STRUCTURAL FLUCTUATIONS IN THE STEADY STATE OF MUSCULAR CONTRACTION

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ABSTRACT Recent studies of the intensity fluctuation spectra of coherent light scattered from striated muscle have demonstrated the existence of large scale fluctuations in position and polarizability at the level of the myofibrillar sarcomere and its major structural subunits during the steady state of contraction. The existence of these fluctuations implies a fluctuating driving force. Various possible fluctuating motions of the thick and thin filaments, A and I bands, and entire sarcomeres are described. The magnitude of the fluctuating forces associated with the making and breaking of cross bridges is estimated. A mechanical model is proposed for coupling structural elements of a single sarcomere to one another and for coupling myofibrillar sarcomeres to one another. It is shown that the fluctuating force generated by the spontaneous making and breaking of cross bridges in conjunction with the model accounts for some of the features of the observed intensity fluctuation spectra.

INTRODUCTION

In his applications of steady-state thermodynamics to problems of biology, Aharon Katchalsky concentrated on systems which involved the transport of matter across membranes or sequences of chemical reactions. In more recent years he and his colleagues began the investigation of the structural instabilities that occur in steady-state systems. In this paper we review and discuss some of our intensity fluctuation spectroscopic studies which show that when a striated muscle passes from a state of rest into a state of contractile activity a steady state of structural fluctuations develops. These fluctuations may involve thick and thin filaments, A and I bands, or even entire myofibrillar sarcomeres and myofibrils. Such large scale structural fluctuations are in no way included in current muscle models. A mechanical model of the myofibrillar sarcomere is described and its dynamics are discussed in terms of the fluctuating tension developed by the myosin cross bridges of muscle.

There is ample evidence for believing that both resting and contracting muscle constitute two different levels of steady-state activity. In resting muscle there is a steady-state of metabolism; ATP is hydrolized at a low steady rate and there is a steady rate of oxygen consumption. Heat production proceeds at a very low but steady rate, there is no tension developed, and the sarcomeres which make up the repeating structural elements in striated muscle show a constant mean length and a stable nonfluctuating structure. In the contracting muscle heat production, ATP splitting, and oxygen con-

sumption increase markedly and reach high steady-state values during the plateau of a tetanus. The muscle develops tension which reaches a constant and steady-state value and although the mean sarcomere length achieves a constant steady-state value, it is now clear that the structural elements of the sarcomere show fluctuating optical properties during contraction.

According to our current views, tension development is the result of the asynchronous cyclic interaction of myosin cross bridges of the thick filaments with the actin of the thin filaments accompanied by ATP splitting. Although the kinetics of the cross-bridge cycle are still being developed the basic features seem to be clear. The major structural events that are believed to occur involve the movement of an ATP activated myosin cross bridge away from the thick filament to the surface of the thin filament where it combines with actin and undergoes a tension producing conformational change, releases ADP, disassociates from the actin in the presence of ATP, and reverts to its activated conformation to complete the cycle. According to this scheme the primary and possibly the only structural fluctuation that occurs in the steady state of an isometric contraction is that due to the cyclic movement of the cross bridges from the surface of the thick filament out to the thin filament and back. Recent results obtained in our laboratory definitely indicate that the structural fluctuations in muscle involve larger structural elements than the myosin cross bridges themselves. The observed fluctuations must stem from the basic force generating process; for example, from fluctuation in the number of attached cross bridges that exist at any instant of time. If the cyclic cross-bridge model for the contractile mechanism of muscle is correct, then the finding that other structural elements fluctuate during contraction must be a result of the coupling of the cross-bridge fluctuations to these other structures. It is essential to an understanding of the physics of muscular contraction, therefore, to examine various ways in which this coupling between the cross-bridge dynamics and the structural fluctuations of larger elements of the sarcomere can arise.

INTENSITY FLUCTUATION SPECTROSCOPY OF MUSCLE

The development of the coherent laser has made it possible to study dynamics of microscopic elements which make up matter. This area of modern optics, known as intensity fluctuations spectroscopy, is sometimes referred to as photon correlation spectroscopy or quasi-elastic light scattering. A comprehensive review of the subject including some of its applications to biological problems is given in Cummins and Pike (1974). If a plain polarized monochromatic coherent beam of light, such as one produced by a helium-neon laser, is incident upon a sample of matter some of the incident beam will be scattered in different directions and produce a scattered optical field. If, as is the case with muscle, the matter contains spatially periodic variations in its polarizability (refractive index) with dimensions of the order of magnitude of the wavelength of light, the spatial distribution of the intensity of the scattered light will show spatially periodic diffraction bands. If the position and polarizability of the microscopic regions of the scattering object are stationary in time, then the intensity at a

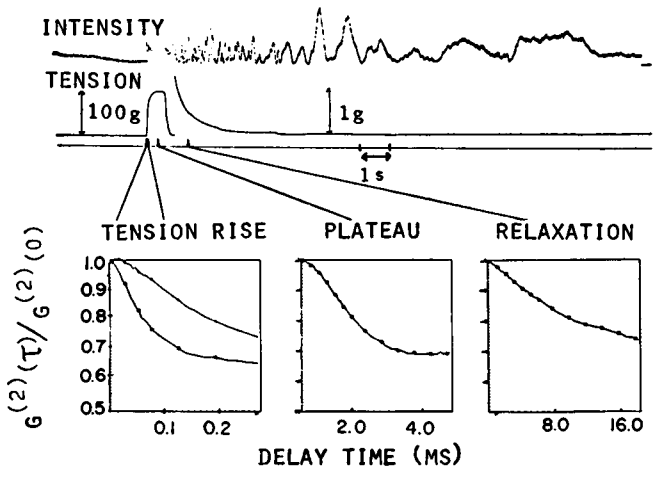

FIGURE 1 Representative time course of scattered light intensity, tension, and intensity autocorrelation function during 0.7 s isometric tetanus. Top: Intensity at detector smoothed by inertia of the strip chart recorder. Zero intensity marker at far right. Immediately below: Simultaneous tension record with 100 g indicated at left. Break in tension curve occurred when sensitivity increased 100-fold during relaxation of tetanus. Lower graphs show representative prescaled intensity autocorrelation functions with maximum set equal to 1 for comparison. Tension rise autocorrelation: contiguous 50-ms samples during the rise of isometric tetanic tension, 4–54-ms and 54–104-ms sample periods, respectively. Tension plateau autocorrelation: sample period is 0.2–0.7 s from first stimulus of isometric tetanus. Late relaxation autocorrelation: sample period is 0.5–1.5 s after last stimulus.

