Structural Dynamics of Frog Muscle during Isometric Contraction

ROBERT F. BONNER and F. D. CARLSON

From The Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218. Dr. Bonner's present address is the Max-Planck-Institut für Biophysikalische Chemie, Abt. Molekulare Biologie, Göttingen, W. Germany.

ABSTRACT Intensity fluctuation autocorrelation functions of laser light scattered by actively contracting muscle were measured at points in the scattered field. They were reproducible and showed characteristics which depended on the physiological state of the muscle and the parameters of the scattering geometry. The autocorrelation functions had large amplitudes and decay rates that varied significantly with the phase of the contraction-relaxation cycle. The dependence of the autocorrelation function on scattering geometry indicated many elements with diameters on the order of 0.5 μm (presumed to be myofibrillar sarcomeres or their A bands or I bands) undergo independent random changes in their axial positions and their internal distribution of optical polarizability during the plateau of an isometric tetanus. The experimental results are interpreted in terms of a model in which most of the scattering elements in isometrically contracting muscle have random fluctuating axial velocities of average magnitude 20 nm/ms that persist for a few milliseconds at least. In addition to these axial motions there are local fluctuations in polarizability. Similar intensity fluctuation autocorrelation functions were observed throughout the active state on two muscle preparations, whole sartorius muscle and small bundles of single fibers (three to eight) of semitendinosus muscle. These results imply that the tension developed during an isometric tetanus contains a fluctuating component as well as a constant component.

INTRODUCTION

An essential feature of the cyclic cross-bridge mechanism of force generation by contracting striated muscle is the stipulation that the heavy meromyosin subfragment of myosin must move away from the core of the thick filament toward the thin filament where its S-1 component combines with actin. There then follows a sequence of chemical interactions and conformational changes which result in the development of an impulsive contractile force, the hydrolysis of ATP, the dissociation of the actomyosin link, and the subsequent restoration of myosin to its initial activated but unbound state to complete the cross-bridge cycle. The evidence for the myosin cross-bridge movement comes...
largely from the X-ray studies of Elliott et al. (1965), Huxley and Brown (1967), and Haselgrove and Huxley (1973). It is indirect because the X-ray diffraction technique necessarily gives information about differences in the space-time average structure between a relaxed muscle and a contracted or rigor muscle. The actual dynamics executed by the structural elements of muscle in a given physiological state or during the transition from one physiological state to another are not directly observable by existing X-ray diffraction techniques. In vitro kinetic studies of the myosin and actin-activated myosin ATPase (Taylor, 1972; Lymn and Taylor, 1971) have lead to a chemical kinetic scheme for the cross-bridge cycle but these results allow no precise statements about the dynamic structural changes of myosin and its organized aggregates that occur during the cycle. Finally, Huxley and Simmons (1972) and Podolsky and Nolan (1972) have accepted the validity of the myosin cross-bridge cycle and developed kinetic models on this basis. They have calculated the various kinetic parameters of the assumed model from experimentally observed mechanical responses of contracting muscle to small, rapid changes in tension or length. Thus, it is fair to say that the acquisition of detailed factual knowledge about the structural dynamics of the myosin cross bridge and other structural components of muscle during contraction is a central problem of muscle physiology and a proper subject for direct experimental investigation.

We report here the results of a study of the structural dynamics of tetanically contracting muscle using a technique that is primarily sensitive to changes or fluctuations in the microscopic structure of matter including translational and rotational motions of one region of a sample relative to another. The technique, Intensity Fluctuation Spectroscopy, is based on the fact that the scattering of coherent (laser) light by matter is closely coupled to its space-time structural organization. Space-time fluctuations in structure are accompanied by corresponding fluctuations in the space-time distribution of the intensity of the scattered coherent light. A study of these intensity fluctuations yields information about structural fluctuations that occur within the scattering material.

We have found that: (a) The dominant light-scattering elements have the dimensions of the myofibrillar sarcomeres or their A and I band subunits. (b) During the plateau of an isometric tetanus myofibrillar sarcomeres attain a structural steady state during which they execute, more or less independently of one another, random displacements back and forth along the muscle fiber axis with velocities that are narrowly distributed and persist for several milliseconds at 18–20°C. (c) During the transient phases of tension development and relaxation in the twitch and tetanus the myofibrillar sarcomeres execute, in concert, rapid and synchronous axial and radial displacements consistent
with a constant volume internal shortening process. (d) In addition to the random displacements there are also random fluctuations in the polarizability of the scatterers that occur within a narrow range of decay times near 1 ms at 18-20°C. These experimental findings are incompatible with the hypotheses that: (a) the only structural elements in muscle that move during a steady state of contraction are the myosin cross bridges, and (b) the axial forces acting across or possibly within a single myofibrillar sarcomere are in exact balance at every instant during the steady state. A preliminary report of these results was given by Carlson and Fraser (1974).

METHODS

Intensity Fluctuation Spectroscopy

When a plane polarized, collimated, beam of coherent monochromatic light (such as is produced by a continuous wave Ar laser) is incident upon matter, some of the light is scattered (diffracted) out of the main beam to produce a scattered optical electromagnetic field, $E_s(t)$ at the field point, $R$, at time, $t$. Such scattering is due to spatial variations in refractive index of the material about its mean. This is why striated muscle produces its characteristic diffraction bands. The scattered field is the sum of the scattered fields of each of the microscopic scattering elements in the scattering object and the space-time distribution of the scattered field depends on how these fields interfere.

If the spatial distribution of scattering elements and their refractive index variations are invariant in time the intensity will be constant. If the spatial distribution of refractive index fluctuates with time or if there are relative movements between elements within the scattering object, or both, the field intensity will fluctuate on a time scale determined by the dynamics of the structural fluctuations. The magnitude of the intensity fluctuations is a measure of the extent to which fluctuation processes occur in the scatterer. Examples of processes that produce intensity fluctuations are: translational and rotational diffusion, chemical reactions, phase transitions, turbulent flow, and random translational movement of motile organisms.

