen plane so that the single fiber fills the X-ray aperture. The X-ray source was a rotating anode generator (Elliott Automation, Borehamwood, England, Type GX3) operated at 35 kv with a 25 mA beam current directed at the copper target (nominal focal size 100 μ × 1 mm).

The fiber was placed vertically in the beam and aligned so that the X-rays passed through the same area of the fiber which had been measured with light diffraction. The X-ray diffraction patterns were recorded on Ilford Industrial G film at a specimen-to-film distance of approximately 25 cm in 1 ¼-2-hr exposures. After each exposure, the specimen-to-film distance was recorded and the diffraction orders on the developed film (Fig. 3) were measured with a Microcomparator (Nikon, Model 6C). The distance of the first equatorial order of diffraction from the incident spot is inversely related to the distance between the $\bar{1}$, $\bar{0}$ lattice planes according to Bragg's law:

$$n\lambda = 2d \sin\theta$$

When the ($\bar{1}$, $\bar{0}$) lattice spacing ($d_{\bar{1},\bar{0}}$) has been determined, the myosin-to-myosin distance (d_{m-m}), the cross-sectional area, and the volume of the unit cell are calculated as before (section on measurement of lattice volumes of fixed fibers).

Measurement of the Diameters of Whole Fibers

Single intact and skinned fibers were observed with the light microscope at a variety of sarcomere lengths. The diffraction patterns were recorded and the fibers photographed through the microscope. The fiber diameters were measured, from which an approximation of the cross-sectional area could be calculated.

RESULTS

Lattice Volumes of Fixed Fibers

The interfilament distances of single muscle fibers fixed with 1% OsO_4 at various sarcomere lengths were determined by optical transform analysis of the electron micrograph plates (Fig. 2). The sarcomere lengths were determined by light diffraction (Fig. 1). Plastic-embedded thick sections (0.5 μ) were cut as near to the parallel axis of the fiber as possible in the area in which the light diffraction measurements had been made prior to fixation. These sections were stained with toluidine blue, and the sarcomere lengths were measured with a phase microscope. The sarcomeres were approximately 3% shorter after fixation and embedding, which agrees with reports by Carlsen et al. (5), A. Huxley and Peachey (21), and Page and H. Huxley (29). In the determination of the unit-cell volumes of the fixed fibers, the sarcomere lengths obtained by light diffraction prior to fixation were reduced by 3% and then used to calculate the unit-cell volume after fixation.

The mean unit-cell volume of fibers fixed in control saline solution was 21.5 ($\pm$1.1) × 10^{-3} μ^3 and consistent over the range of sarcomere

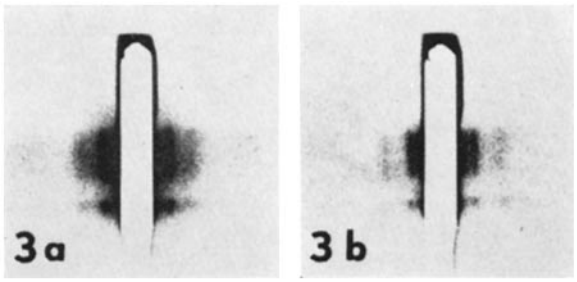

FIGURE 3 X-Ray diffraction patterns from living single muscle fibers at a sarcomere length of 9.6 μ. Fig. 3 *a*, control saline; Fig. 3 *b*, isosmotic potassium propionate with the sarcolemma removed. In each figure, the upper reflections are focused only in one plane while the lower reflections are focused in mutually perpendicular planes. The specimen-to-film distance is approximately 280 mm and the prints are reproduced here at × 4.4.

