THE RELATIONSHIP BETWEEN MYOFILAMENT PACKING DENSITY AND SARCOMERE LENGTH IN FROG STRIATED MUSCLE

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ABSTRACT

In cross-sections of single fibers from the frog semitendinosus muscle the number of thick myofilaments per unit area (packing density) is a direct function of the sarcomere length. Our data, derived from electron microscopic studies, fit well with other data derived from in vivo, low-angle X-ray diffraction studies of whole semitendinosus muscles. The data are consistent with the assumption that the sarcomere of a fibril maintains a constant volume during changes in sarcomere length. The myofilament lattice, therefore, expands as the sarcomere shortens. Since the distance between adjacent myofilaments is an inverse square root function of sarcomere length, the interaction of the thick and the thin myofilaments during sarcomere shortening may occur over distances which increase 70 A or more. The "expanding-sarcomere, sliding-filament" model of sarcomere shortening is discussed in terms of the current concepts of muscle architecture and contraction.

INTRODUCTION

With contraction, striated muscle changes its shape but maintains its volume almost perfectly. Consistent with this fact, the sarcomere of a myofibril thickens as it shortens (1), and also appears to maintain a constant volume. The striation changes which accompany changes in sarcomere length have been fitted to a sliding-filament model (2, 3) which has formed the basis for a hypothesis on the mechanism of muscle contraction (4). The model, however, does not take into account either the earlier light microscope observation of the concomitant thickening and shortening of the sarcomere or the X-ray diffraction data which demonstrate that the myofilament lattice maintains a constant volume during changes in sarcomere length (5–8). This paper reports electron microscopic studies on the relation between myofilament "packing density" and sarcomere length in single fibers of the semitendinosus muscle of the frog. The data show close agreement with the X-ray findings, and require an expanding-sarcomere, sliding-filament model for striated muscle.

METHODS

Single fibers from the frog semitendinosus muscle were isolated by dissection. They were maintained at different lengths in frog Ringer solution or were exposed to a sequence of experimental solutions. In some of the solutions the chloride ion was replaced isosmotically by propionate, which only transiently affects the fiber volume (9). Other fibers were swollen

by immersion in a hyposomotic solution in which the NaCl concentration was reduced to one-half, or in isosomotic K-enriched media in which the Na^+ was replaced by K^+. Some fibers were exposed to hyposmotic propionate media, and after return to the isosmotic media their volumes were as much as 50% smaller than their initial control volumes. The decrease in volume indicates a loss of intracellular material, "loss phenomenon", (9). One fiber was shrunk in a medium made hyperosmotic by doubling the NaCl concentration. The control and the experimental fibers were fixed by replacing the experimental solution with cold (ice bath) Palade's osmium-tetroxide fixative (10).

TABLE I

Packing Density, Sarcomere Length, and Relative Lattice Volume

Exp. No.	Average sarcomere length	Packing density	No.	Relative lattice volume after fixation
	μ	$(No_4\mu^2)$	SD	(FL/N)
1. Single fibers, Palade's OsO^4 fixative				
A. Isomotic saline				
59–10	2.78	700	78	116
59–11	2.23	680	97	96
59–12	2.86	790	58	106
61–1	2.64	904	83	81.5
78–4	1.0	320	56	91
91–2	1.89	555	48	102
95–7	2.26	645	74	98
95–8	2.64	853	87	90.5
			Avg	97.6%
B. Loss phenomena, shrunken				
59–14	2.19	675	37	95
73–2	3.06	778	57	115
74–1	2.78	827	46	98.5
76–1	2.26	790	64	98.5
95–3	2.33	804	49	84.5
			Avg	98.2%
C. -60 Sodium, swollen				
91–4	1.7	618	29	80.5
95–6	2.31	690	29	98
95–9	2.28	738	53	90.5
			Avg	89.6%
D. Swollen and reversed				
59–13	2.66	683	43	87
59–15	2.19	714	45	89.5
59–16	2.26	645	23	102
73–1	2.55	593	40	110
74–2	2.99	818	67	106
76–2	2.24	634	39	103
			Avg	99.5%
E. Isosmotic KCL, swollen				
91–3	2.33	510	70	133%
F. 2 × NaCl, shrunken				
95–10	2.19	928	58	69%