The theory and techniques used to interpret intensity fluctuation spectra in terms of the underlying fluctuation processes are on solid mathematical and experimental grounds, see Cummins and Pike (1974). The theory yields an exact expression for $E_s(t)$ at time, $t$, in terms of the scattered fields of a set of appropriate microscopic structural elements which collectively make up the scattering sample. This is achieved by subdividing the sample into microscopic volume elements each with a local refractive index different from the mean or by representing the sample as a collection of different species of discrete scatterers each with a characteristic polarizability, such as solution of macromolecules. In the case of muscle Rome (1967) showed that individual myofibrillar sarcomeres act as identical discrete scattering elements, a result confirmed in these studies. In the case of X-ray diffraction, individual myosin and actin molecules are regarded as the discrete scatterers. The scattered field due to $M$ scattering elements each of which produces a scattered field, $E_j = A_j \exp \{i(q \cdot r_j -}$
\[
E_j(t) = \sum_{j=1}^{M} A_j \exp \{i(q \cdot r_j - \omega_j t)\}.
\]

\(\omega_j\) is the frequency of the incident light and \(q\) the scattering vector with a magnitude, \((4\pi n \sin \psi/2)/\lambda_\text{vac}\). \(n\) is the mean refractive index of the scattering sample, and \(\lambda_\text{vac}\) the wavelength of the incident beam in vacuo. \(q = k_\text{o} - k\), where, \(k_\text{o}\), the wave vector of the incident beam has a magnitude \(2\pi n \lambda_\text{vac}\) and the direction of the incident beam.

For scatterers with velocities much less than the velocity of light the wave vector of the scattered light, \(k\), also has a magnitude \(2\pi n \lambda_\text{vac}\), but its direction is that of the scattered beam which makes an angle, \(\psi\), with the incident beam. The average intensity, \(\langle I \rangle\), of the scattered light is defined as:

\[
\langle I \rangle \equiv \langle |E_\text{s}(t)|^2 \rangle = \left\langle \sum_{j=1}^{M} \sum_{j'=1}^{M} A_j^* A_{j'} \exp \{i\mathbf{q} \cdot (\mathbf{r}_j - \mathbf{r}_{j'})\} \right\rangle.
\]

An ideal photomultiplier at \(R\) will have an average current proportional to \(\langle I \rangle\) and an instantaneous current proportional to the instantaneous intensity, \(I(t)\):

\[
I(t) = E_\text{s}^*(t) \cdot E_\text{s}(t) = \sum_{j=1}^{M} \sum_{j'=1}^{M} A_j^* A_{j'} \exp \{i\mathbf{q} \cdot (\mathbf{r}_j - \mathbf{r}_{j'})\}.
\]

The field autocorrelation function, \(G^{(1)}(\tau)\), is defined as:

\[
G^{(1)}(\tau) = \langle E_\text{s}^*(t) \cdot E_\text{s}(t + \tau) \rangle.
\]

The intensity autocorrelation function, \(G^{(2)}(\tau)\) is defined as:

\[
G^{(2)}(\tau) = \langle I(t) \cdot I(t + \tau) \rangle = \langle E_\text{s}^*(t)E_\text{s}(t)E_\text{s}^*(t + \tau)E_\text{s}(t + \tau) \rangle.
\]

When \(E_\text{s}(t)\) has a Gaussian distribution about a mean of zero, it is related to \(G^{(2)}(\tau)\) as, \(g^{(2)}(\tau) = 1 + |g^{(1)}(\tau)|^2\). Where \(g^{(1)}(\tau) = G^{(1)}(\tau)/\langle I \rangle\) and \(g^{(2)}(\tau) = G^{(2)}(\tau)/\langle I \rangle^2\), \(g^{(1)}(\tau)\) and \(g^{(2)}(\tau)\) are the normalized field and normalized intensity autocorrelation functions, respectively.

The quantity \(G^{(2)}(\tau)\) can be measured directly by photon correlation, or by photomultiplier current correlation or spectral analysis. The point is, \(g^{(2)}(\tau)\) is related through Eqs. 4 and 1 to \(A_j\), the amplitude and \(\{i\mathbf{q} \cdot \mathbf{r}_j - \omega_j t\}\), the phase, components of \(E_j\). \(A_j\) depends on the instantaneous size, shape, orientation, and refractive index of a scatterer. The phase depends only on \(\mathbf{r}_j\), which can change in time and with \(\mathbf{q}\). From measurements of \(g^{(2)}(\tau)\) it is possible to determine \(g^{(1)}(\tau)\) and obtain information about the dynamics of the scatterers: Cummins et al. (1969) Cummins and Swinney (1970).

The generation of intensity fluctuations by the interference of the scattered fields of moving particles is illustrated by considering two particles, with equal and constant amplitudes, \(A\), and velocities \(\mathbf{v}_1\) and \(\mathbf{v}_2\). From Eq. 3 for \(M = 2\) we obtain:
\[ I(t) = 2A^2 (1 + \cos(q \cdot v_{12} t)), \text{ since } (r_1 - r_2) = (V_1 - V_2)t = V_{12} \cdot t, \text{ where, } V_{12} = (v - v_2), \text{ the relative velocity of the particles. Thus } I(t) \text{ varies from } 0 \text{ to } 4A^2 \text{ about a mean of } 2A^2 \text{ with a period } 2\pi/q \cdot v_{12}, \text{ or a “time scale,” } \pi/2q \cdot v_{12}, \text{ to make a maximum fluctuation regardless of sign. The “rate of decay” of a fluctuation, } 2q \cdot v_{12}/\pi, \text{ is faster the higher } v_{12}. \]

For many nonidentical particles with a distribution of velocities there is a corresponding distribution of “decay rates,” weighted according to the scattering power of different scatterers. Since \( q \) varies as \( \sin \psi/2 \) so does the “decay rate” of intensity fluctuations as measured by \( G^{(2)}(\tau) \). A small fluctuating component in the scattered field means that only a small fraction of the scattering material in muscle is experiencing structural fluctuations of some kind.