point in the scattered optical field will be constant or stationary in time. Thus, light scattered from a resting frog's sartorius muscle will produce a scattered field containing characteristic diffraction bands which show a speckled appearance, but which at any point in the field will have an almost constant intensity because the regions of local refractive index variation within the resting muscle fluctuate only slightly if at all. If, however, the scattering material contains elements that move relative to one another or exhibit fluctuations in their optical polarizability then the intensity at a point in the scattered optical field will not be constant but will fluctuate. These intensity fluctuations arise because the resultant optical field, $E_s(t)$, due to all microscopic scattering elements, is constantly changing in time as the elements move with respect to one another or change their polarizability, producing changing phase differences between the scattered optical field produced by one element and that produced by another. Fig. 1 shows the intensity fluctuations that occur in the light scattered from contracting muscle during various phases of a tetanic contraction and the subsequent return to the resting state as reported by Carlson et al. (1972, 1974, *a*, *b*) and Bonner and Carlson (1974, 1975). Whereas in the resting muscle there are virtually no appreciable fluctuations in the intensity of the scattered light, the contracted muscle shows extremely large and rapid fluctuations, the rapidity of which depends on the particular phase of contraction. In fact the fluctuations in intensity persist beyond the contraction

phase on into the post-relaxation phase. This then is the basic optical phenomena which we have used to study the structural dynamics of contracting muscle.

It is beyond the scope of this paper to present a detailed account of the theoretical basis and experimental techniques used to relate intensity fluctuations in the scattered optical field to the internal fluctuating forces and structural dynamics of the scattering material. These subjects are treated by various authors in Cummins and Pike (1974). Suffice it to say that the experimental quantity of interest is the intensity autocorrelation function $G^{(2)}(\tau) = \langle I(t) \cdot I(t + \tau) \rangle$ where $I(t)$ and $I(t + \tau)$ are the intensities ($I(t) = |E_s(t)|^2$) at a field point at times t and $(t + \tau)$, respectively, and $\langle \ldots \rangle$ indicates the time average. The normalized intensity autocorrelation function $g^{(2)}(\tau) = G^{(2)}(\tau)/G^{(2)}(0)$ is, for Gaussian optical field with zero mean, related to the normalized field autocorrelation function, $g^{(1)}(\tau) = \langle E_s^*(t) \cdot E_s(t + \tau) \rangle / \langle I(t) \rangle$ by the relation $g^{(2)}(\tau) = 1 + |g^{(1)}(\tau)|^2$, where $E_s(t)$ is the optical field at time t. Thus if $g^{(2)}(\tau)$ is known $g^{(1)}(\tau)$ can be determined and from it the dynamics of the scattering elements can be inferred through the use of scattering theory.

CHARACTERISTICS OF THE STRUCTURAL DYNAMICS OF CONTRACTING MUSCLE

Our intensity fluctuation spectroscopic studies on contracting frog's sartorius and semitendinosis muscle have led to the following conclusions regarding the identity of the scattering elements in muscle and the dynamic fluctuations which they undergo during contraction.

(*1*) The dominant scattering elements in striated muscle are the individual myofibrillar sarcomeres or one or more of their structural components; that is, the thick filaments and thin filaments which collectively make up the A-band–M-line complex and the I-band–Z-line complex, respectively. The precise extent of the relative contribution of the A- and I-band components to $g^{(2)}(\tau)$, the normalized intensity autocorrelation function, remains to be determined.

(*2*) $g^{(2)}(\tau)$ measurements on resting muscles have decay amplitudes, $(g^{(2)}(0) - 1)$, of about 0.01 and decay times of about 10 ms. The intensity fluctuation spectra of resting muscle might possibly be explained by the bound diffusion model of Carlson and Fraser (1974 *b*).

(*3*) $g^{(2)}(\tau)$ measurements on muscle in rigor (glycerol extracted) are flat from 10 μs to 100 ms with decay amplitudes, close to zero. This result means that there is virtually no significant fraction of the scattering material in rigor muscle that is experiencing significant movement or polarizability fluctuations.

(*4*) $g^{(2)}(\tau)$ measurements made during the plateau of an isometric tetanus are stationary and showed large decay amplitudes almost equal to those expected for free scatterers. Further, $g^{(2)}(\tau)$ approached $\tau = 0$ with zero slope and a decay time of about 1 ms at 18–20°C for a sarcomere length near 2.3 μm as shown in Fig. 2. Also, the decay time is a strong function of sarcomere length. A model that assumes: (*a*) that cross bridges behave as independent, bound Brownian particles constrained to execute