TABLE I—*Continued*

Exp. No.	Average sarcomere length	Packing density	No.	Relative lattice volume after fixation
	μ	$(No.\mu^2)$	SD	(FL/N)
2. Whole muscles, glutaraldehyde fixative				
110–1c	1.60	625	62	75
110–2c	1.77	800	30	65
117–1a	1.90	767.5	34	73
117–2a	2.12	842.5	56	73.5
110–3f	2.21	840	63	75
117–3	2.46	820	37	84
110–7g	2.85	1030	99	80.5
117–4	3.03	1272.5	87	72.5
			Avg	74.8%

The packing density, sarcomere length, and relative lattice volume are listed for groups of single fibers exposed to a variety of experimental solutions then fixed in Palade's osmium tetroxide fixative, and also for isolated fibers teased from the surface of whole muscles fixed in glutaraldehyde dissolved in Ringer solution. In part 1, the data refers to single fibers under the following experimental conditions: (*A*) exposed to isosmotic chloride or propionate salines, (*B*) showing a volume loss averaging 40% before fixation obtained by exposure to hyposmotic propionate media then return to control propionate media, see *Methods*, (*C*) swollen about 60% in hyposomotic media prior to fixation, (*D*) swollen and then reversed to normal volume before fixation, (*E*) swollen by exposure to iosomotic KCl prior to fixation, (*F*) shrunken in media made hyperosmotic by doubling the NaCl concentration. The whole muscles utilized for obtaining the data in part 2 were held at various lengths in control Ringer solution and were fixed by substituting Ringer solution containing glutaraldehyde After this fixation, single fibers were teased from the surface, postfixed in osmium tetroxide, and then treated like the single fibers in part 1. The relative lattice volume after fixation is the ratio of the lattice volume of the fiber, calculated from the data in the Table, to the in vivo lattice volume, calculated from the X-ray data (6). These volumes are calculated according to the relationship $V = FL/N$.

The effects of another fixative on the packing density also were examined. Glutaraldehyde solutions were used to fix whole semitendinosus muscles, and single fibers were teased from the surface of the muscle after 30 min fixation. Two different conditions of fixation were used in these experiments. The control Ringer solution bathing the muscle was replaced for 30 min by a Ringer solution to which 0.2% glutaraldehyde was added. Then the muscles were fixed in Palade's fixative immediately or after an intermediate 30 min step in 2% glutaraldehyde in Ringer solution. There was no visible contraction of the muscles with either method of glutaraldehyde fixation, whereas the single fibers placed directly into Palade's fixative usually contracted slightly.

After 1–2 hr of osmium tetroxide fixation the fibers were dehydrated in graded ethanols to pure ethanol, washed with propylene oxide, and embedded in an Epon mixture (11) containing 4% dibutyl phthalate. The blocks were cut in half and the adjacent parts of the fibers were cut, respectively, in cross-section and in longitudinal section; the direction of the stroke was normal, to the long axis of the fiber. The sections were stained in uranyl acetate solutions followed by lead hydroxide solutions, and were photographed in an RCA EMU 3C or Phillips 200 electron microscope. The instrument was calibrated each time it was used and the micrographs of the replica grating were printed along with the micrographs of the fiber sections. In both cases measurements were made on the prints. Average lengths for the sarcomere were determined with a light microscope on 0.5 μ thick longitudinal sections stained with toluidine blue. 20–30 sarcomeres were averaged into each measurement. These measurements agreed within $\pm$ 10% with those made on single sarcomeres photographed in the electron microscope.