The photon correlation technique for measuring \( G^{(2)}(\tau) \) is based on the fact that the probability that a photodetector will detect \( n(t) \) photons in a time interval, \( T \), centered at \( t \) is proportional to the intensity \( I(t) \). From this it can be shown that:

\[ G^{(2)}(\tau) = \langle n(t) \cdot n(t + \tau) \rangle, \]

where \( n(t) \) and \( n(t + \tau) \) are the number of photons detected in the interval, \( T \), about \( t \) and \( (t + \tau) \), respectively. This result reduces the measurement of \( G^{(2)}(\tau) \) for a given \( t \) to counting the number of photons, \( n(t) \) and \( n(t + \tau) \), detected in the sample interval, \( T \), centered at \( t \) and \( (t + \tau) \), respectively; multiplying these two numbers, and then repeating the process for different values of \( t \) to obtain a time average. The evaluation of \( G^{(2)}(\tau) \) can be simplified considerably by employing the technique of “prescaling,” Koppel and Schaefer (1973). Prescaling correlates \( n(t) \) with a scaled version of \( n(t) \), \( n_p(t) \), instead of correlating \( n(t) \) and \( n(t + \tau) \). \( n_p(t) \) is obtained by generating one count for every \( p \)th photon. The scaling factor, \( p \), must be chosen large enough so that the probability of detecting more than one scaled photon during \( T \) is vanishingly small. When this is so, the prescaled correlation approaches \( G^{(2)}(\tau)/p \) with negligible error regardless of the statistics of the field. Prescaling produces values of \( n_p(t) \) that are either zero or one selected at random but does not alter \( n(t + \tau) \). This reduces the multiplication of \( n_p(t) \) by \( n(t + \tau) \) to the trivial process of multiplying by zero or one.

**Spectrometer and Autocorrelator**

The spectrometer and autocorrelator used in these studies were the same as previously described in Fraser (1971) and Carlson et al. (1972) except that the digital autocorrelator was modified to compute the prescaled autocorrelation function (Koppel and Schaefer, 1973).

**Analysis of Data**

Typically, contracting muscle gave intensity autocorrelation functions like that shown in Fig. 1. Each point has an ordinate equal to \( G^{(2)}(\tau) \). The labeled parameters in Fig. 1 were evaluated from experimentally measured quantities after smoothing the data with an iterative least-squares fit of the form:

\[ G^{(2)}(\tau) = B + A(s \exp \{-\tau/t_2\} - \exp \{-sr/t_2\})/(s - 1). \]
Fitted value of decay amplitude, $G^{(2)}(0) - G^{(2)}(\infty)$. B fitted experimental background, $G^{(2)}(\infty)$. C theoretical estimator of background. $T_{1/2}$: decay time, the value of $\tau$ at which the fitted function, $G^{(2)}(\tau)$, equals $B + A/2$. For a correlator with essentially zero dead time $A$ 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 $0 < (A/B) < 1$. 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 value of $(A/B)$ for polystyrene spheres ranged from 0.55 to 0.60.

This function with four parameters ($s$, $t_2$, $B$, and $A$) gave excellent fits to the autocorrelation data, see Fig. 1. The constant background, $B$, at long delay times is $G^{(2)}(\infty)$. A theoretical estimate of $B$ (designated by $C$ in Fig. 1) was calculated from the relation $C = (N^2/S \cdot p)$ where $N$ is total number of photons counted in $S$ sample times at a prescale level, $p$. $N$, $S$, and $p$ are all measured or predetermined quantities. The absence of a significant difference between $B$ and $C$ indicated the absence of an unmeasured, long-term correlation in the signal. The autocorrelation decay amplitude, $A$, is defined as: $A = (G^{(2)}(0) - G^{(2)}(\infty))$. The ratio, $(G^{(2)}(0) - G^{(2)}(\infty))/G^{(2)}(\infty)$, evaluates $g^{(2)}(0) - 1$, the normalized decay amplitude. It is a

FIGURE 1. Typical nonnormalized, prescaled intensity autocorrelation function, $G^{(2)}(\tau)$ from plateau of an isometric tetanus. Points: measured values of $G^{(2)}(\tau)$ for the delay times, $\tau$, indicated. Solid line through data: best least-squares fit of data to smoothing function, Eq. 7. A Fitted value of decay amplitude, $G^{(2)}(0) - G^{(2)}(\infty)$. B fitted experimental background, $G^{(2)}(\infty)$. C theoretical estimator of background. $T_{1/2}$: decay time, the value of $\tau$ at which the fitted function, $G^{(2)}(\tau)$, equals $B + A/2$. For a correlator with essentially zero dead time $A$ 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 $0 < (A/B) < 1$. 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 value of $(A/B)$ for polystyrene spheres ranged from 0.55 to 0.60.
measure of the magnitude of the intensity fluctuations. Since the measured autocorrelation functions had a characteristic sigmoidal shape, the time to decay to half the decay amplitude, \( T_{1/2} \), called the decay time, was used to characterize the time-course of the decay of the autocorrelation function. Plots of normalized autocorrelation function decays were used to compare the shape and time-course of individual intensity autocorrelation functions. Frequently, the results are presented or discussed in terms of the decay rate which is defined as \( 1/T_{1/2} \).

**Muscle Dissection and Mounting**

The sartorius muscle of *Rana castebiana* has a much less diffuse sarcomere diffraction pattern than *Rana pipiens* (A. Fraser, personal communication). Moreover, Fraser and Carlson (1973) described a pinned mounting which holds a 1-mm² central region of a sartorius muscle in a fixed position within limits of 0.25 mm throughout an isometric tetanus. For these reasons, sartorius muscles from small (80-150 g) bullfrogs were used in most of our experiments. The tissue preparation and muscle mounting followed the procedure of Fraser and Carlson (1973). When scattering was observed in the plane perpendicular to the muscle fiber axis (denoted as vertically oriented muscle), the Lucite muscle mount was placed in a snug-fitting cylindrical cuvette (Phoenix Precision C-105, Phoenix Precision Instrument Div., Virtis Co., Inc., Gardiner, N. Y.) filled with Ringer's solution. In order to observe scattering in a plane containing the muscle fiber axis (denoted as horizontally oriented muscle) the muscle mount, without the cuvette, was mounted horizontally on a translatable stage. Horizontally mounted muscles were periodically flushed with fresh Ringer's solution to prevent their deterioration.