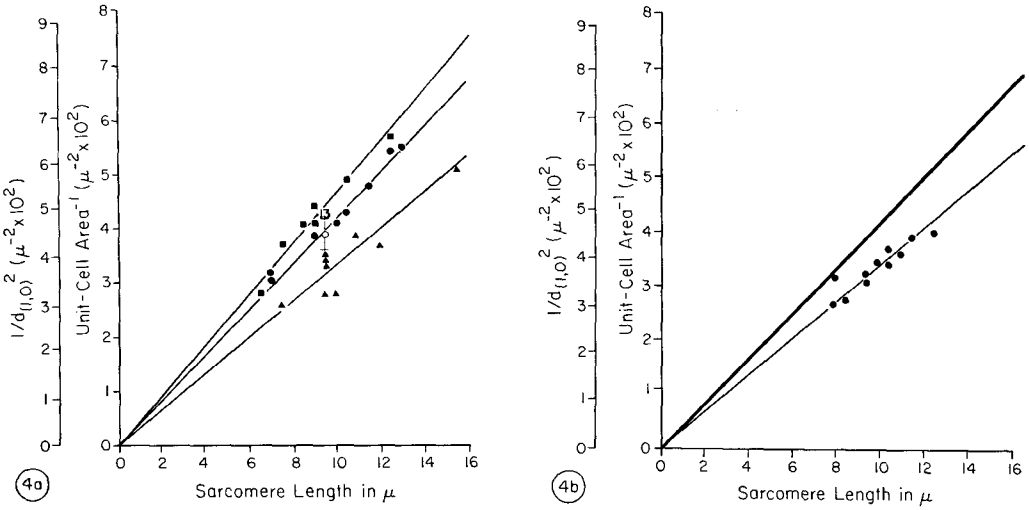

FIGURE 4 Unit-cell area as a function of sarcomere length. (*a*) The reciprocal of the area of the unit cell is plotted against the sarcomere length for single muscle fibers studied by electron microscopy in the fixed state (squares), by X-ray diffraction in the living state (circles), and with the sarcolemma removed (triangles). The fit of the data by the method of least squares to a straight line with an origin intercept indicates a constant lattice volume. The open symbol represents a mean value with the standard deviation from the mean indicated by brackets. (*b*) The reciprocal of the area of the unit cell is plotted against the sarcomere length for single fibers in a swollen state (hypotonic medium) studied by X-ray diffraction. The line through the data is fit by the method of least squares and indicates a constant volume. The heavier line is the result of the X-ray for control fibers taken from Fig. 4 *a*.

lengths investigated. Fig. 4 *a* shows that the area of the unit cell is inversely proportional to the sarcomere length: $L_s = K_v$ (1/A). When a least squares regression line ($r = 0.98$) is fitted to the points (solid squares), the line passes through the origin (0.00 ± 0.01). Since the volume of the unit cell (the slope) is constant, the average interfilament spacing of the thick myofilaments at the selected standard sarcomere length of 9.6 μ is 507 ($\pm$13) A. The interfilament spacing, sarcomere length, and unit-cell volumes are listed in Table I.

Lattice Volumes of Intact Living Fibers

The lattice volumes of intact single fibers bathed in control saline solution were determined at various sarcomere lengths by X-ray diffraction, and the results are listed in Table II. The unit-cell volume is 24.1 ($\pm$1.7) $\times$ 10^{-3} μ^3, it is constant over the range of sarcomere lengths investigated, and it is larger than the volume of the fixed fiber. Fig. 4 *a* shows that the area of the unit cell determined by X-ray diffraction is also inversely proportional to the sarcomere length. A least squares regression line ($r = 0.98$) fitted to the points (solid circles) passes through the origin. Thus, at the standard sarcomere length of 9.6 μ the average in vivo interfilament distance is 538 ($\pm$19) A, in contrast to 507 ($\pm$13) A for the fixed fibers.

Lattice Volumes of Skinned Living Fibers

The unit-cell volumes of skinned single muscle fibers were determined at various sarcomere lengths by X-ray diffraction, and the results are listed in Table III. Upon exposure to the isosmotic skinning solution (potassium propionate), the freshly dissected fiber swells by approximately 24%. There is, moreover, an additional swelling in the unit-cell volume upon removal of the sarcolemma (2). The area of the unit cell of the skinned fiber determined by X-ray diffraction is inversely proportional to the sarcomere length (Fig. 4 *a*). When a least squares regression line ($r = 0.97$) is fitted to the experimental points (solid triangles), the line passes essentially through

TABLE I

Lattice Parameters Determined by Electron Microscopy as a Function of Sarcomere Length