The packing density (number per μ^2, termed "spacing" in an earlier report) (12) of the thick myofilaments was determined from the cross-sections through the A bands by counting the thick myofilaments appearing in a square aperture representing 0.04 μ^2 of the original section. When one-half, or less, of a filament was exposed it was counted as one-half a filament. 10 such areas were counted, with five different micrographs as sources. The number of thick myofilaments in each area, usually between 20 and 40, was multiplied by 25 to obtain the value for 1.0 μ^2. This figure was averaged with the other measurements to obtain the packing density, and standard deviations were calculated.

RESULTS

Table I summarizes the measurements of the average sarcomere length, packing density, and calculated relative myofilament lattice volumes

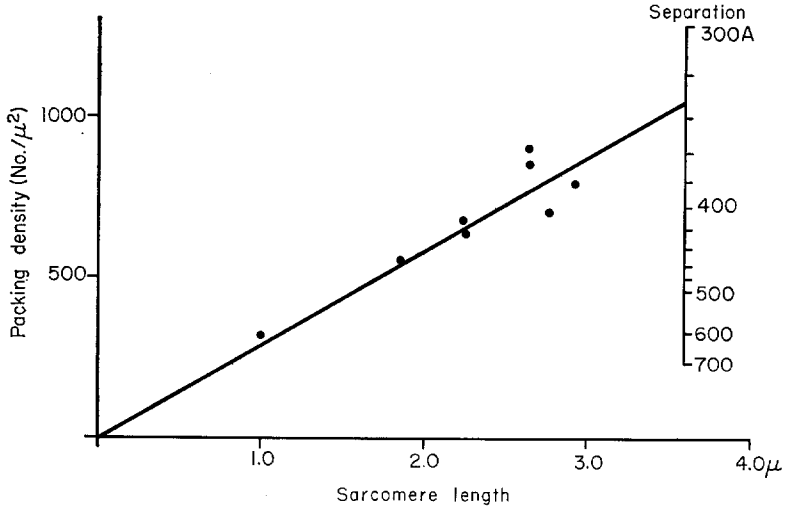


FIGURE 1 The packing density of the thick myofilaments (No. /μ^2) in cross-sections of A band is plotted against the sarcomere length (in μ) for single fibers exposed to isosmotic salines and fixed in Palade's fixative. The data are close to or on a line drawn from the origin which agrees with a plot of the X-ray diffraction data for living semitendinosus muscles of the frog (6). The data demonstrate that the myofilament lattice is constant in volume with changes in the sarcomere length and that the absolute volume is conserved by this method of preparation for electron microscopy. On the right ordinate the center to center separation of thick myofilaments is given in angstroms.

after fixation obtained on 24 isolated fibers subjected to different experimental conditions and fixed in osmium tetroxide, and on eight fibers teased from glutaraldehyde-fixed whole muscles. The packing density for the isolated fibers exposed to isosmotic Na salines, with either propionate or chloride as the anion, is plotted against the sarcomere length in Fig. 1. The data are close to or on a line drawn from the origin which agrees with a plot of the X-ray diffraction data for living whole semitendinosus muscle of the frog (6). The right ordinate of Fig. 1 gives the separation between adjacent thick myofilaments as a function of the sarcomere length.

Longitudinal and cross-sections of a fiber with an average sarcomere length of 2.23 μ are presented in Figs. 2 *a* and 3 *a*, respectively. Fig. 2 *b* and 3 *b* are similar electron micrographs of a fiber with an average sarcomere length of 2.86 μ. The differences in packing density are readily apparent in Figs. 3 *a* and *b*. Fig. 3 *c* is a micrograph of a cross-section of a fiber with an average sarcomere length of 1.0 μ. It is again obvious that the thick myofilaments are separated from one another increasingly as the sarcomere shortens.