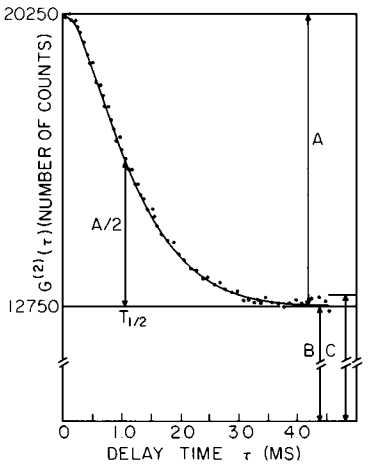

FIGURE 2 Typical non-normalized, prescaled intensity autocorrelation function, $G^{(2)}(\tau)$ from plateau of an isometric tetanus. Points: measured values of $G^{(2)}(\tau)$ for the delay times, τ, indicated. Solid line through data: best least-squares fit of data to the function, $G^{(2)}(\tau) = B + A[\exp\{-s/t_2\} - \exp\{-s/t_2\}]/(s - 1)$. A: fitted value of decay amplitude, $(G^{(2)}(0) - G^{(2)}(\infty))$. B: fitted experimental background, $G^{(2)}(\infty)$. C: theoretical estimator of background. $T_{1/2}$: delay time, the value of τ at which the fitted function, $G^{(2)}(\tau)$ equals $B + A/2$. For a correlator with essentially zero dead time and a scattered field that is Gaussian with zero mean, A is an instrumental constant determined by the degree of spatial coherence which the collection optics produce at the detector (photomultiplier) and the ratio (A/B) is $0 < (A/B) \leq 1$. The maximum value of (A/B) was evaluated for a particular set of collection optics by measuring $g^{(2)}(\tau)$ for scatterers known to produce a Gaussian field with zero mean namely a dilute solution of monodisperse polystyrene latex spheres. The maximum values of (A/B) for polystyrene spheres ranged from 0.55 to 0.60.

10 nm or so displacements away from a stationary thick filament core and (*b*) that cross bridges are the only moving elements in muscle, can not produce intensity autocorrelation functions with decay amplitudes as large as those observed ($(g^{(2)}(0) - 1) = 0.5$ to 0.6). Small decay amplitude intensity correlations are to be expected if the cross bridges are the only moving elements because light scattered from the static thick filament cores, the I band, and the Z line would show no intensity fluctuations, since these elements do not move. Thus the fraction of the fluctuating component of the scattered light would not be as great as it would be if all of these structures moved also. Further, restricting the displacement of the cross bridges to 10 nm or less is equivalent to severely constraining their diffusive motion which according to the bound diffusion theory of Carlson and Fraser (1974*a*, *b*) leads to a further reduction in the autocorrelation decay amplitude. This interpretation implies that the intensity fluctuations observed *do not* arise directly from the cross-bridge motion. If cross-bridge motion does occur it must produce movements of larger elements in the sarcomere such as the thick filaments, thin filaments, A band, or I band which in turn produce the large observed values of the autocorrelation decay amplitude.

(*5*) The dominant scattering elements attain, during the plateau of a tetanus, axial

velocities of 20 nm/ms which they maintain for a few milliseconds at least. The random to and from axial movements of the myofibrillar sarcomere, or its subunits, in one myofibril are more or less independent of the movements of the sarcomere components in other myofibrils during a tetanus plateau.

(*6*) During the plateau of an isometric tetanus myofibrillar sarcomeres show no detectable radial velocities. This is to be expected if the lattice volume of a myofibrillar sarcomere is constant, and the axial fluctuations of the sarcomere in one myofibril are independent of those in other myofibrils.

(*7*) During the transient phases of tension development or relaxation of a short tetanus or a twitch, when shortening or lengthening of the sarcomeres occurs, individual myofibrils shorten in concert and synchronously attain radial velocities consistent with the hypothesis of a constant sarcomere lattice volume. This result indicates that the sarcomere lattice volume is constant under both dynamic and equilibrium conditions.

(*8*) In addition to the axial movements of the elements of single myofibrillar sarcomeres there are fluctuations in the polarizability of these elements which are isotropic with respect to the muscle, have large decay amplitudes and approximately 1 ms decay times. These polarizability fluctuations could be due to changes in shape or polarizability of the thick or thin filaments, the A band, the I band, or the M and Z lines. Their origin is not yet understood.

(*9*) The fact that elements as large as an entire myofibrillar sarcomere, an A or I band, or a thick or thin filament execute such rapid random displacements can only mean that these structures are subject to fluctuating forces during contraction. The tension across the moving element, whether it be a sarcomere, A band, I band, or thick or thin filament can not be a constant either in time or along the length of a myofibril.

There are two other lines of evidence that are consistent with our finding of rapid axial fluctuations of the elements of myofibrillar sarcomeres. The observation of Kawai and Kuntz (1973) that the intensity of the first order diffraction maxima of single striated muscle fibers decreases significantly during contraction is consistent with the existence of random fluctuations of the A bands and the I bands or entire sarcomeres about their mean positions. Such fluctuations in the scattering elements of a lattice introduce a temporal disorder of the lattice that results in a reduction in the intensity of the diffraction maxima produced when light is diffracted by the lattice elements. This phenomenon, known as the Debye-Waller effect, is well known to X-ray crystallographers, see Ziman (1967).

Further corroboration of our results are found in the X-ray diffraction studies of Huxley and Brown (1967). These workers established that the thick myosin filaments of the A band which are organized on a three-dimensional superlattice undergo changes during contraction that result in a considerable decrease in the intensity of the X-ray reflections associated with the thick filament cross-bridge positions. Such a fall in the intensity of the thick filament reflections would occur if their lattice spacings fluctuate in space and time as they would with fluctuations in sarcomere dimensions under the constraint of constant sarcomere lattice volume.

A DYNAMIC MODEL OF THE MYOFIBRILLAR SARCOMERE

Having established the existence of rapid fluctuations in the positions of myofibrillar sarcomeres or their structural subunits it is instructive to develop and analyze a dynamical model of this system. An exact approach to this problem would require the development of the structure of the sarcomere from its constituent molecules: myosin, actin, M protein, etc. This is not yet possible. Furthermore, since our dynamical studies employ light with wavelengths ranging from 450 nm to 650 nm, we shall confine our attention to structural elements of the myofibrillar sarcomere with dimensions of this order. There are two major structures which make up the bulk of the contractile and light-scattering material in the myofibrillar sarcomere: (*1*) The thick filament designated here by *m*, which contains myosin, and the M- and C-line proteins, and (*2*) The thin filament designated here by *a*, which contains actin, tropomyosin, troponin, and at one end the Z-line substance which connects the thin filaments to one another. These two elements, *a* and *m*, are regarded as the elementary dynamic structural and dynamic elements of our model of the myofibrillar sarcomere. That is to say, the *a* elements and *m* elements that make up a single myofibrillar I or A band, respectively, are assumed to be able to make both radial and axial displacements relative to one another. We assume that there are three simple dynamical fluctuations possible for an *a* or an *m* element. These simple fluctuations are defined as follows.

