SARCOMERE LENGTH DISPERSION IN SINGLE SKELETAL MUSCLE FIBERS AND FIBER BUNDLES

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ABSTRACT Light diffraction patterns produced by single skeletal muscle fibers and small fiber bundles of *Rana pipiens* semitendinosus have been examined at rest and during tetanic contraction. The muscle diffraction patterns were recorded with a vidicon camera interfaced to a minicomputer. Digitized video output was analyzed on-line to determine mean sarcomere length, line intensity, and the distribution of sarcomere lengths. The occurrence of first-order line intensity and peak amplitude maxima at approximately 3.0 μm is interpreted in terms of simple scattering theory. Measurements made along the length of a single fiber reveal small variations in calculated mean sarcomere length (SD about 1.2%) and its percent dispersion (2.1% ± 0.8%). Dispersion in small multifiber preparations increases approximately linearly with fiber number (about 0.2% per fiber) to a maximum of 8–10% in large bundles. Dispersion measurements based upon diffraction line analysis are comparable to SDs calculated from length distribution histograms obtained by light micrography of the fiber. First-order line intensity decreases by about 40% during tetanus; larger multifibered bundles exhibit substantial increases in sarcomere dispersion during contraction, but single fibers show no appreciable dispersion change. These results suggest the occurrence of asynchronous static or dynamic axial disordering of thick filaments, with a persistence in long range order of sarcomere spacing during contraction in single fibers.

INTRODUCTION

Single skeletal muscle fibers exhibit regularly spaced light and dark transverse striations—the I and A bands—and consequently behave like simple one dimensional diffraction gratings when illuminated by monochromatic light (Hill, 1953). The early investigations of Sandow (1936) on whole sartorius frog muscle and of Buchthal and Knappeis (1940) on single fibers revealed that the diffraction line spacings satisfied the Bragg equation $n\lambda = d \sin \theta$ (where n = diffraction line order, λ = light source wavelength, d = the "grating," or sarcomere spacing, and θ = the diffracting angle). Following the demonstration of Marikhin and Myasnikova (1970) that sarcomere length dispersion could be estimated from diffraction line width, Kawai and Kuntz (1973) measured the dispersion in small (two to nine cell) resting semitendinosus fiber bundles as 1–2% before and 3–4% after tetanic contraction. Kawai and Kuntz observed 0–50%

decreases in first-order line intensity upon contraction, with the decrease greatest at approximately 2.8 μm.

Recently, Fujime (1975) has examined the light diffraction pattern produced by stretched "skinned" single fibers from *R. pipiens* sartorius muscle. Using a simple model of a unit cell scattering element within a muscle, Fujime derived an equation expressing the dependence of scattered light intensity upon overlapping thick and thin myofilament lengths and upon densities of myosin and actin. The observed diffraction line intensity decrease upon contraction was ascribed solely to small random fluctuations in thick filament position in the longitudinal direction.

The experiments described in this report were undertaken to determine the variation in sarcomere length and its dispersion within a single fiber, and to estimate the relative intrafiber and interfiber contributions to the observed dispersion of multifiber preparations. We also examined the dependence of first order diffraction line intensity upon sarcomere length. A preliminary description of our results has been presented at a recent scientific congress (Paolini et al., 1975).

MATERIALS AND METHODS

Whole semitendinosus muscles from double-pithed, small grass frogs (*R. pipiens*) were exposed and tied at the tendons with silk thread. *In situ* (reference) lengths were measured to the nearest 0.5 mm with the limb joints at right angles. The ventral head and exterior fibers of the dorsal head of the excised muscle were removed prior to mounting of the remaining 100–200 fiber bundle in a muscle chamber. Subsequent dissection was performed carefully under a dissecting microscope to obtain the size of preparation desired. Preparations were repeatedly washed and allowed to equilibrate 30 min in oxygenated Ringer's solution (composition in grams per liter: NaCl, 6.50; KCl, 0.14; $NaHCO_3$, 0.20; NaH_2PO_4, 0.011; $CaCl_2$, 0.12; pH adjusted to 7.4). Data from eight viable single fibers were analyzed in addition to 12 multifiber preparations ranging from 3 to approximately 150 fiber bundles.