The data in Figs. 1, 2, and 3 demonstrate that packing density is directly proportional to sarcomere length. This proportionality follows if the sarcomere of a myofibril maintains a constant volume throughout changes in sarcomere length. The volume (V) of a sarcomere or any larger segment of the myofilament lattice is determined by $V = FQL$, where F is the number of myofilaments, Q is the cross-sectional area occupied by each, and L is the sarcomere length. Since it is assumed that the volume is constant, and because the number of myofilaments does not change, then $Q \approx 1/L$ and the packing density (N) which is the reciprocal of Q is directly proportional to the sarcomere length. Because the packing density and the sarcomere length are sufficient to determine the volume of the myofilament lattice, data which fall above the control line and beyond the anticipated variation in Fig. 1 must come from fibers in which the myofilament lattice has lost volume, whereas data which fall below the line in a similar manner must come from fibers in which the lattice has gained volume.

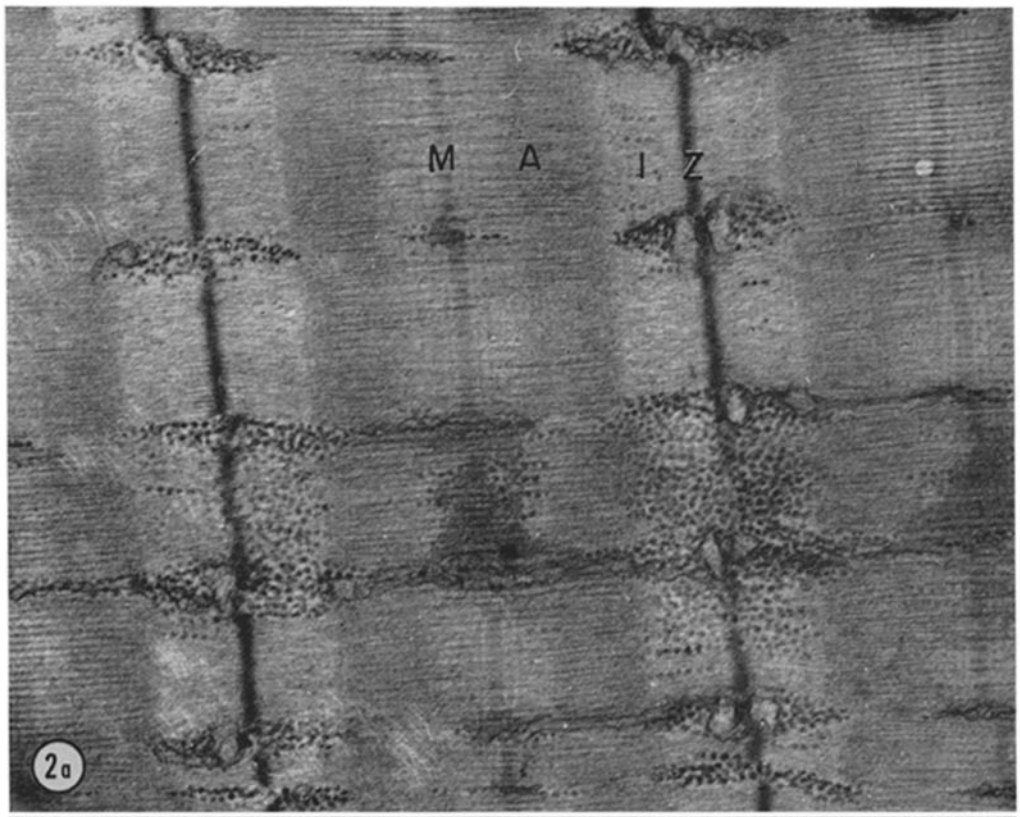
It might be supposed that any appreciable volume change in the fiber must involve the lattice since this represents at least 90% of the total cell volume. However, fibers which had lost intracellular material (9) and had decreased in volume by about 50% in vivo showed no significant change in lattice volume after fixation (Table I, group 1 *B*). Single fibers swollen by removal of half the NaCl from the Ringer solution were found to have essentially the same lattice volume as the control fibers (Table I, group 1 *C*). Fibers swollen by the same procedure and then returned to control volume prior to fixation showed no consistent change in the lattice volume (Table I, group 1 *D*). One fiber, shrunk by doubling the NaCl concentration, was found to have a lattice volume about 70% of the control volume (Table I, group 1 *F*; and Fig. 3 *d*), and another fiber swollen in isosmotic KCL sloution had a lattice volume 33% greater than the control volume (Table I, group 1 *E*). Data on single fibers teased from the surface of whole muscles fixed in glutaraldahyde are given in part 2 of Table I. In these fibers the volume of the myofilament lattice averages 75% of the in vivo volume.