Let $\mathbf{r}_a(t)$ be the position vector of the center of mass of a single particular *a* element located in the I band of a particular myofibrillar sarcomere, then $\mathbf{r}_a(t) \equiv \mathbf{r}_{oz} + \mathbf{r}_z(t) + \mathbf{r}_{za}(t)$. Where $\mathbf{r}_{oz}$ is a time-independent constant, $\mathbf{r}_z(t)$ is the time-dependent displacement of the center of mass of the collection of *a* elements that make up the I band in which the particular *a* element is located, and $\mathbf{r}_{za}(t)$ is the time-dependent displacement of the particular *a* element from the center of mass of the I band. Further $\langle \mathbf{r}_a(t) \rangle = \mathbf{r}_{oz}$ and $\langle \mathbf{r}_z(t) \rangle = \langle \mathbf{r}_{za}(t) \rangle = 0$. $\mathbf{r}_{oz}$ is a time-independent constant that corresponds to the mean position of the Z line (center of mass of an I band). Again, $\langle \ldots \rangle$ denotes the time average.

Accordingly, the three simple fluctuations of an *a* element are given by (*1*) $\mathbf{r}_z(t) \equiv 0$ for all values of t and $\mathbf{r}_{za}(t)$ is a time-dependent quantity, (*2*) $\mathbf{r}_z(t)$ is a time-dependent quantity and $\mathbf{r}_{za}(t) \equiv 0$ for all values of t, and (*3*) both $\mathbf{r}_z(t)$ and $\mathbf{r}_{za}(t)$ are time-dependent quantities. Condition *1* corresponds to no fluctuation in the Z-line position and *a* elements fluctuate about the Z-line mean. Condition *2* corresponds to no fluctuation of *a* elements about their Z line but the Z line (center of mass of all *a* elements) fluctuates as a unit about its mean $\mathbf{r}_{oz}$. Condition *3* corresponds to fluctuations in *both* the mean position of the Z-line and in the positions of the *a* element about this mean.

Similarly three simple fluctuations can be defined for the position vector of an *m* element, $\mathbf{r}_m(t) = \mathbf{r}_{om} + \mathbf{r}_m(t) + \mathbf{r}_{ma}(t)$ and the center of mass of the *m* elements in an A-band (the M line). These simple fluctuations and the no fluctuation case, labeled relaxed muscle, for which $\mathbf{r}_2(t) \equiv \mathbf{r}_{za}(t) \equiv \mathbf{r}_m(t) \equiv \mathbf{r}_{Mm}(t) \equiv 0$ for all t, are illustrated in Fig. 3.

Composite fluctuations involving both the *a*, and *m* elements and/or their respective Z line and M lines are also illustrated in Fig. 3.