The muscle holder, which was horizontally positioned during experiments, consisted of a small rectangular Lucite-walled chamber with a microscope glass bottom (see Fig. 1). The tendon at one end of the muscle was impaled upon the pin of a high sensitivity strain gauge force transducer. Evaluated tetanus-twitch ratios were used as a criterion of fiber viability. Muscle length could be set by a micrometer screw adjustment which positioned a clamp fastened to the other tendon. Muscle lengths were read from a millimeter scale mounted on the chamber bottom as the spacing between the tendon clamp and the transducer pin. Tetanic stimulation was applied by means of submersed parallel platinum electrodes mounted a few millimeters on either side of the muscle within the chamber.

The chamber was mounted on an optical bench 25 cm from a 3 mW continuous wave He-Ne laser of 6,328 Å wavelength. The diffracted beam fell on a concave cylindrical translucent screen located 8 cm from the muscle. A vidicon camera with a f0.95, 25-mm lens was mounted 30 cm from the screen and focused upon it.

The television camera was operated through a digital interface or controller to a small minicomputer (Paolini and Rhodes, 1973; Paolini and Roos, 1975). Programs resident in the computer loaded the controller with 9-bit x and y coordinates specifying a point anywhere within the video field. With the x coordinate held at a fixed value, y coordinates were incremented so that a 480 point resolution vertical section through the diffraction pattern could be obtained in 33.3 ms.

Camera video and tension transducer output signals were digitized and stored in a minicom-

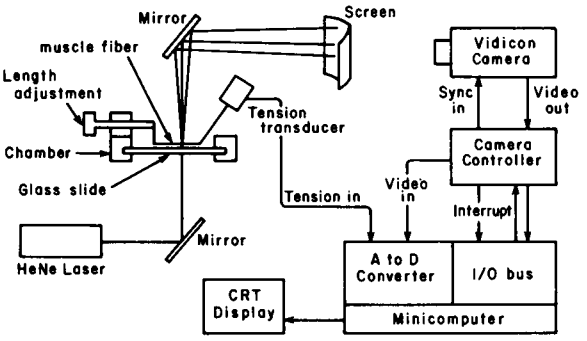

FIGURE 1 Experiment control and data acquisition system.

puter. Diffraction patterns were displayed on-line during the experiment on a CRT interfaced to the computer. Data waveforms were also stored on magnetic tape for later analysis.

Diffraction pattern waveforms were corrected for background level by subtraction of a camera dark current signal and were then subjected to a five-point smoothing program. Sarcomere lengths were calculated by computer evaluation of the zero-to-first order line peak spacing, using the formula

$$S = \lambda/\sin[\tan^{-1}(PD \cdot SF)], \tag{1}$$

where S is sarcomere length in micrometers, λ is the wavelength of the laser light source, 0.6328 μm, PD is the zero-to-first order line peak distance in horizontal coordinate units, from 0 to 480, and SF is a geometrical scale factor which depends on muscle-to-screen and screen-to-camera distances.

Diffraction line parameters were then estimated, using the equations applied to muscle diffraction phenomena by Kawai and Kuntz (1973). Parameters included the intensity, or zero moment M_0 of each line, and the first and second moments M_1 and M_2, where

$$M_0 = \sum_{n_1}^{n_2} A_i; \quad M_1 = \sum_{n_1}^{n_2} i \cdot A_i; \quad M_2 = \sum_{n_1}^{n_2} i^2 \cdot A_i; \tag{2-4}$$

with A_i = amplitude of the ith coordinate from n_1 to n_2, at which the line has fallen to background scattering level; the line width,

$$W = [M_2/M_0 - (M_1/M_0)^2]^{1/2}, \tag{5}$$

and the percent dispersion,

$$\%D = W/PD \cdot 100. \tag{6}$$

The derived relationship between line width and sarcomere length assumes that the ideal grating equation can be validly applied to muscle: specifically, it is assumed that each myofibril consists of identical sarcomeres, and that these uniform myofibrils exhibit a gaussian distribution of sarcomere lengths. Furthermore, each myofibril is assumed to scatter light independently of all other myofibrils: multiple scattering effects are neglected (Rome, 1967). To our knowledge, a general theory of light diffraction by muscle, taking into account the consequence of multiple diffraction and effects of long or short range disordering of sarcomeres, remains to be derived.