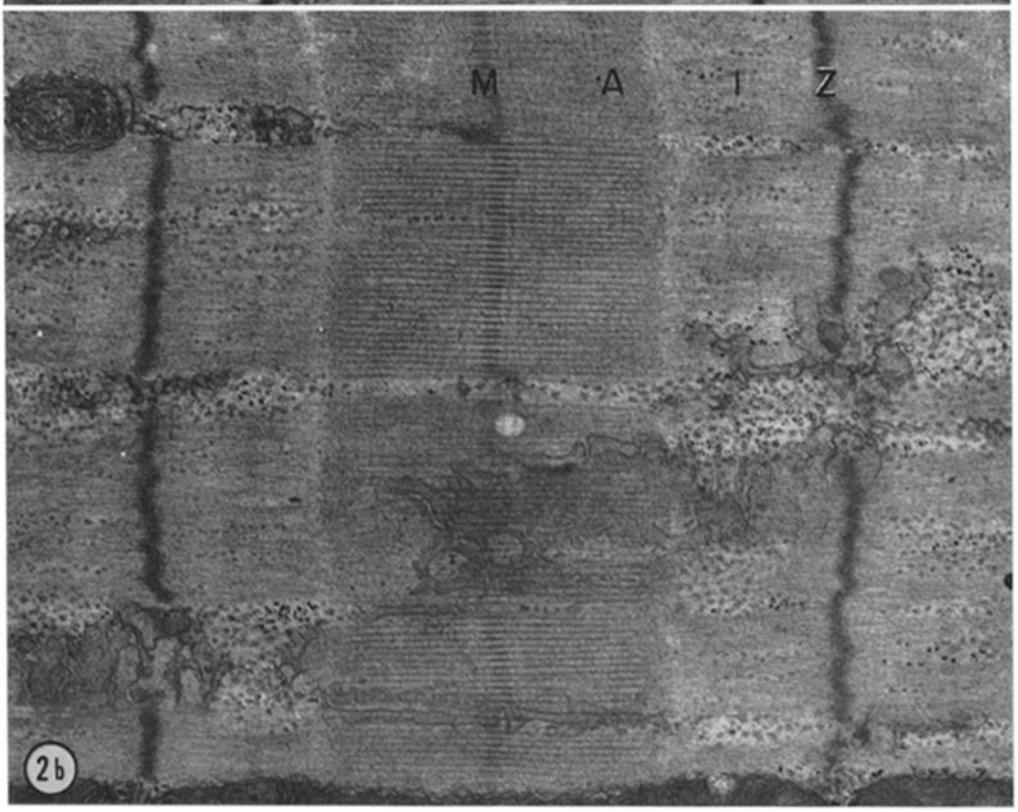
DISCUSSION

Single fibers exposed only to isosmotic sodium salines, fixed in Palade's fixative, and prepared for electron microscopic study have a myofilament lattice volume essentially identical with the in vivo volume (Table I and Fig. 1) as inferred from X-ray diffraction data. In the case of whole muscles fixed in several ways in glutaraldehyde, then teased into single fibers before postfixation in Palade's fixative, these single fibers have a lattice volume about 25% smaller than the in vivo volume. However, data on fibers fixed with either fixative support the conclusion that the packing density varies directly with the sarcomere length.