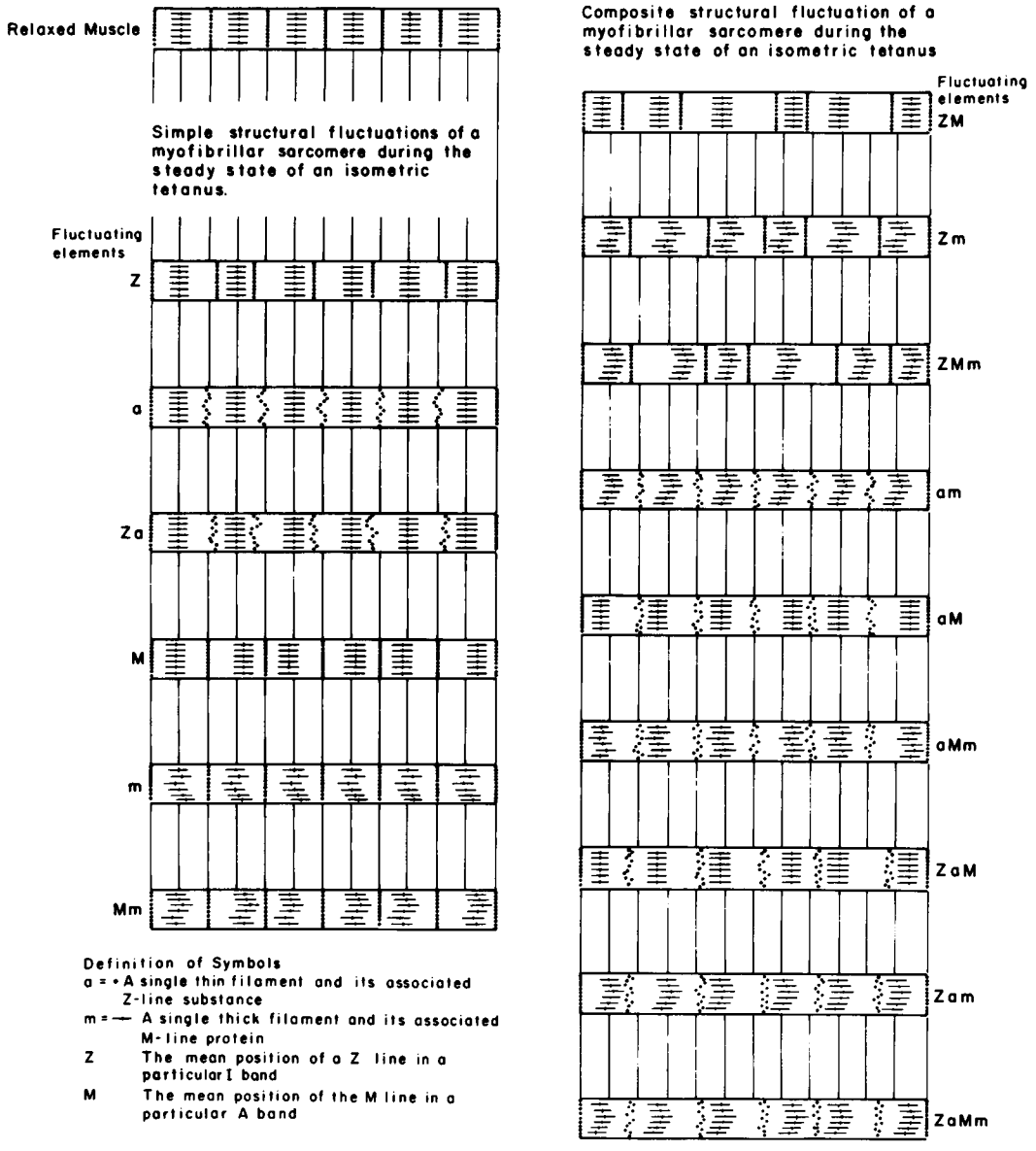

FIGURE 3 Simple and composite fluctuations that can arise in the thick and thin filaments and the A and I bands of a single myofibril during an isometric tetanus. Each of the 16 illustrations of these fluctuating motions represents an instantaneous configuration of the elements involved. See text for formal definitions of the simple and composite fluctuating motions. Vertical lines radiate positions of Z and M lines for resting muscle (no fluctuating motions).

Note that the mean positions of Z lines and M lines need not be the same in the tetanically contracting muscle as they are in a resting muscle. Such perturbations in the sarcomere spacing could lead to a loss of long or short range order in the sarcomere periodicity. All of the 16 fluctuating modes shown (including relaxed muscle) have the same average sarcomere length. In Fig. 3 the sarcomere periodicity of the resting muscle is taken to be perfect and hence possessing a high degree of long range order. In point of fact we do not know the extent of long range order in resting or contracting myofibrils.

The existence of positional fluctuations in the scattering elements of a myofibrillar sarcomere such as those schematized in Fig. 3 *implies that the fluctuating element is subjected to a fluctuating force.* What is the origin of this fluctuating force? What is its magnitude, its autocorrelation function or power spectrum, and how is it coupled on the one hand to the observed intensity autocorrelation function and on the other to the elementary contractile mechanism? These are the questions we seek to answer from the analysis of the intensity fluctuation spectra of light scattered from contracting muscle.

FLUCTUATING FORCES IN MUSCLE

Our experimental observations indicate the following:

Resting muscle. While there appears to be a small amount of motion of scattering elements in resting muscle with decay times in the 10 ms range, we have too little factual information on which to base reliable conclusions. It is possible, however, that the intensity fluctuation spectra obtained on resting muscle arise from the bound Brownian movement of the *a* elements, *m* elements either individually or collectively as I and A bands respectively, see Carlson and Fraser (1974 *b*). This possibility requires further examination.

Tetanically contracting muscle. From our intensity fluctuation spectra studies we know that $g^{(2)}(\tau)$ is essentially flat below 0.1 ms, and above 5.0 ms. Consequently, the fluctuation time scale of the contractile forces must include this range of times. While shorter or longer relaxation times may be present, they could be filtered out as a result of the properties of the coupling between the force generator and the scattering elements.

Since the decay time of $g^{(2)}(\tau)$ increases with stretch it is possible that either the autocorrelation function of the force fluctuations or the coupling of the force to the scattering elements, or both, are strongly dependent on the sarcomere lattice constants or some other structural parameter.

Recovery state. Following a brief tetanus and relaxation to a state of zero tension there are long-lasting very slow intensity fluctuations. These fluctuations appear to correlate in time with the recovery of the X-ray diffraction pattern to that of the more ordered state characteristic of the resting muscle reported by H. E. Huxley (1972). These slowly fluctuating forces may have a different origin from the contractile force. Perhaps they involve the lattice forces responsible for the long range structural order within the relaxed muscle's sarcomeres.

Fluctuations in the Position of a Elements and m Elements

To estimate the magnitudes of the fluctuating forces that might arise in an isometric tetanic steady state and produce the simple and composite fluctuation modes diagrammed in Fig. 3, and to relate them to the various proposed schemes for the kinetics of the cross-bridge cycle we shall make use of the dynamical model diagrammed in Fig. 4. For the present, we shall concentrate only on the *m*, M, and *m*M fluctuations, a similar treatment would also apply to *a* elements.

Let $\mathbf{T}_m(t)$ be the net force acting on a single *m*-element at time, t. It is the vector sum of $\mathbf{T}_l(t)$ and $\mathbf{T}_r(t)$, the forces acting on the left and right arms, respectively, of the *m* element. $\mathbf{T}_l(t)$ and $\mathbf{T}_r(t)$ are assumed independent. Thus, $\mathbf{T}_m(t) = \mathbf{T}_l(t) + \mathbf{T}_r(t)$. Further let $\mathbf{T}_l(t)$ and $\mathbf{T}_r(t)$ be given by the sum of a constant term and a time-dependent fluctuating term such that: $\mathbf{T}_l(t) = \mathbf{T}_o + \mathbf{T}_{fl}(t)$ and $\mathbf{T}_r(t) = -\mathbf{T}_o + \mathbf{T}_{fr}(t)$, where $\langle \mathbf{T}_l(t) \rangle = -\langle \mathbf{T}_r(t) \rangle = \mathbf{T}_o$ and $\langle \mathbf{T}_{fl}(t) \rangle = \langle \mathbf{T}_{fr}(t) \rangle = 0$. Again $\langle \ldots \rangle$ indicate time averages. $\langle \mathbf{T}_m(t)^2 \rangle$, the mean square fluctuating force acting on a single *m* element is $\langle [\mathbf{T}_l(t) + \mathbf{T}_r(t)]^2 \rangle$. For independent, statistically identical fluctuating force components, $\langle \mathbf{T}_{fl}(t)^2 \rangle = \langle \mathbf{T}_{fr}(t)^2 \rangle = \langle \mathbf{T}_f(t)^2 \rangle$, and $\langle \mathbf{T}_m(t)^2 \rangle = \langle 2\mathbf{T}_f(t)^2 \rangle$.