Observed line widths in unstimulated fibers were assumed to consist of only two components,

the first due to the intrinsic length dispersion among sarcomeres, and the second due to instrumental factors, principally to the finite size of the incident beam. Sarcomere length distributions appear to be gaussian. Depending on the assumed shape of the beam cross-section intensity profile (e.g., gaussian, cauchy), a relatively simple equation can be used to subtract this contribution (Klug and Alexander, 1974). If the instrument error contribution is also gaussian, the variances of the two components are additive, so that

$$\sigma_{\text{muscle}} = (\sigma^2_{\text{observed}} - \sigma^2_{\text{beam}})^{1/2}, \tag{7}$$

where σ designates the standard deviations of each curve. Line width calculations were corrected by such a subtraction of incident beam line width contributions according to this formula.

Extensive testing of the camera detector system indicated its ability to consistently resolve the position of diffraction line amplitude peaks as better than ± 1 point in 480, corresponding to sarcomere length variations of between 50 and 150 Å, depending on the absolute sarcomere length being measured. The linearity of the camera's response to light level was tested by observing camera output response to a uniformly illuminated gray wedge. The camera's output voltage to input light level correlation coefficient was 0.996. Spatial linearity of the camera was measured to be better than 1%.

Direct light microscopy was utilized to allow evaluation of sarcomere length distributions at the same values of muscle length or at the same positions along the muscle as examined in the diffraction experiments. The muscle chamber with its micrometer length adjustment was transferred from its holder on the optical bench to the x-y stage of a trinocular microscope fitted with a 40× water immersion objective and a 35-mm photomicrography attachment. Developed negatives were projected onto a screen approximately 5 m away so that 50–200 sarcomere lengths per photograph could be measured. Nine of the 20 preparations were examined (with an average of 1,408 measurements per preparation) to obtain direct estimates of length dispersion for comparison with diffraction dispersion estimates.

RESULTS

Fig. 2 illustrates the appearance of a computer generated CRT display of a vertical section through the light diffraction pattern obtained from a single frog semitendinosus fiber. Large bundles of fibers show the diffuse first-order line structure characteristic of whole muscles. For example, the mean sarcomere length of a 20 fiber bundle was calculated to be 2.967 μm, with an estimated average dispersion of 4.93%. The more sharply defined first-order lines in Fig. 2 were obtained from a single muscle fiber, and correspond to an estimated sarcomere dispersion of ± 1.57%. Dispersion estimates were corrected for the contribution from finite beam width by evaluating the undiffracted laser beam line width at the conclusion of each experiment.

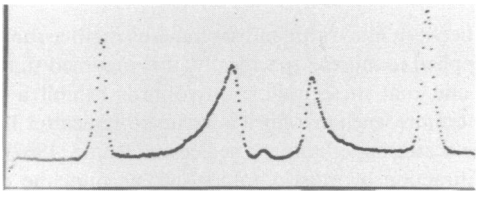

FIGURE 2 CRT display of a video scan through the diffraction pattern of a single semitendinosus fiber at rest.

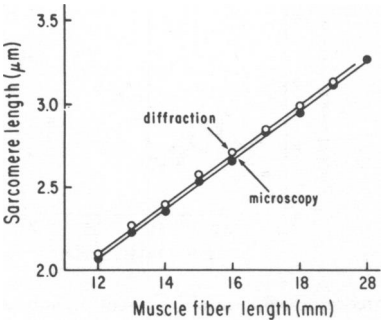

FIGURE 3

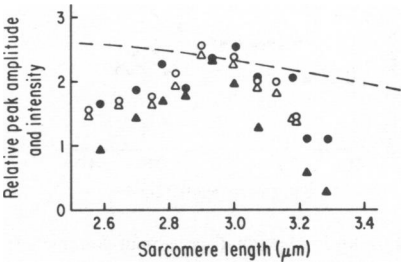

FIGURE 4

FIGURE 3 Data fitted by least squares method to fiber length-sarcomere length values obtained from diffraction line spacing (○) and from light microscopy (●) measurements on the same semitendinosus fiber. Slopes, 0.1496 and 0.1497; intercepts, 0.3091 and 0.2752; correlation coefficients, 0.9992 and 0.9992, for diffraction and microscopy data, respectively.

FIGURE 4 Dependence of first-order line peak amplitudes (○, ●) and intensities (△, ▲) upon sarcomere length of two different single semitendinosus fibers at rest.