During sarcomere shortening, the I band diminishes in width until it disappears at approximately 65% of the resting length (2, 3). In draw-

FIGURE 2 Electron micrographs of longitudinal sections of two fibers with average sarcomere lengths of 2.23 (*a*) and 2.86 μ (*b*). The examples shown are not the exact average length. Labels indicate the A band (*A*); I band (*I*); M band (*M*); and the Z disc (*Z*). X 29,000.


M A I Z
2a


M A I Z
2b

ings of the sliding-filament model this sequence of events (Fig. 4 *a* and *b*) commonly is portrayed as a decrease or a disappearance of the volume occupied by the I band (13). On the basis of the evidence the third, or thickness, dimension must be added to the sliding-filament model. Part of the I band volume, including the thin myofilaments, moves into the A band and the thick myofilaments separate to accommodate the added volume (Fig. 4 *d*). As a result, the sarcomere becomes thicker as it becomes shorter.

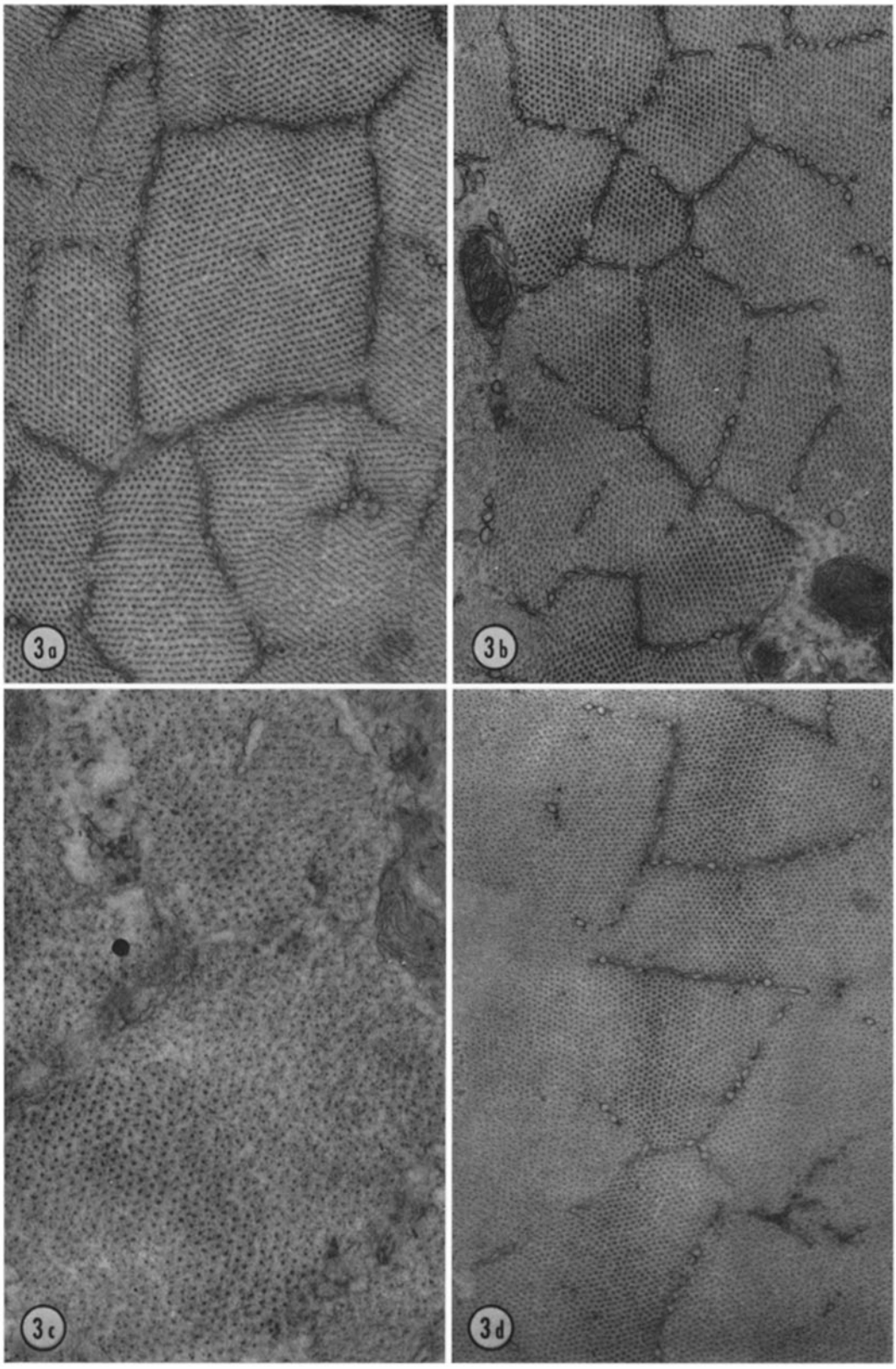
It is surprising that the lattice volume obtained from electron microscopic data on single semitendinosus fibers fixed in Palade's fixative agrees with X-ray diffraction data on whole living semitendinosus muscles (6). The myofilaments are thought to be shortened markedly by Palade's method of fixation (14), and single fibers are reported to lose 20–30% of their volume during osmium tetroxide fixation and ethanol dehydration (15). However, in our study the average lattice volume of single fibers was unchanged after osmium tetroxide fixation, ethanol dehydration, and embedding in Epon (Table I). If lattice volume is lost during the first two steps of preparation for electron microscopy, perhaps analogously to lattice shrinkage which accompanies drying of the muscle in X-ray diffraction studies (5), it must be recovered in the embedding procedure. It, therefore, would be of considerable interest to have X-ray diffraction data on the lattice dimensions before, during, and after preparation of fibers for electron microscopy.