Live fiber bundles were dissected from the semitendinosus muscle of *R. castebiana*. The whole muscles were rapidly dissected and transferred to ice-cold Ringer's solution for the dissection of small fiber bundles. First, a single fascicle (10-20 fibers) free of discernible nerve fibers was obtained. This was then carefully reduced to a smaller bundle or a single fiber. A thread with a loop in it was tied to one tendon and used for attachment to a tension transducer (RCA 5734, RCA Electronic Components, Harrison, N. J.). After completion of the dissection, the fibers were stored in Ringer's at 4°C for 2-10 h, and then mounted between fixed forceps and the tension transducer. The preparation was then transferred to the scattering cuvette and positioned at the center of the rotary table of the spectrometer in the horizontal orientation. The scattering angle, \( \psi \), was usually about 30°, near the third-order sarcomere diffraction maximum for a muscle with mean sarcomere length of 3.0 \( \mu \)m.

**Stimulation and Programming of Autocorrelator**

One channel of a dual-channel stimulator (Ortec 910, Ortec Inc., Oak Ridge, Tenn.) provided the desired stimulus routine, and the second channel triggered the autocorrelator at the desired time during the period of stimulation. A square-wave output was electronically differentiated to give experimental stimulus pulses of alternating sign, amplitudes of 0-12 V, and a decay time of approximately 0.5 ms depending on the electrical resistance of the muscle. A twitch stimulus consisted of one such exponential pulse. A tetanic stimulus consisted of a series of these exponential pulses of alter-
nating polarity delivered at a frequency of 67/s. The amplitude of tetanic stimulus was slightly supramaximal for a maximum twitch response as determined at the beginning of the experiment.

The autocorrelator could be triggered on or off at any time relative to the start of the stimulus pattern or interrupted during the time span of the autocorrelation measurement. The memory of the autocorrelator could be subdivided into two parts so that autocorrelation functions during two different time samples in a stimulus pattern (tetanus or twitch) or alternatively, two different time samples from different stimulus patterns could be measured. This feature made it possible to avoid effects due to variations in the state of fatigue of a muscle.

Since the accuracy of measured autocorrelation functions depends on the duration of an experiment, measured autocorrelations were necessarily time averages over some small range of physiological states and not instantaneous. For example, a typical measurement would be the average autocorrelation function during an isometric tetanus plateau from 0.2 to 0.7 s after the first stimulus. Data with the same statistical accuracy could also be obtained from the cumulated measurement of five successive tetanic plateaus sampled over the shorter time period from 0.4 to 0.5 s after the first stimulus.

The muscles were tetanized at 6- to 10-min (or longer) intervals, or twitched at 1-min (or longer) intervals, until the tension waveform showed signs of deterioration. The criteria for judging fatigue of a muscle were: (a) failure to attain 90% of initial maximal tension production, or (b) an irregular tension waveform (e.g., a tension plateau with a nonzero slope). Typical muscles produced 10–30 1-s tetani without signs of fatigue. All experiments were done in the range 15–22°C, most in the range of 18–20°C. The tension-time course was displayed on a Tektronix 547 oscilloscope (Tektronix, Inc., Beaverton, Ore.) and recorded with a Sanborn strip-chart recorder (Hewlett-Packard Co., Waltham Div., Waltham, Mass.). A second channel recorded a voltage proportional to the photon count rate averaged over approximately 5 ms and gave a graphic record of the intensity of the scattered light detected by the photomultiplier of the spectrometer.

RESULTS

Dependence of Intensity Fluctuation Autocorrelation Function on the Physiological State of Muscle

Isometrically contracting muscles were systematically studied because the tension and length of these muscles reach reproducible steady-state values. A display depicting the general relationships between the actual intensity fluctuations at a point detector and the average intensity autocorrelation function are shown in Fig. 2 for different sample times during an isometric tetanus. All of these autocorrelation functions show normalized decay amplitudes, approximately 0.6. The values of $T_{1/2}$, however, are strongly dependent on the time of observation during the period of stimulation. Slow fluctuations in intensity persist for many seconds after the last stimulus.

In Fig. 3, measured values of $1/T_{1/2}$ at different sample periods of succes-
sive tetani in the same muscle are plotted. As the tension reached a constant plateau, $1/T_{1/2}$ decreased slightly. During the maximum rate of tension relaxation, $1/T_{1/2}$ increased by more than an order of magnitude and approached the high decay rates observed during the maximum rate of tension rise at the onset of contraction. After the maximal rate of decrease of tension during relaxation, $1/T_{1/2}$ decreased roughly exponentially for approximately 0.5 s. The time constant of the decrease of $1/T_{1/2}$ during late relaxation was approximately 100 ms. Large amplitude autocorrelation functions with changing characteristics were observed more than 1 s after the last stimulus. Thus during tetanic stimulation, intensity fluctuation autocorrelation functions have decay times that depend strongly on the tension and rate of change of tension. Only during the tetanus plateau do the autocorrelation functions approach essentially constant or stationary values in contiguous time samples (Fig. 3, dotted lines) indicating that during this phase of contraction the underlying processes have attained a steady state. This is the only time during isometric contraction in which there is a constant mean sarcomere length (Cleworth and Edman, 1972). The intensity fluctuation autocorrelation studies
discussed below were for the most part conducted during the plateau of the tetanus.

The autocorrelation functions from different time samples gave virtually constant mean autocorrelation amplitudes throughout the first 1.5 s of isometric tetani in 121 experiments (Fig. 4). This result means that the amount of material involved in the fluctuating properties remains the same throughout the tetanus plateau.