Mean sarcomere lengths calculated from peak spacing measurements using Eq. 1 were observed to vary quite linearly with resting muscle length: correlation coefficients from the least squares analyses of this dependence exceeded 0.999 for single fibers and 0.990 for multifiber preparations. Fig. 3 illustrates the length-length relationship for a preshortened single semitendinosus fiber stretched passively from 0.86 to 1.43 of its reference length. Direct microscopic determination of mean sarcomere lengths over this same range of extension routinely yielded virtually identical data for single fiber preparations, with only slightly larger discrepancies in multifiber bundles.

The length dependence of the first order diffraction line peak amplitude and intensity for two typical single fiber preparations is graphed in Fig. 4. As with virtually all of the single fibers tested, peak amplitude and intensity exhibit relative maxima at 2.9–3.0 μm sarcomere length. Multifiber preparations exhibited maxima within the range of 2.5–3.0 μm, with the larger fiber bundles exhibiting relative maxima at correspondingly shorter sarcomere lengths.

There was no significant correlation seen between sarcomere length dispersion calculated from the standard deviation of length distribution histograms plotted from single fiber and fiber bundle lengths measured microscopically, nor was there any apparent length correlation of resting dispersion measured from diffraction line width of single fibers (Fig. 5 *a*). However, sarcomere dispersion in multiple fiber bundles was positively correlated with length (Fig. 5 *b*).

Table I summarizes the percent dispersion measurements made over a wide range of lengths (typically 2.3–3.7 μm) on eight single fibers and four multifiber bundles. With the exception of muscle 40, which was most likely a damaged preparation, mean percent sarcomere dispersion measurements calculated from diffraction line width of single fibers averaged 2%.

There was clearly a correspondence between the number of fibers present in a fiber

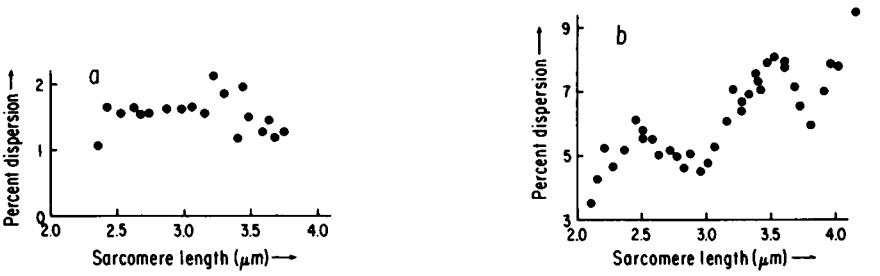

FIGURE 5 Percent dispersion in sarcomere length vs. sarcomere length in a single semitendinosus fiber (*a*) and in a 45 fiber bundle (*b*).

bundle and the sarcomere dispersion within that bundle. Fig. 6 *a* summarizes the mean dispersion data for all resting lengths examined (2.3–3.7 μm) in 12 muscle preparations as a function of the number of fibers per bundle. In order to eliminate variability from differences (in age, mean fiber diameter, amount of connective tissue, etc.) between preparations, a bundle of 20 cells was reduced in stepwise fashion to a single fiber preparation, with diffraction measurements made at each step of the dissection. The results of this experiment (Fig. 6 *b*) indicate a relatively linear dependence (correlation coefficient, 0.995) of dispersion upon fiber number. A least squares fit of data from this serial dissection yielded a slope of 0.21% dispersion increase per fiber.

Microscopic analysis of sarcomere length distribution was performed on nine of the muscle preparations for which diffraction dispersion data was available. Table I shows the correspondence in mean dispersion of sarcomere length calculated from length distribution histograms to dispersion data evaluated from diffraction line width.

TABLE I

COMPARISON OF SARCOMERE LENGTH DISPERSION MEASUREMENTS ON SINGLE SEMITENDINOSUS FIBERS AND FIBER BUNDLES AT REST

Mean values in percentages, ± 1 SD. (Muscles 40, 43, and 50 were not examined by microscopy because these single fibers sustained damage during diffraction experiments.)