In contrast to the lattice in osmium tetroxide-fixed fibers, the lattice in glutaraldehyde-fixed fibers is or remains shrunken after the same procedures. This is a particularly important point since it has been argued that the aldehyde method is superior to other methods of fixation (14) because it maintains the myofilaments at a nearly constant length independent of sarcomere length. Such results conflict with earlier reports (16, 17) that the thick myofilament length and diameter vary with sarcomere length. It is only by using techniques for accurately measuring the in vivo width of the A band or the lengths of the myofilaments that this point can be directly settled.

Fibers that had undergone loss of contents in vivo showed no measurable change in the average lattice volume. Since the lattice occupies such a large proportion of the fiber, the volume change measured before fixation somehow was erased by the preparative procedures. Fibers swollen by removal of one-half the NaCl also failed to show a consistent increase in lattice volume. Harris (18) reports that swelling frog fibers osmotically increases their extrafibrillar space and not the fibrillar compartment. Although the sample is too small to be significant, in the present study one fiber swollen by isosomotic substitution of the NaCl by KCl did have a swollen lattice (33%), and one fiber shrunk by doubling the NaCl concentration did have a lattice shrunken to about the expected degree (30%). In a preliminary study on crayfish single muscle fibers, a quantitative correlation was found between osmotically induced changes in fiber volume and thick myofilament packing density. Since the distribution of water between lattice and extracellular compartments after experimentally induced volume changes cannot be determined unequivocally by electron microscopic studies, it is important to study these changes in vivo with X-ray diffraction techniques.