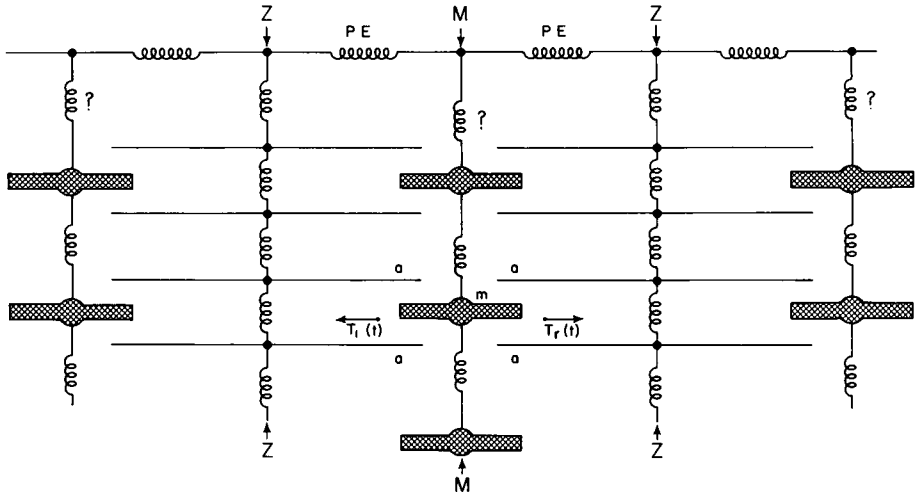

FIGURE 4 A possible dynamical model for the structural elements *a*, *m*, Z line, and M line of the myofibrillar sarcomere *a* and *m* indicate *a* elements (thin filaments) and *m* elements (thick filaments), respectively. M and Z indicate M and Z lines respectively. Springs (undamped elastic elements) between *a* and *m* elements indicate coupling of elements for both axial and radial displacements. PE indicates parallel elastic element to which the M line is coupled with a question mark indicating uncertainty. Damping due to the viscous drag associated with the displacement of *a* and *m* elements is not schematized with a dashpot although its existence is included in the model. As stated in the text, but not shown, Huxley and Simmons nonlinear Voigt elements are assumed to connect an *m* element with its associated *a* elements in their region of overlap. See text for definitions of $T_l(t)$ and $T_r(t)$. Not shown but not excluded from the model are elastic elements between each half a element and its junction with the Z line. Similarly an elastic element might be included between each arm of an *m* element and the M line.

That is, the mean square fluctuating force acting on an m element is the sum of the mean square fluctuating forces developed by each half of the element assuming statistical independence of the two halves.

These expressions for the fluctuating force can be translated into corresponding expressions for fluctuations in the instantaneous number of tension developing cross bridges between myosin and actin once one knows the coupling equations that link tension development to cross-bridge kinetics. For the cross-bridge kinetic scheme originally proposed by A. F. Huxley (1957) $|\mathbf{T}_l(t)| = k \cdot n_l(t)$ and $|\mathbf{T}_r(t)| = k \cdot n_r(t)$ where k is the force developed per attached cross bridge and $n_l(t)$ and $n_r(t)$ are the number of attached cross bridges at time t. Further, in the isometric steady state Huxley's scheme gives for each half m element: $dn(t)/dt = (n_m - n(t)) \cdot k_f - n(t) \cdot k_g$, where k_f and k_g are the rate constants for making and breaking cross bridges, respectively, (corresponding to A. F. Huxley's f and g) and n_m is the total number of myosin cross bridges on one arm of an m element. In the steady state $\langle dn/dt \rangle = 0$ and we obtain $\langle n(t) \rangle = n_m \cdot k_f/(k_f + k_g)$. Consequently, $\langle \mathbf{T}_l(t) \rangle = -\langle \mathbf{T}_r(t) \rangle = \mathbf{T}_o = k\langle n(t) \rangle$. The fluctuating force has a magnitude, $|\mathbf{T}_f(t)| = k \cdot n_f(t)$, where $\langle n_f(t) \rangle = 0$, $n_f(t)$ being the fluctuation in the number of attached cross bridges, about $\langle n(t) \rangle$. Therefore, for the entire m element, $\langle \mathbf{T}_m(t)^2 \rangle = 2k^2\langle n_f(t)^2 \rangle$. Assuming cross-bridge attachment statistics are given by the binomial distribution, it follows that, $\langle n(t) \rangle = n_m \cdot p$ and $\langle n_f(t)^2 \rangle = \langle n(t) \rangle (1 - p)$, where p is the probability of forming a cross-bridge attachment and taken to be $\frac{1}{4}$ in our calculations.

Recently, Huxley and Simmons (1971, 1972) have revised the original scheme in order to explain the mechanical response of contracting muscle to rapid changes in length or tension. The revised scheme includes not only a reaction for making and breaking cross bridges and its associated relaxation time, which is several tens of milliseconds, but it also specifies that each cross bridge contains an undamped elastic element attached to each cross-bridge head. The head has a small number(s) of combining sites (M_1, M_2, M_3, etc.) each of which combines reversibly with a site (A_1, A_2, A_3, etc.) on the actin filament. The relaxation times associated with the undamped elasticity and the equilibration of the M and A combining sites are in the 1–5 ms range depending on the length transient involved. We shall not attempt to completely analyze the force fluctuations that this revised model might produce. It could account for the fluctuation times in the millisecond range, the range of our observed intensity correlation function decay times, in the following way.

According to the Huxley-Simmons model, there is at any time a distribution of *attached* cross bridges in which two consecutive binding sites on a myosin head (M_1,M_2, etc.) are simultaneously combined with two corresponding consecutive sites on an actin filament (A_1,A_2, etc.). The tension in the undamped elastic element of an attached cross bridge varies with the particular pair of attachment sites occupied because of configurational differences assumed to exist for cross bridges associated with different pairs of combining sites. The affinity for the sites is smallest for M_1A_1, larger for M_2A_2, and steadily increases up to the $(s - 1)$th stable position at which the myosin

can be detached from the actin site by processes that involve the hydrolysis of ATP. Accordingly, when a muscle is in the steady state of contraction the allowed distributions of the thick filament cross bridges among the M_1A_1, M_2A_2, etc., attached sites are those compatible with the tension developed by the muscle. The transient responses of contracting muscle observed by Huxley and Simmons were explained by them in terms of the readjustment of the cross-bridge attachments among the M_1A_1, M_2A_2, etc., combining sites and the corresponding change produced in the tension of the undamped series elastic element. The transient behavior of this model is equivalent to a spring in series with a parallel combination of a nonlinear spring and nonlinear viscous element. Such a combination of springs and a viscous element is termed a nonlinear Voigt element. I propose that if, during the steady state of a tetanus, an imbalance in the force develops across a single m element due to the occurrence of a spontaneous difference between the number of *attached* cross bridges on the left and right arms of an m element, the situation is equivalent to imposing a rapid small tension transient on the element.