Sample no.	No. of fibers	Microscopic analysis	Diffraction analysis
37	41	9.35 ± 1.25	7.66 ± 3.76
36	21	7.69 ± 1.29	7.25 ± 2.17
38	15	8.29 ± 1.36	7.00 ± 1.15
39	12	6.97 ± 0.60	4.17 ± 0.92
40	1	—	4.86 ± 0.84
41	1	1.69 ± 1.89	2.63 ± 1.11
42	1	3.97 ± 0.77	1.94 ± 1.20
43	1	—	2.43 ± 0.89
45	1	2.19 ± 2.19	1.57 ± 0.47
46	1	3.99 ± 1.17	1.90 ± 0.86
47	1	2.88 ± 2.11	2.70 ± 0.98
50	1	—	2.01 ± 0.92

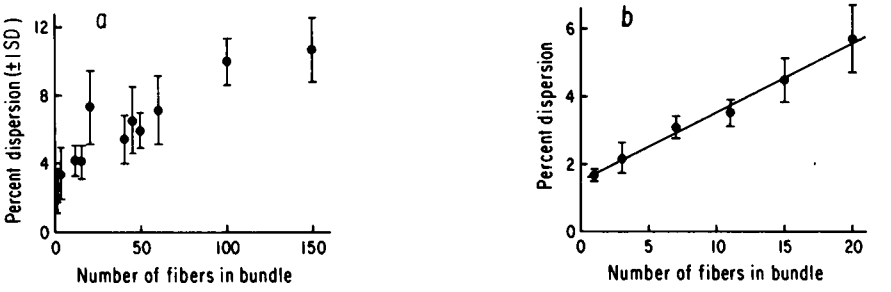

FIGURE 6 (*a*) Average dispersion in sarcomere length: dependence upon number of fibers in a bundle. Summary of 12 experiments: verticle bars represent SDs of measurements (mean = 27) taken from each preparation. (*b*) Mean dispersion in sarcomere length: its dependence upon number of fibers present in a semitendinosus preparation dissected serially from 20 fibers to 1 fiber. Vertical bars represent SDs of measurements (mean = 6.3) taken at each step of the dissection.

The changes in diffraction pattern line spacing, amplitude and width accompanying tetanic contraction of large and small bundles of fibers are illustrated in Fig. 7. Single fiber preparations yielded patterns virtually identical to that of Fig. 7 *b*. The contraction of large fiber bundles was associated with a substantial line broadening and intensity decrease, as well as the expected decrease in zero-to-first order line spacing. For the example shown in Fig. 7 *a*, dispersion increased from a resting value of 5.65% to 10.58% during tetanus, while the smaller bundle recording representing in Fig. 7 *b* corresponds to a calculated dispersion increase from 5.35% at rest to 5.93% during tetanus.

Table II summarizes the diffraction data obtained from three multiple fiber and two single fiber preparations which were stimulated tetanically. Line width and dispersion increased most substantially (by 59%) in the largest bundle; there was no significant change in line width or dispersion in single fibers. The first-order diffraction line intensity decrease was quite variable, ranging from 16 to 67% resting intensity. The smaller decrease magnitudes are probably the more reliable: large apparent decreases in some experiments seemed to accompany a very slight lateral movement of the con-

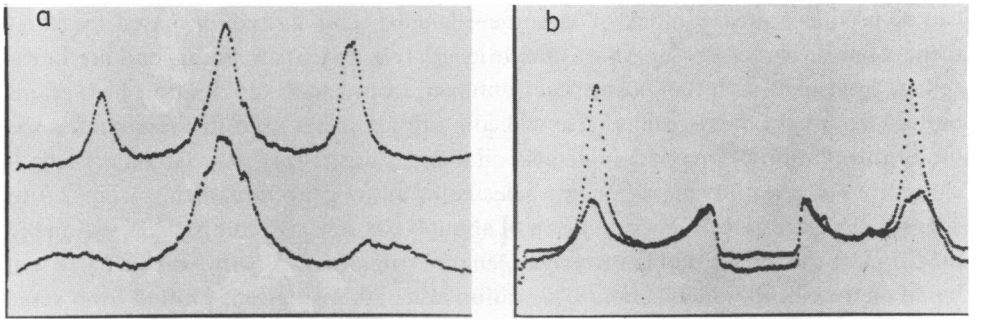

FIGURE 7 Change in the diffraction pattern from rest (upper traces) to tetanic contraction (lower traces) in large (*a*) and small (*b*) multifiber bundles. (*a*) 41 fibers: mean sarcomere length, 2.596 μm at rest and 2.144 μm during contraction. (*b*) 12 fibers: mean sarcomere length, 2.97 μm at rest and 2.86 μm during contraction.