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at $L_s = 9.6\ \mu$
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69008B	190 mM/liter NaCl (0.4455 Osm)	6.40	566	3.69	23.7	533
68130C	" " " "	7.40	485	2.71	20.1	491
68133D	" " " "	8.60	462	2.46	21.2	504
68073A	" " " "	8.90	460	2.44	21.7	511
68130B	" " " "	9.00	446	2.29	20.7	498
69008C	" " " "	10.30	422	2.05	21.2	504
69007A	" " " "	12.30	392	1.77	21.8	512
	Average				21.5 (±1.1)	507 (±13)

TABLE II

Lattice Parameters Determined by X-Ray Diffraction as a Function of Sarcomere Length

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at $L_s = 9.6\ \mu$
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
68350A1	Control (0.4455 Osm)	7.10	537	3.32	23.6	533
69129B1	" "	7.10	514	3.05	21.7	510
68350A1	" "	8.80	475	2.60	22.9	525
[13]	" "	9.60	470 (±18)	2.55 (±0.20)	24.5 (±1.9)	542 (±21)
69175A1	" "	9.80	460	2.44	23.9	536
69130A1	" "	10.30	452	2.35	24.3	540
69132B3	" "	11.40	426	2.09	23.9	536
69132B1	" "	12.60	399	1.83	23.2	527
69129A1	" "	12.90	398	1.82	23.6	532
	Average				24.1 (±1.6)	538 (±19)

the origin (-0.05 ± 0.18) and has a volume constant (slope) of 30.8 (± 2.7) $\times\ 10^{-3}\ \mu^3$, about 128% of the control unit-cell volume. In light of later experiments in which the pH of the medium was varied (to be reported in a subsequent paper[1]), we think that, owing to inadequate buffering, the effective pH of the skinning solution in this particular series of experiments was somewhat lower than pH 7.4.

[1] E. W. April, P. W. Brandt, and G. F. Elliott. The myofilament lattice: studies in isolated fibers. II. The effects of osmotic strength, ionic concentration, and pH upon the unit-cell volume. In preparation.

The Effect of Tonicity upon Isovolumic Shortening

The unit-cell volume of intact fibers can be adjusted by varying the osmotic pressure of the bathing medium.[1] The unit-cell volumes of fibers swollen in a hyposmotic saline solution (-90 mM/liter NaCl) were determined at various sarcomere lengths by X-ray diffraction, and the results are listed in Table IV. The reciprocal of the unit-cell area is plotted against the sarcomere length in Fig. 4 *b*. Even though the fiber is in a swollen condition, the area of the unit cell is inversely proportional to the sarcomere length, and the least squares regression line ($r = 0.93$) fitted to

TABLE III

Lattice Dimensions of Intact and Skinned Fibers Determined by X-Ray Diffraction

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at $L_s = 9.6\ \mu$
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
	Intact					
69194A1	200 mM/liter K propionate	9.10	532	3.26	29.7	598
69205A1	" " "	9.10	554	3.54	32.2	622
69204A1	" " "	9.60	521	3.13	30.1	601
69207A1	" " "	9.60	492	2.79	26.8	568
69212B1	" " "	9.60	503	2.92	28.0	580
69133A1	" " "	9.90	500	2.88	28.6	586
69214A1	" " "	10.10	498	2.86	28.9	589
69216A1	" " "	10.40	488	2.74	28.6	586
69210B1	" " "	10.60	513	3.03	32.2	622
	Average				29.4 (±1.8)	595 (±18)
	Skinned					
68351B1	200 mM/liter K propionate	7.60	587	3.97	30.2	603
69133A2	" " "	9.60	498	2.86	27.5	575
69133A3	" " "	9.60	505	2.94	28.3	583
68349B1	" " "	9.60	510	3.00	28.8	588
69188A1	" " "	9.60	558	3.59	34.5	644
69191A1	" " "	9.70	560	3.62	35.1	650
69134A1	" " "	11.30	479	2.64	29.9	600
68352A1	" " "	11.90	485	2.71	32.3	623
69135A1	" " "	15.40	417	2.00	30.9	609
	Average				30.8 (±2.7)	608 (±26)

TABLE IV

Lattice Parameters as a Function of Sarcomere Length Determined by X-Ray Diffraction in an Intact Swollen Fiber