Thus, to couple the Huxley and Simmons scheme to the dynamics of the m, and a, elements we must connect the left and right arm of each m element to its overlapping a elements with $n(t)$ of the nonlinear Voigt elements which Huxley and Simmons (1972) proposed as models to describe cross-bridge viscoelastic behavior and dynamics. Of course, $\langle n(t) \rangle$ will not be the same for the two halves of an m element. The average number of attached Voigt elements (cross bridges) $n(t)$ is then determined by the kinetics of cross-bridge making and breaking and the fluctuations in the number of Voigt elements attached at any instant is determined by the statistics of $n_f(t)$, assumed independent in the two halves of an m element.

With the continuous spontaneous making and breaking of cross bridges single m elements, a elements, and entire A and I bands will experience fluctuating forces and displacements such as those schematized in Fig. 3. The velocities associated with these displacements will be determined not only by the magnitude of the fluctuating forces and their recovery transients, but they will also depend on the frictional factors of the moving elements and the coupling between elements. The frictional factor, of course, depends on the size and shape of the moving element, the viscosity of the medium in which it is moving, and on mechanical and hydrodynamic restraints imposed by the proximity of other moving or stationary elements present in the sarcomere. Even though it is not yet possible to quantitatively develop all aspects of this scheme for relating cross-bridge kinetics to the intensity fluctuation spectra of muscle, it is instructive to pursue it as far as existing data allow.

Table I shows estimates for the fluctuations in number of active cross bridges and the tension acting on a single m element and a single myofibrillar A band.

The basic data used in deriving these quantities was obtained from the literature as follows: The number of myosin cross bridges per m element has been reported to be 400 by Morimoto and Harrington (1974), giving $n_m = 200$. Haselgrove and Huxley (1973) give upper bounds of $0.15n_m$ and $0.5n_m$ for $\langle n(t) \rangle$, hence lower values are not rigorously excluded. For our purposes we have assumed $\langle n(t) \rangle = 0.25n_m$, 50 per

TABLE I

ESTIMATED FLUCTUATING FORCES ACTING ON THICK FILAMENTS AND A BANDS

Structural unit	No. of myosin cross bridges	Average no. of attached cross bridges $\langle n \rangle$	Average force $k \cdot \langle n \rangle$	Rms fluctuation in no. of attached cross bridges $\langle n_f(t)^2 \rangle^{1/2}$	Rms fluctuating force $\sqrt{2}\, k \langle n(t)^2 \rangle^{1/2}$	Force needed to produce constant velocity of 2×10^{-3} cm/s in water at 27°C = T_v	$\frac{\sqrt{2}\, k \langle n_f(t)^2 \rangle^{1/2}}{T_v}$
			dyn		*dyn*	*dyn*	
Half a single *m* element	$2 \times 10^2 (= n_m)$	50	5×10^{-5}	7	10^{-5}	5.6×10^{-9}	1,800
Half a myofibrillar A band = 800 *m* elements	16×10^4	4×10^4	4×10^{-2}	2×10^2	3×10^{-4}	4.5×10^{-6}	67

half m element. To calculate the number of m elements per myofibrillar sarcomere the unit cell area, $(\sqrt{3}/2) \cdot 1.6 \times 10^3$ nm^2 of the thick filament lattice reported by Huxley and Brown (1967) was used. Our own results, Bonner and Carlson (1975), indicate that the average myofibrillar diameter for the frog's sartorius muscle is 600 nm. To be on the safe side I have used a diameter of 1,200 nm. Hence, an A-band should contain less than 8×10^2 m elements. Tensions developed by a frog's sartorious muscles are in range of 2.0 to 3.5 $\times$ 10^6 dyn/cm^2 or 0.6 to 1.0 $\times$ 10^{-6} dyn/attached cross bridge. Since there is a fair fraction of noncontractile material in a muscle I have used k = 10^{-6} dyn/attached cross bridge in the calculations of the fluctuating forces.

As already noted, our experimental observations have shown that the scattering elements achieve speeds of 20 nm/ms (2×10^{-3} cm/s) which they maintain for periods of a millisecond or more. Neglecting inertial terms, in a viscous medium the force, $\mathbf{T}_v$, required to produce a constant velocity, v, is $f \cdot v$ where f is the frictional factor of the moving element. The value of f for an m element moving along its long axis in water (viscosity = 0.01 poise) can be obtained from its translational diffusion constant, D_T, and the fact that the frictional factor for a long rod translated parallel to its long axis is one-half that for translational motion perpendicular to the long axis. From rough measurements of D_T on native thick filaments in water (unpublished results from our laboratory) a value of 2.8 $\times$ 10^{-6} dyn/s $\cdot$ cm^{-1} was obtained for f at 27°C. The frictional factor for a myofibrillar A band was estimated by assuming the A-band to be free draining, hence since it contains 800 m elements its frictional factor is just 800 times that of an m element. While the assumption that an A band is free draining is plausible because the thin filaments and the sarcoplasmic fluid "flow" through it as it moves it remains to be either rigorously justified or revised. The assumption that the A band is not free draining but completely impenetrable seems less reasonable. Table I lists the values for the force, T_v, required to move an m element and an A band with a velocity of 2×10^{-3} cm/s and the ratio of the corresponding root mean square (rms) fluctuating force to $\mathbf{T}_v$. The fact that the fluctuating force is 1,800 times greater than the $\mathbf{T}_v$ for a single m element and 67 times greater than the $\mathbf{T}_v$ for an A band means that the effective frictional factors or other restraints acting on these elements in the muscle cell could be 1,800 times and 67 times their value in water and still the fluctuating force would be great enough to produce velocities equal to those derived from our intensity fluctuation spectra. We conclude, therefore, that it is reasonable to suppose that the moving elements that give rise to the observed intensity fluctuation spectra are either m elements (thick filaments), or A bands or both and that their dynamic characteristics as reflected in the intensity autocorrelation function will be determined by the viscoelastic forces acting on the moving element including those that are developed by the nonlinear Voigt element proposed by Huxley and Simmons as a mechanical equivalent of a single attached cross bridge.