The expanding - sarcomere, sliding - filament model (Fig. 4 *d*) may explain partially the function of the Z band structure. In the various models of the Z band (19, 20) each thin filament splits into four at the edge of the Z band, and each Z filament attaches to a Z filament from the abutting I band. The angle that the Z filaments make with the parent thin myofilaments may vary with

FIGURE 3 Electron micrographs of cross-sections of four fibers. (*a*) and (*b*) correspond to the two fibers shown in Fig. 2. It is evident that the thick myofilaments are packed more closely in (*b*) than in (*a*). The fiber in (*c*) was made to contract by depolarization with KCl for a few seconds before fixation, and in subsequent longitudinal sections the sarcomeres averaged about 1.0 μ in length. The packing of the thick myofilaments is obviously less dense than in (*a*) or (*b*). Although the fiber in (*d*) had a sarcomere length of about 2.2 μ, its packing density is high and resembles that of longer sarcomeres (about 3.1 μ). This high density indicated that the loss of volume (30%) induced in the fiber in vivo by doubling the NaCl concentration of the bathing Ringer solution prior to fixation was sustained in this instance throughout the preparative procedure. × 29,000.


3a
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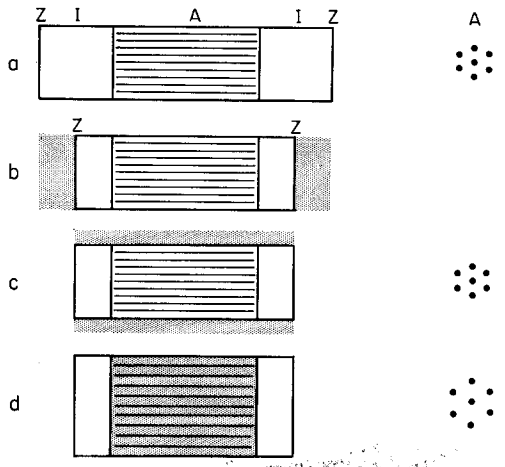

FIGURE 4 Diagram showing the volume displacements from the I band which may accompany sarcomere shortening. Longitudinal sections (left) and cross-sections (right) are represented. *a* represents a control sarcomere at rest length (left) and the corresponding packing density (right). *b* shows a shortened sarcomere, and the stippled area represents the I band volume not accounted for in the current sliding-filament model. In *c* this displaced volume theoretically is moved to the region between the myofibrils (left) and it is apparent that packing density (right) would be unaffected in this instance by sarcomere shortening. In *d* the displaced volume is redistributed in the sarcomere, and the thickening of the longitudinal section (left) and the change in packing density of the cross-section (right) are noted. This expanding-sarcomere, sliding-filament model (*d*) is in agreement with the evidence from both electron microscopic analysis of fixed fibers and X-ray diffraction analysis of living fibers. It is also in agreement with earlier light microscopic observations.

sarcomere length and allow the Z band diameter to follow the diameter changes of the fibril which accompany changes in sarcomere length. Hoyle et al. (21) report that in barnacle muscles spaces open up in the Z band as the sarcomere shortens and that these spaces are sufficiently large to permit bundles of thick myofilaments to pass through upon supercontraction of the fibers. These authors anticipated a passive expansion of the Z disc, in apparent recognition of the constant volume principle, but argued from their observations that it was in fact active. We also have observed (unpublished) thick myofilaments passing through the Z band when relaxed frog fibers are fixed. Other changes in the geometry of the myofibril are to be expected as a result of the deformation of the sarcomere as its shortens. The sarcoplasmic reticulum collar around the fibril must become thicker, and the transverse tubules will be lengthened or straightened and perhaps diminished in diameter.

It is important to examine how the separations of the thin myofilament from the thick myofilament are effected during changes in sarcomere length, for the constant approximation of these myofilaments has been assumed in the construction of a hypothesis of muscle contraction (4). Elliott et al. (6) report that the separation between actin and myosin is a function of sarcomere length and conclude that the interaction of thick and thin myofilaments must be capable of operating over distances which vary up to 70 A. To date, we have not been able to quantify with the electron microscope the changes in the separation of thick from thin myofilaments.

Several models of contractility based on the principle of interdigitating sets of sliding filaments have been proposed (4, 22, 23). However, none appears to predict both the shortening of the sarcomere and the concomitant proportional separation of the myofilaments which are required in the expanding-sarcomere, sliding-filament model of Fig. 4 *d*.

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