TABLE II

CHANGES IN DIFFRACTION LINE WIDTH AND INTENSITY, AND THE CALCULATED DISPERSION IN SARCOMERE LENGTHS, FROM REST TO SUSTAINED TETANIC CONTRACTION

Summary of experiments on five preparations at various lengths from approximately 2.0 to 3.5 μm.

Muscle no.	No. of fibers	No. of waveforms analyzed	Mean shortening	Line width at rest	Line width during tetanus	$\%D$ at rest	$\%D$ during tetanus	Intensity decrease on contraction (I_{tet}/I_{rest})
37	41	16	13.6%	16.35 ± 1.42	25.93 ± 6.57	6.50 ± 1.39	8.82 ± 2.97	0.84 ± 0.36
49	20	6	7.1%	20.72 ± 2.17	25.03 ± 8.55	5.38 ± 0.47	6.17 ± 2.14	0.39 ± 0.26
39	12	20	3.4%	9.99 ± 0.63	13.27 ± 2.59	3.91 ± 0.71	5.34 ± 0.78	0.57 ± 0.16
50	1	4	1.9%	12.91 ± 1.39	13.02 ± 1.56	2.24 ± 0.68	1.74 ± 1.01	0.83 ± 0.01
42	1	15	3.5%	10.29 ± 1.31	11.53 ± 2.39	4.04 ± 1.17	4.85 ± 2.13	0.33 ± 0.23

tracting muscle, causing a decrease in the number of sarcomeres illuminated by the light source.

The optical diffraction pattern of several single fiber preparations were examined at intervals along the fiber length to determine uniformity in first-order line spacing, magnitude, and width. The small variations in mean sarcomere length were calculated for three different muscle lengths of one of the fibers studied. At a muscle length of 15 mm, the standard deviation in the mean length of 2.45 μm was ± 1.1%; at 17 and 19 mm, the mean values ± 1 SD were 2.75 μm ± 1.0% and 3.07 μm ± 1.6%, respectively. Peak amplitude and intensity of the line remained reasonably constant along the fiber. Standard deviations in normalized intensities at 2.45, 2.75, and 3.07 μm mean lengths were, in order, 13.9, 7.2, and 10.1%. The percent dispersion variations at the same three consecutive mean sarcomere lengths were 0.49, 0.45, and 0.07%.

DISCUSSION

It is well established (Sandow, 1936; Buchthal and Knappeis, 1940; Rome, 1967; Kawai and Kuntz, 1973; Paolini et al., 1974) that optical diffraction line peak spacing can be used as a precise index of sarcomere length. Our diffraction-based measurements of mean sarcomere length vs. muscle length (e.g. Fig. 3) are linear, and are in excellent agreement with the mean values obtained from length distribution histograms plotted from light micrographs. The vidicon diffractometer used in these studies exhibits a resolution of one part in 480 (Paolini and Roos, 1975); at a sarcomere length close to 2.4 μm, with camera position selected to allow peak monitoring from 2.0 to 4.0 μm, this corresponds to a resolution of about 0.008 μm. By comparison, estimated precision of the micrographic sarcomere length estimates at 2.4 μm was ± 0.018 μm based on the calculation of standard deviation in length histograms plotted from stage micrometer calibration markings. Diffractometric estimates of sarcomere length distributions should be more realistic than estimates from microscopy since the statistics are so much better, i.e., based on more than 10^6 sarcomeres (in a 1 mm length of single fiber) rather than on the 200 or so sarcomeres viewed through the microscope.

The variation of mean sarcomere length along the axis of a single fiber is quite small (Huxley and Peachey, 1961), and corresponds typically to a standard deviation of $\pm$ 1.25%.