Similar time-dependent studies of $T_{1/2}$ were conducted on a total of 11 muscles. Time sampling was done over a wide range in these experiments in order to explore properties of both the very early and the late phases of the plateau. The decay rate, $1/T_{1/2}$ decreased with time and reached a constant value at about 0.9 s from the initial stimulus (Fig. 5). Since values of $1/T_{1/2}$ changed significantly during the 0.2- to 0.5-sec experimental samples in the first second of the tetanus, measured average values of $1/T_{1/2}$ made during this time interval increased more slowly than would instantaneously measured values. An exponential increase of the instantaneous values of $T_{1/2}$ with a time
constant of 0.17 s would have given the results shown in Fig. 5. We conclude that during the constant tension phase of an isometric tetanus, there are structural fluctuations whose decay rates decrease approximately exponentially with time into the tetanus. These fluctuations continue to occur at a stabilized rate after approximately 0.5 s into the tetanus.

**Identity of the Dominant Scattering Elements**

The work of Rome (1967) on glycerol-extracted rabbit psoas muscle established that the dominant light-scattering elements in that preparation are the individual myofibrils within a single muscle fiber and not the muscle fibers themselves. The results described in this section show that in contracting frog's sartorius muscle the myofibril is also the dominant independent light-scattering element. Since the myofibril is composed of sarcomeres, the myofibrillar sarcomere can be regarded as the dominant scatterer but myofibrillar sarcomeres need not always act independently.

Information about the spatial dimensions and distribution of light-scattering elements in a scattering object can be obtained from measurements of the spatial distribution of the intensity of the light scattered by the object. The prominent diffraction bands produced by striated muscle arise from the organization of scattering material into sarcomeres, which have a characteristic spacing of the order of a few microns. Other scattering elements in muscle
Figure 5. The dependence of decay rate, $1/T_{1/2}$, on sample period during the plateau of an isometric tetanus. Data from 11 muscles were scaled to the mean long-time asymptote to remove variations among muscles. Dashed line indicates dependence of $1/T_{1/2}$ on the mean time from first stimulus of the interval of measurement, assuming the instantaneous values decayed exponentially to a steady-state rate with a time constant of 0.17 ms. The mean steady-state asymptote of $T_{1/2}$ was $2.9 \pm 0.9$ ms SD. Symbols denote data from same muscle.

make only a minor contribution to the muscle diffraction pattern because the intensity of the scattered light outside the diffraction band is extremely low. The spatial distribution of the intensity of scattered light in directions normal to the fiber axis is determined by the radial dimensions and the spatial organization of the scattering material normal to the fiber axis. To obtain information about the radial dimensions and spatial distribution of scattering material the muscle was mounted in the vertical orientation and the angular distribution of the scattered light intensity was measured in the horizontal scattering plane of the zeroth-order diffraction maximum (the equatorial plane). Relative intensity measurements were made at angles, of 10, 20, 30, and 40° by recording the average photomultiplier current during the plateau of a tetanus and then normalizing each current measurement by dividing the average current measured at $\psi$ by the average current measured at $\psi = 10^\circ$. These normalized quantities are tabulated in Table I along with calculated values of $P(\psi)$ for randomly packed cylindrical myofibrils with the diameters indicated. The functional form of the angular dependence of the scattered light intensity, called the form factor, is given for randomly packed, smooth cylinders by Bear and Bolduan (1950) as:

$$P(\psi) = \left| 2J_1(b \cdot q)/bq \right|^2$$

where $b$ is the cylinder radius and here $q = 4\pi n / \lambda \sin \psi/2 n$, is the average refractive index of the myofibril (assumed to be 1.38), $\lambda_0$, the wave length of
the incident light in vacuo, $\psi$ the scattering angle, and $J_1$ denotes the Bessel function of the first order. Rome (1967) included the axial variation in $n$, due to sarcomere structure and obtained an expression for the intensity distribution in the diffraction bands as well as along the bands ($P(\psi)$).

Experimental values of $P(\psi)$ fall between the theoretical values for a uniform distribution of diameters of 0.45 and 0.7 μm. Good agreement with the experimental data was obtained by assuming 30% of the myofibrils had a diameter of 0.45 μm and 70% had a diameter of 0.70 μm. If all the myofibrils had a diameter of 1.0 μm, $P(\psi)$ would have been 0.05 at 20° and negligible at higher angles, whereas a uniform diameter of 0.1 μm would have given a $P(\psi)$ that had dropped only to 0.91 at 40°. These results indicate that the dominant scattering element in the contracting frog's sartorius muscle has a diameter in the range 0.45-0.70 μm. This range of diameters corresponds closely to that reported by Rome's (1967) finding on glycerol-extracted rabbit psoas muscles, and her observation that living frog's sartorius muscles were similar to glycerol-extracted psoas muscle.

The conclusion that the myofibrillar sarcomere is the dominant independent scattering element is strengthened further by the additional finding (see below) that during the plateau of a tetanus there is no coordinated radial movement of the scattering elements in the muscle such as occurs during the rising and falling phases of tension development in the twitch and tetanus when all myofibrillar sarcomeres in the muscle fiber shorten or lengthen synchronously as the series elastic element is stretched and then released. The intensity fluctuations during a tetanus arise primarily from the fluctuations in the optical properties and/or positions of the myofibrillar sarcomeres and not the whole muscle fiber. Although the myosin cross bridges or the sarcoplasmic reticulum may produce intensity fluctuations that arise directly from their dynamic behavior during contractions these fluctuations must be very small compared to those arising from the myofibrillar sarcomere and its A and I band dynamics. This is not to say that cross-bridge dynamics are not reflected

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### Table I
A Comparison of Experimentally Observed Values of $P(\psi)$ Obtained from Tetanically Contracting Muscle with Theoretical Values Calculated for Model Consisting of Randomly Packed, Smooth Cylinders with Diameters Indicated

| $\psi$ | Observed $P(\psi)$ | $2\theta = 0.45$ μm | $2\theta = 0.70$ μm | $2\theta = 0.45$ μm - 30% | $2\theta = 0.70$ μm - 70% |
|--------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 10     | 1.00              | 1.00              | 1.00              | 1.00              |
| 20     | 0.50              | 0.71              | 0.33              | 0.47              |
| 30     | 0.14              | 0.38              | 0.02              | 0.15              |
| 40     | 0.06              | 0.14              | 0.01              | 0.06              |
in the sarcomere dynamics, they must be coupled since tension is developed by the cross bridges.