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at $L_s = 9.6\ \mu$
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69201A1	100 mM/liter NaCl (0.2655 Osm)	8.10	528	3.21	26.1	560
69196A1	" " "	8.20	569	3.73	30.6	607
69202B2	" " "	8.60	562	3.64	31.4	614
69224B1	95 mM/liter NaCl (0.2555 Osm)	9.60	520	3.12	30.0	600
69225A1	" " "	9.60	526	3.19	30.7	607
69177A1	100 mM/liter NaCl (0.2655 Osm)	9.90	505	2.94	29.2	592
69179A2	" " "	10.60	484	2.70	28.7	587
69179A1	" " "	10.60	507	2.96	31.5	615
69178A1	" " "	10.80	497	2.85	30.8	608
69180A1	" " "	11.60	475	2.60	30.2	602
69181A1	" " "	12.60	467	2.51	31.7	617
	Average				30.1 (±1.5)	601 (±16)

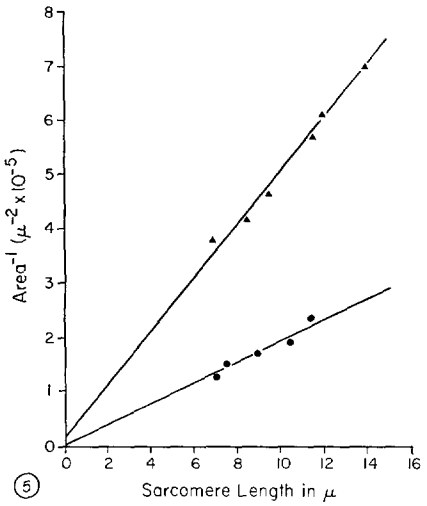

FIGURE 5 Cross-sectional area as a function of sarcomere length. The reciprocals of the cross-sectional areas are plotted against sarcomere length for an intact single fiber (diam at 9.6 μ = 264 μ; circles) and a skinned fiber (diam of 9.6 μ = 165 μ; triangles). The fit of the data to least square regression lines indicates constant-volume shortening both in the presence of or absence of the sarcolemma.

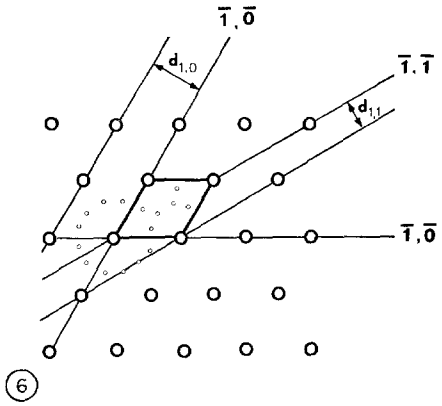

FIGURE 6 Schematic representation of the crayfish myofilament array. The large circles represent the myosin filaments. These filaments lie in planes, forming a lattice with the distances between the lattice planes indicated. The small circles represent the actin filaments, 12 of which are arranged more or less equidistant around each thick filament (shown completely for one thick filament and partially for three others). The heavy solid lines delineate the unit cell, containing one thick filament ($\frac{1}{6} + \frac{2}{6} + \frac{1}{6} + \frac{2}{6}$ of the included thick filaments) and six thin filaments. This results in a 1:6 unit-cell ratio of thick filaments to thin.

the data passes close to the origin (0.57 $\pm$ 0.15) with a volume constant of 30.1 ($\pm$1.5) $\times$ 10^{-3} μ^3 which is about 124% of the volume of the control unit cell.

Constant-Volume Shortening in the Whole Muscle Fiber

The reciprocals of the cross-sectional areas of an intact and a skinned fiber of different control diameters (determined by light microscopy), are plotted against the respective sarcomere lengths in Fig. 5. Since the regression slopes are linear and pass through or close to the origin, it is clear that the entire muscle fiber, whether or not the sarcolemma is present, shortens in an isovolumic manner.