The resting first order intensity vs. sarcomere length dependence illustrated in Fig. 4 is worthy of comment. Umazume and Fujime (1975) have derived an expression for the diffracted light intensity from a unit cell scattering element, i.e. an idealized sarcomere. The first-order line resting intensity can be written as

$$I_1 = C_1 [n_1^o \sin (2a\pi/S) - n_A^o \sin (\pi b/S)]^2, \quad (8)$$

for a sarcomere length S, where a and b are the lengths, and n_1^o and n_A^o the densities (i.e. protein concentrations), respectively, of the thin and thick filaments in a precisely aligned interdigitating myofilament array. The coefficient C_1 is dependent upon N^2, where N is the number of scattering centers irradiated by the beam; C_1 must also be inversely proportional to S, since the number of scatterers illuminated by a beam of fixed cross-sectional area will decrease as the sarcomeres are extended. If values of a = 1 μm, b = 1.5 μm (Fujime, 1975) and n_1^o/n_A^o = 0.35 (Huxley and Hanson, 1957) are assumed, the length dependence of resting intensity will exhibit the form shown on Fig. 4 (dotted line). The experimental values of first-order intensity show more dependence upon sarcomere length than can be explained by Eq. 8. These data also do not agree with the results of Kawai and Kuntz (1973), who reported a monotonic increase in intensity upon semitendinosus fiber bundle passive stretch from 2.5 to 4.0 μm.

Our observation of a marked decrease in first-order line intensity during tetanic contraction is in agreement with the reports of Kawai and Kuntz (1973) and Fujime (1975): magnitude of the decrease is variable, and can be as great as 50% or more at some sarcomere lengths (Fig. 7 *b*).

What is the origin of this intensity decrease? Any increase in line width (i.e. length dispersion) during tetanus would be expected to cause an observable intensity decrease assuming other contributing factors remain constant. However, no significant line width increases are observed to occur in single fibers and small multifiber bundles (Table II). In agreement with these results, Cleworth and Edman (1972) saw no evidence of first-order line broadening during tetanus of a single semitendinosus fiber. The experiments of Kawai and Kuntz (1973) "... indicate a general loss of diffraction intensity ... *without* a major increase in the line width of the diffraction lines" (italics theirs) produced by 2–11 fiber bundles. Fujime (1975) has suggested that the intensity decrease during tetanus may arise solely from changes in some dynamic state of the thick filaments. In particular, Fujime invokes use of the so-called "Debye-Waller factor" from X-ray crystallography; this is the amount by which a crystal's X-ray diffraction spot intensities are reduced by thermal vibrations of the scattering structures. Fujime suggests that first-order line intensities from a resting muscle are reduced by such a factor during tetanus, because of small random fluctuations in thick filament position along the axis of a fiber. The root mean square displacement of scatterers (calculated from Fujime's Eq. 8) based on intensity decreases observed during tetanus in the experiments reported here was about 0.17 μm: this is the average magnitude of axial displacement which thick filaments may exhibit during contraction.

The likelihood of such axial shifts being associated with contractile activity is further corroborated by Bonner and Carlson (1975). They recorded variations in intensity fluctuation autocorrelation functions of laser light scattered by tetanically contracting frog whole sartorius muscles and semitendinosus fiber bundles. Because of the dependence of these functions upon geometry of the scattering source, Bonner and Carlson (1975) and Carlson (1975) explained their data by proposing a model in which there occurred rapid independent random changes in axial position of some fiber component having an approximate 0.5 μm diameter; these were presumably the *A* or *I* bands if not the myofibrillar sarcomeres themselves. The scattering elements exhibited randomly fluctuating axial velocities of 200 Å/ms. The axially oscillating scattering source seen during tetanus in Bonner and Carlson's experiments is possibly the thick filament array. These investigators observed intensity fluctuation changes in fiber bundles one or two cells thick as well as in whole muscle, so that the changes must arise from within single fibers, rather than as a consequence of fiber-to-fiber interactions, i.e. a multiple scattering artifact.

The occurrence of static or dynamic asynchronous axial thick filament displacements would have major implications for current theories of the contraction mechanism. For example, the time frame in which dynamic fluctuations may occur is the same as phases one and two of the transient tension response (ascribed to the cross-bridges) exhibited by single fibers subjected to small, abrupt length changes in the experiments of Huxley and Simmons (1973).

First-order diffraction line intensity does not vary greatly along the length of a single resting semitendinosus fiber if the fiber has been carefully dissected and cleansed of connective tissue. The standard deviation of normalized intensity measurements taken along a fiber was about 10%. Kawai and Kuntz (1973) reported threefold variations in resting intensity along the length of a fiber bundle (2–11 cells) and suggested that a major source of the variation was a small amount of nerve tissue on the fibers. The single fiber preparation does not appear to suffer from this variability, however.