Here and in what follows when we refer to a fluctuating displacement of a myofibrillar sarcomere we do not exclude fluctuations in the positions of the thick or thin filaments, A or I bands, or M and Z lines within a single myofibrillar sarcomere. In general, displacements of any of these elements would produce a displacement in the optical center of mass of the myofibrillar sarcomere.

Form of the Intensity Autocorrelation Function

Measurements of \( g^{(2)}(\tau) \) for isometric tetani were obtained from short tetani sampled for the first half-second after constant tension was attained. Generally, 0.75-s isometric tetani were sampled from 0.2 to 0.7 s after the start of the stimulus. By using short tetani the maximum number of successive tetani having flat plateaus with at least 90% of the initial maximal tension were obtained and reliable comparisons were possible. A single tetanus at rest-length, when sampled for 0.5 s, usually gave a smooth monotonically decaying autocorrelation function (Fig. 1).

When an appropriate range for the delay time scale was used, \( g^{(2)}(\tau) \) characteristically has a slope that approaches zero at both very short and very long delay times. An experiment with a 20-fold shorter delay time scale, sampled for the same 0.5-s period of the tetanus, showed that for the shorter delay times \( g^{(2)}(\tau) \) is essentially constant. Similarly, the use of a longer delay time scale, revealed no discernible slowly varying changes in \( g^{(2)}(\tau) \). Thus the intensity fluctuations observed during the plateau of an isometric tetanus are characterized by intensity autocorrelation functions which have a narrow, continuous band of delay times.

This point was further secured by a series of 104 experiments in which the accuracy of each point of the autocorrelation function was better than 1% and the experimental background was clearly observable. The difference between the observed background, \( B \), and the theoretical background, \( C \) (Fig. 1), was calculated and found to be 0.2% (±1% SD) of the theoretical background. This result ensures that an autocorrelation measurement, with a range of delay times selected so that the initial (short delay time) flattening is apparent for the first few delay times selected so that the initial (short delay time) flattening is apparent for the first few delay times, will have decayed to within 1% of the theoretical background by the longest delay times available for the range selected (Fig. 1).

Consequently, no significant unmeasured long-time intensity fluctuations are present in the scattered field and a narrow range of delay time is sufficient to characterize the time scale of the intensity fluctuations and their underlying processes. In other words, the myofibrillar sarcomeres within the muscle move and/or change their optical properties in a manner that produces intensity fluctuations with decay rates which are limited to a rather narrow range of distribution. Little if any of the scattering material in muscle contributes to
intensity fluctuations in the scattering field which have faster or slower decay rates than those included in this narrow distribution.

Scattering Angle Dependence of Intensity Autocorrelation Function

For measurements of the dependence of $g^{(0)}(\tau)$ on $q$ or the scattering angle, the angle was changed between successive tetani.

$T_{1/2}$ for Muscle in Vertical Orientation

For vertically oriented muscles, relative motions of scattering elements along the fiber axis cannot directly cause a “phase” or $q$ dependency in $g^{(0)}(\tau)$. For this orientation, only relative motions in the scattering plane, which is perpendicular to the fiber axis, and changes in intrinsic scattering properties produce intensity fluctuations. Measurements of the angular dependence of $1/T_{1/2}$ were made on five muscles with sarcomeres at mean lengths slightly above rest length for scattering angles, $\psi$, of 10°, 20°, 30°, and 40°. Linear least-squares fits of the dependence of $1/T_{1/2}$ on $q$ were made for all the measurements on each muscle individually. In no case was there a large variation of $1/T_{1/2}$ over the nearly fourfold change in $q$. The largest individual variation observed was a 22% decrease in $1/T_{1/2}$ as $q$ increased fourfold.

Forty-seven experiments on five muscles gave the plot of the $q$ dependence of $1/T_{1/2}$ shown in Fig. 6. Within the margin of experimental error $1/T_{1/2}$ does not show a significant $q$ dependence for vertically mounted muscle.

![Figure 6](image-url)

**Figure 6.** (Left) The dependence of $1/T_{1/2}$ on $q$ for vertically oriented muscle for the first 0.5 s of the plateau of isometric tetanus. Solid line: linear least-squares fit of $1/T_{1/2}$ to data (solid vertical bars indicate standard error and dotted vertical bars the standard deviation). Data were obtained from 47 experiments on five muscles. Data from each muscle were scaled to the mean of all muscles assuming linear dependence of the slope and zero intercept in order to eliminate variations among muscles. Dashed horizontal line: linear least-squares fit of same data (solid circles) scaled assuming independence of slope and zero intercept variations among muscles. (Right) The dependence of $1/T_{1/2}$ on $|q| \cdot \cos \psi/2$ for horizontally oriented muscles during the first 0.5 s of the plateau of isometric tetanus. Points with error bars are data from 62 experiments on four muscles with data from each muscle scaled assuming independence of slope and zero intercept in single muscle data. Dashed line: computed fit of model of axial velocities with mean of 20 nm/ms using Eqs. 7 and 9.
The lack of a \( q \) dependence for \( 1/T_{1/2} \) and \((g^{(2)}(0) - 1)\) for autocorrelations measured in the scattering plane perpendicular to the axis of the muscle indicates that myofibrillar sarcomeres do not execute relative radial displacements as large as 10 nm over a time interval as short as \( T_{1/2} \) during the plateau of an isometric tetanus. The fluctuations in intrinsic scattering properties randomize the scattered field more rapidly than any phase randomization due to relative motion between scattering elements. This orientation was chosen so that axial velocities projected onto the scattering plane would have zero magnitude.