DISCUSSION

This work was undertaken in order to determine whether the constant-volume restriction (3, 10–12, 22, 24, 25) to the sliding filament model of sarcomere shortening demonstrated with vertebrate muscle extends to crayfish muscle. While there are similarities in the myofilament lattice structure between crayfish muscle and vertebrate muscle, there are also very significant differences (4, 16; April and Brandt, manuscript in preparation). In crayfish, the myosin filaments are surrounded by 12 actin filaments which appear to be equidistant from one another and lie on either side midway along imaginary lines connecting the myosin filaments. The lattice thus has a 6:1 thin-to-thick myofilament ratio in the unit cell. This is illustrated schematically in Fig. 6.

The light microscopic observations of the intact and skinned fiber diameters at various sarcomere lengths indicate that crayfish muscle shortens in an isovolumic manner (see Fig. 5). The data obtained both on fixed crayfish fibers by electron microscopy and on living crayfish fibers (intact, swollen, and skinned) by X-ray diffraction are consistent with the isovolumic relation, in that the reciprocals of the spacing between the lattice planes under all conditions (and hence the reciprocals of the unit-cell areas) are linear functions of the sarcomere length which extrapolate to zero (see Fig. 4). These results are in substantial agreement with the X-ray diffraction studies of whole amphibian muscle reported by Elliott et al. (10–12), H. Huxley (23, 24), and Rome (33, 34).

The volume shrinkage incurred by the lattice

during preparative procedures for electron microscopy was 11% of the in vivo unit cell, and the interfilament spacing shrinkage was 5½%.

The lattice volumes of skinned fibers are also constant (see Fig. 4 *a*), but in this instance, the unit-cell volume is 30.8 ($\pm$2.7) $\times$ 10^{-3} μ^3 which is 28% greater than the control volume. This increase is due in part to a swelling in the isosmotic potassium propionate solution which has not been fully explained (32). There is an additional variable increase in the lattice volume upon subsequent removal of the sarcolemma.[1] It is evident that the sarcolemma is not essential for the isovolumic behavior of the fiber over the range of sarcomere lengths investigated. Since the lattice has been demonstrated to behave in an isovolumic manner at two different "equilibrium" volumes in the intact fiber and at other volumes in the skinned fiber (see Figs. 4 *a* and 4 *b*), it is also evident that this basic isovolumic behavior of the myofilament lattice is independent of the equilibrium volume of the lattice. The isovolumic property of the lattice is, therefore, intrinsic to the lattice.

The isovolumic relationship appears to be consistent with one aspect of the electrostatic hypothesis of Elliott et al. (9, 11–12) and Worthington (37, 38) and the more recent electrostatic-hydraulic hypothesis of Elliott et al. (13, 14). However attractive a general electrostatic theory might be to explain the isovolumic behavior of the lattice, there are certain differences between the pH and ionic strength behavior of the skinned fiber lattice (to be reported later[1]) and that of the glycerol-extracted rabbit muscle studied by Rome (33, 34) which require detailed reexamination of some of the aspects of the theory in its present form.

From evidence obtained on skinned fibers, it is also unlikely that the isovolumic nature of the lattice is due to the redistribution of cytoplasmic fluid as has been proposed by Carlsen et al. (5) and Harris (19). The fluid might be environmentally constrained, however, if the solvent is in an anomalous state (2, 6, 13, 14, 28); and it is possible that the isovolumic behavior is due to the gel nature of the myoplasm (5, 19). In light of more recent experiments,[1] this latter possibility appears quite plausible and is intimately related to the possible role of electrostatic forces within the myofilament lattice.

Although the constant-volume relationship is an integral part of muscle shortening, it has never been properly explained in physical-chemical terms. A subsequent paper[1] will cover further experiments on the behavior of the myofilament lattice with changes in osmolarity, ionic strength, and pH.

We wish to thank Mr. Robert Demarest for the graphics and Drs. Harry Grundfest, Jean Hanson, John P. Reuben, Robert V. Rice, and Elizabeth Rome for their valuable discussions.

This work was supported in part by National Institutes of Health grants (NB-03270, NB-03728, NB-05328, NS-05910, and 2T01GM-00256) and a National Science Foundation grant (GB-6988X). Dr. April is also grateful to the Grass Foundation for a Grass Fellowship in Neurophysiology. Dr. Elliott is grateful to the Marine Biological Laboratory for the Rand Fellowship and to Carnegie-Mellon University for a visiting professorship.

Received for publication 14 December 1970, and in revised form 12 May 1971.

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