Fluctuations in Sarcomere Length and Position

In what has been discussed so far only the m elements and A band within a single myofibrillar sarcomere have been considered. However, the m elements of one sarcomere

are coupled to those of neighboring sarcomeres through the *a* elements and Z lines. As a consequence, fluctuations in the tension developed by the *m* elements and A band of a single sarcomere will be transmitted to its nearest neighbors, next nearest neighbors, etc. and produce changes in both the length and position of these neighboring sarcomeres in the same myofibril. By combining the simple fluctuations of the *a* elements and *m* elements it is possible to produce fluctuations in the length and displacements of the individual sarcomeres in a myofibril. Such length fluctuations and displacement fluctuations are illustrated in Fig. 3. Note that only those combinations involving both Z and M fluctuations produce both changes in length and large displacements of entire sarcomeres. Such large scale length changes and displacements would have profound effects on the intensity autocorrelation function of light scattered from contracting muscle. The dimensional fluctuations of single sarcomeres would produce polarizability fluctuations. Large fluctuating displacements would produce a statistical distribution of axial and radial velocities among different sarcomeres in the same and adjacent myofibrils which would in turn give rise to intensity fluctuations. A rigorous analysis of the dynamics and light-scattering characteristics of a myofibrillar model with adjacent sarcomeres mechanically coupled in some way (for example shown in Fig. 4) is required in order to estimate the manner and extent to which cross-bridge number fluctuations propagate and produce whole sarcomere displacements and deformations which in turn contribute to the intensity fluctuation spectra. We shall report the results of such a dynamical analysis in a future publication. From what we now know it is possible to develop plausible models whose intensity fluctuation spectra will contain contributions from single myofibrillar sarcomere displacements and deformations. For the purposes of this presentation I shall discuss briefly an extreme case which would produce axial velocities of the order of 2×10^{-3} cm/s in large numbers of myofibrillar sarcomeres. A single myofibril of a 4 cm frog's sartorius muscle contains about 1.7×10^4 sarcomeres in series. The rms fluctuating force per sarcomere is from Table I, 3×10^{-4} dyn, and the average force is 4×10^{-2} dyn corresponding to a percentage fluctuation of 0.75%, or about 1%. According to the model of Huxley and Simmons (1971) a 1% change in tension will produce a change of 0.17 nm in the length of each sarcomere. Consider the following highly simplified model. Assume that the tension in any of the 1.7×10^4 sarcomeres in a myofibril held isometrically by fixing its terminal Z lines is either 1% greater or 1% less than the average tension each with a probability of $\frac{1}{2}$. This corresponds to the one-dimensional random walk problem and the rms excess of the number of sarcomeres having a tension 1% greater (less) than the average tension over those having a tension 1% less (greater) than the average tension is $(1.7 \times 10^4)^{1/2} = 130$. If all these 130 sarcomeres happened to be consecutive (an event of very low probability) the segment of the myofibril which they occupy will very rapidly increase or decrease its length by 130×0.17 nm = 22 nm in response to the tension transient and will then readjust its length according to the transient behavior of the Huxley and Simmons model and in doing so it will shorten or lengthen 22 nm in 2–4 ms at 4–6°C. The terminal sarcomeres of the segment of 130 sarcomeres will approach one another at 5–11 nm/ms at 4°C. These velocities are less than those we have

measured for the light-scattering elements in tetanically contracting muscle at 20°C. Possibly at 20°C the transient response times of the Huxley and Simmons model would be smaller, if so higher velocities would be obtained.

Obviously this highly contrived model is at best marginally successful in accounting for the observed velocities in terms of independent fluctuating forces acting on entire myofibrillar sarcomeres. Indeed it suggests that only if force fluctuations propagate or are correlated over many sarcomeres, say 10 to 100, would regions along the myofibril attain velocities of the magnitude of those observed. In other words, coupling between neighboring sarcomeres is a more likely possibility as a basis for explaining our results.

Fluctuating Displacements of Entire Myofibril

It is possible that the myofibril as an entirety is displaced by the fluctuating forces. If, for example, we assume that *a* elements are rigid elements that couple the *m* element of one sarcomere to the *m* element if its nearest neighbor, then the resultant rms fluctuating force acting on the myofibril is just that due to the left and right terminal half sarcomeres alone. This force would displace the center of mass of the entire myofibril slightly and the dynamics of these displacements would depend on the forces, frictional and elastic, acting at the surface and at the ends of the myofibril. Quantitative estimates of these forces and displacements are yet to be made.

SUMMARY

The discovery of rapid large amplitude fluctuation in the intensity of coherent light scattered from contracting muscle demands an examination of the dynamic behavior of the structural elements of the myofibrillar sarcomere and the mechanical coupling between and propagation of forces along the sarcomeres of a single myofibril. The structural elements involved are the thick and thin filaments, the A and I bands, M and Z lines, the single myofibrillar sarcomere itself, and its interaction with neighboring sarcomeres. Although our findings of large scale steady-state structural fluctuations seem to correlate with dynamic structural studies of contracting muscle obtained by X-ray diffraction techniques and quick-release transient studies, the mere existence of these structural fluctuations may very well lead to revisions or reinterpretations of the conclusions based on these other studies. We believe that further theoretical and experimental investigations of the intensity fluctuation spectra obtained from contracting muscle will provide new insight into the details of the molecular dynamics and origin of the contractile force of muscle.

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