**\( T_{1/2} \) for muscle in horizontal orientation** When the scattering plane is chosen to include the muscle axis, relative axial velocities due to fluctuations in sarcomere position and length can be observed. To detect such axial displacements and ascertain whether the intensity fluctuations observed for vertical orientation were isotropic, \( g^{(2)}(\tau) \) was measured on horizontally oriented muscles. Sixty-two experiments on four isometrically tetanized muscles fixed near rest length were performed during the first 0.5 s of the plateau of tension at scattering angles between 10 and 55°. Each muscle showed a significant increase in \( 1/T_{1/2} \) with increasing \( q \), and when these data were averaged the plot shown in Fig. 6 was obtained. The significant linear dependence of \( 1/T_{1/2} \) on \( q \) indicates that there must be relative axial motions of the scattering elements which influence \( g^{(2)}(\tau) \) on the same time scale as the \( q \)-independent "intrinsic" fluctuations in the scattering properties (see Discussion). The finite intercept at \( q = 0 \) indicates the existence of a fluctuation process involving the intrinsic scattering properties of the scattering elements which dominates \( g^{(2)}(\tau) \) at low angles, where the \( q \)-dependent fluctuation process becomes much slower.

In order to clearly demonstrate that the \( q \)-independent (zero-\( q \) intercept) process was independent of the scattering plane, it was necessary to eliminate the effects of variations among muscles. Low-scattering-angle experiments were performed on the same muscle when mounted in both the horizontal and vertical orientations at the identical sarcomere length. The results of these experiments given in Table II show that at low angles the values of \( T_{1/2} \), were essentially the same. Since the \( q \)-independent term is dominant at these low

| Scattering angle | Vertically mounted muscle | Horizontally mounted muscle | Scattering angle |
|------------------|--------------------------|----------------------------|-----------------|
| IDD4             | 1.04                     | IDD5                       | 1.05            |
| IDD5             | 1.05                     | 11°                         |
| 2DD2, 3, 4       | 1.70, 0.07               | 2DD7, 8, 9, 10              | 1.78            |
|                  | ± 0.07                   | ± 0.08                      | 22°             |
angles for horizontally oriented muscle, the quality of \( g^{(2)}(\tau) \) at \( \varphi = 0 \) for the two orientations in the same muscle means that the \( q \)-independent process is spatially isotropic. In contrast, the \( q \)-dependent process is significant only in horizontally mounted muscle which leads to the conclusion that the \( q \)-dependent process is caused by relative movements of the sarcomeres along the muscle fiber axis.

In general, the decay amplitude, \( g^{(2)}(0) - 1 \), was large at all angles observed, azimuthal as well as equatorial, with respect to fiber axis. These large-amplitude decay amplitudes, with mean values between 0.42 and 0.55, were slightly less than amplitudes measured on a dilute aqueous (0.1\% SDS) solution of 0.1 \( \mu \)m polystyrene latex spheres. When a series of experiments of comparable length were performed with the freely diffusing polystyrene latex particle solution using identical collection optics, a decay amplitude of 0.57 ± 0.04 SD was obtained. In a polystyrene latex sample there are always a large number of independently diffusing scatterers within the scattering volume, and the amplitude of the scattered field is Gaussian distributed with zero mean. Since the decay amplitudes measured on early tetanic plateaus were approximately 90\% of the maximum value obtained on the polystyrene latex sample, we must conclude that most of the scattering elements within the muscle must contribute to the intensity fluctuations in such a way that the field is Gaussian with a mean near zero. An autocorrelation decay amplitude that is only 10\% below that given by Gaussian field can be interpreted to mean that either (a) the scattering from all elements fluctuates, but not with zero mean; or (b) up to 30\% of the scattering mass is static. The fact that \( g^{(2)}(\tau) \) monotonically decreases to the theoretical background is strong additional evidence that the observed field for muscle is the sum of the optical fields of a very large number of independent scattering elements.

**Effect of Scattering Volume**

Fluctuating bulk translations of heterogeneous regions of the muscle into and out of the scattering volume would produce intensity fluctuations with time characteristics determined by the average time taken by the moving regions to traverse the scattering volume swept out by the incident beam. For this case, the transit time and consequently \( T_{1/2} \) should both increase with an increase in scattering volume.

Thirty experiments on two muscles were performed, using two different scattering volumes, with dimensions that differed fourfold in the direction of the fiber axis. The average values of \( T_{1/2} \) and the decay amplitude obtained for each of these two different scattering volumes are shown in Table III. No significant difference exists between them. On the other hand, the decay amplitudes decreased 72 and 59\% with the increase in scattering volume. These decreases compared well the 59\% decrease observed on the poly-
TABLE III
COMPARISON OF T₁/₂ DATA FOR DIFFERENT SCATTERING VOLUMES

| Muscle       | Small volume T₁/₂ ms | Large volume T₁/₂ ms | Small volume A/B | Large volume A/B |
|--------------|----------------------|----------------------|------------------|-----------------|
| IFF (19 exp.)| 1.01 ± 0.02          | 1.00 ± 0.02          | 0.52 ± 0.01      | 0.15 ± 0.003    |
| 2HH (11 exp.)| 1.93 ± 0.05          | 1.77 ± 0.05          | 0.36 ± 0.01      | 0.15 ± 0.003    |

Sytrene latex sample for the same scattering volumes and hence could be attributed to the decrease in the spatial coherence at the detector associated with the increase in scattering volume (Jakeman et al., 1970 b).

Values of T₁/₂ did not change significantly when the incident beam was focused to 100-μm diameter, or when the incident beam was expanded to 10-mm diameter. For these two extreme cases the spatial variation in the intensity of the incident beam over the scattering volume was large yet without any effect on T₁/₂. If the spatial variation in incident intensity were a critical factor, it should have been greatly enhanced in the focused-beam experiments. A comparison of data obtained from one muscle gave T₁/₂ values of 1.25 ± 0.19 ms (SEM) for the focused-beam experiments, and 1.42 ± 0.04 ms (SEM) for the unfocused- (normal) beam experiments. Measurements of T₁/₂ on muscles illuminated with the central portion of a 10-fold expanded beam were the same as those measured on muscles with the normal incident beam. We conclude that variations in the spatial distribution of the intensity of the incident illumination do not contribute significantly to the intensity fluctuations and hence, the intensity fluctuations do not arise from local or large scale bulk movement of heterogeneous regions of the muscle into and out of the scattering volume.