The substantial extent of length dispersion seen in multifibered preparations is clearly associated with variations in mean sarcomere length from fiber to fiber (Fig. 6*b*). Different fibers in the pennate semitendinosus muscle are of somewhat varied length, and probably contain different numbers of sarcomeres. Length changes applied to a fiber bundle would stretch sarcomeres to different fractional amounts, giving rise to increased sarcomere length dispersion at longer muscle lengths. Single fibers, however, exhibit a relatively consistent and small amount of dispersion—about 2%—although this figure may be doubled in damaged fiber preparations. This number agrees well with the estimates of Kawai and Kuntz (1973), indicating 1–2% dispersion before and 3–4% after tetanic contraction in small bundles, and that of Marikhin and Myasnikova (1970), showing 1.3–3% dispersion in frog iliofibularis fibers. It is quite possible that the major source of this "residual" 2% length dispersion could be a variation in mean sarcomere length among myofibrils. The work of Rome (1967) suggested that myofibrils behave as independent scatterers in diffraction experiments: that is, peak spacing, line width, and intensity are governed by myofibrillar rather than fiber geo-

metrical properties. Because single fibers appear to contain uniform lengths of myofibrils and equal numbers of sarcomeres per myofibril, applied length changes should not directly affect sarcomere length dispersion.

Dispersion measurements made along the length of a single resting fiber showed greater (although not very substantial) variability at shorter lengths, and became more consistent as the filter was stretched.

Thus, a single, resting fiber exhibits the greatest regularity of myofilament overlap when it is somewhat extended. As Kawai and Kuntz (1973) have indicated, a dispersion of 2% at a sarcomere length of 3 μm is equivalent to a standard deviation of sarcomere lengths equal to 300 Å, or 150 Å per half-sarcomere, a distance comparable to the 143 Å repeat distance of adjacent cross-bridges along the thick filament (Huxley and Brown, 1967). The fivefold greater dispersion observed in whole sartorius muscle (Paolini and Roos, 1975) is probably the simple consequence of a weighted superposition of fiber-to-fiber variations in mean sarcomere length, with each fiber exhibiting approximately 2% intrinsic dispersion. We have noted during experiments on whole muscles that a slight shift of the muscle's origin held by a tendon clamp could substantially affect the resultant dispersion measurement: presumably any skewing of the muscle introduced systematic variations in mean sarcomere lengths of fibers across the muscle.

It is known that sarcomere length dispersion increases substantially during tetanic contraction of frog whole sartorius muscle (Paolini and Roos, 1975); comparably large increases are seen in large multifibered semitendinosus preparations (Fig. 7*a*). However, small bundles (Fig. 7*b*) and sinble fibers show no marked increase in dispersion (Cleworth and Edman, 1972; Kawai and Kuntz, 1973; Fujime, 1975).

Does the hypothesized disordering of thick filaments accompanying tetanic contraction contribute significantly to the observed length dispersion? We think not. The resting dispersion calculated from first-order line width correlates reasonably well with microscopically evaluated length distribution (Table I). This implies that in the resting case, at least, the line width measurement presents a fairly accurate description of the length distribution. The data indicate that the contribution of multiple scattering interference effects to the observed diffraction line widths (and thus to the estimated dispersions) is apparently small.

There is another reason why we believe that the increased disordering of thick filaments does not contribute greatly to an increased length dispersion during tetanus. The amplitude of intensity fluctuations detected by Bonner and Carlson (1975) in resting muscle were only about 1/100 as great as fluctuations recorded in tetanized muscle. In active muscle, the fluctuations seen at 2.4 μm sarcomere length are faster by an order of magnitude than those recorded at 3.0 μm; that is, the fluctuations are most conspicuous at maximum overlap, and decrease with decreasing tension. If thick filament disordering is an important source of observed dispersion, the dispersion ought to be correlated with overlap, and it is not.

One possibility which cannot be tested with the present diffractometer, because of its slow response time, is whether the slight apparent increase in dispersion during tetanus

in larger fiber bundles is an artifact arising from the blurring of a rapidly oscillating, less broadened distribution. Cleworth and Edman (1972) and Kawai and Kuntz (1973) have reported the absence of any such synchronous length oscillations during tetanus, but 10–20 Hz oscillations have been described in whole muscle by Larson et al. (1968) and Goldspink et al. (1970).

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