Dependence on Sarcomere Length

In preliminary surveys of the intensity fluctuations, it was observed that they became slower by approximately an order of magnitude when the mean sarcomere length was increased from 2.4 μm, to approximately 3.0 μm. Since it took longer to collect data with equivalent statistics from muscles with slower fluctuations, most of the experiments reported in this section were performed near rest length in order to minimize variations due to muscle fatigue. The sarcomere length was varied on four muscles in order to compare autocorrelations measured during the first 0.5 s of the isometric plateau at different sarcomere lengths. These data, summarized in Table IV, show a 6- to 10-fold increase in T₁/₂ with stretch from 2.4 to 2.9 μm.
Twitch Studies

$g^{(2)}(\tau)$ measurements were obtained during the various transient phases of tension development of vertically oriented isometric muscle during a twitch contraction. The general trend of these experiments has been previously reported by Carlson et al. (1972). Individual measurements of $g^{(3)}(\tau)$ were obtained by accumulating data from a series of 10–25 twitches with time samples set relative to the stimulus pulse. $g^{(2)}(\tau)$ measured during the first 2 ms after stimulation in muscle twitches at rest-length were flat, indicating that no large-amplitude intensity fluctuations occurred before the development of tension. Although the values of $T_{1/2}$ were much smaller for $g^{(2)}(\tau)$ measured during twitches than for $g^{(2)}(\tau)$ measured during tetanic plateaus, both the shape and the decay amplitude were similar. Both showed nearly zero slope for short delay times, and both approached the theoretical base line within several decay times. The same functional form which was used to fit tetanus data (Eq. 7) also fits twitch data quite well using very different values of the time constant $t_\tau$. Thus there is a narrow range of decay rates of intensity fluctuations of the various scattering elements in a muscle during a twitch. This rapid, sigmoidal, $g^{(2)}(\tau)$ is similar to that observed during the most rapid changes of tension in the rising and relaxation phases of tetanic contractions.

$g^{(3)}(\tau)$ measurements during the first 25 ms of the rising phase of a twitch in a vertically oriented muscle were obtained in a set of 37 experiments from six muscles at near-rest length. The data (Fig. 7) show a strong $q$ dependence over the range of measurements, and a significant nonzero intercept at $q = 0$. The decay amplitude showed a slight (12%) increase, from 0.49 to 0.55, as the scattering angle increased from 0 to 60°. These results indicate that the $g^{(3)}(\tau)$ measurements from vertically oriented, isometrically twitched muscles are due in part to relative velocities of the scattering elements in the plane of scattering, which is perpendicular to the muscle fiber axis. Similar results were observed during the rising phase of a tetanus. As is shown in the Discussion,
Figure 7. $1/T_{1/2}$ as a function of $q$ for the first 25 ms of the rising phase of twitch tension in 37 experiments on six muscles in vertical orientation. X: mean with standard error bars of experiments with variations among muscles, scaled assuming linear dependence of slope and $q = 0$ intercept. Solid circles: same data, scaled assuming independence of slope and $q = 0$ intercept variations among muscles. Dashed line indicates fit of radial velocity model with a velocity maximum of 3 mm/s.

This result leads to the conclusion that the myofibrillar sarcomeres have high radial velocities during these transient changes in tension which arise from the transient synchronous or coordinated shortening of all myofibrils at constant volume, due to the presence of the series elastic element.

**Relaxed Sartorius Muscle**

Initial experimental observations of the single-clipped autocorrelation on resting muscle indicated low-amplitude intensity fluctuations on a 10-ms time scale (Carlson et al., 1972). These results did not yield a uniform amplitude when corrected for single-clipping (Jakemen, 1970a). Instead the corrected amplitudes varied considerably about a mean of 0.10 ± 0.09 SD. This result could have been obtained if the process involved did not have a Gaussian field. These experiments were repeated using prescaling and they uniformly gave decay amplitudes near 0.01, which indicates either very small fluctuations in the properties of all scatters, or large fluctuations in only 1–2% of the scattering material in the muscle. It is clear, that the effects of structural rearrangement in the resting muscle, if they exist, are minor when compared to the effects observed in the active muscle. Rest muscles gave flat auto-
correlations on the time scale (0.02–3 ms) over which active muscle showed large decay amplitudes.

*Glycerol-Extracted Muscle in Rigor*

g(2)(r) measurements obtained from glycerol-extracted sartorius muscles in rigor were essentially flat over the range from 10 μs to 100 ms. This expected result indicates that in rigor muscle there are no processes that give rise to large amplitude intensity fluctuations.

*Comparison with Semitendinosus Preparations*

g(2)(r) measurements were made on fiber bundles of semitendinosus muscle to determine whether large intensity fluctuations were unique to multifiber sartorius muscles, or a general property of preparations with small numbers of fibers. The fiber bundles from the semitendinosus were generally only one or two fibers thick.

Measurements on six isometrically tetanized semitendinosus fiber bundles with mean sarcomere lengths between 2.8 and 3.1 μm were obtained at scattering angles of 28.2–32.5°. These results, summarized in Table V, showed the characteristic large decay amplitude, sigmoidal shaped g(2)(r) with an average decay amplitudes of 0.59 ± 0.03 SEM and an average T1/2 of 2.1 ± 0.2 ms SEM during the first second of tetanus plateaus. Later in the tetanus plateau average decay amplitudes were 0.55 ± 0.05 SEM, and the average

| T1/2 FOR HORIZONTALLY MOUNTED ISOMETRICALLY TETANIZED FIBER BUNDLES WITH DETECTOR IN THIRD-ORDER MAXIMUM (ϕ = 30°±3) |
|----------------------------------------------------------------------------------------------------------------|
| Muscle | Sample period | Autocorrelation | Amplitude | Number of cells | Sarcomere length |
|--------|----------------|-----------------|------------|----------------|-----------------|
| 1SS    | 0.03–0.8       | 1.2             | 0.38       | 2              | 3.02            |
|        | 1–3            | 1.3             | 0.41       |                |                 |
| 3QQ    | 0.4–1.4        | 2.2             | 0.64       | 20             | 3.08            |
|        | 1.4–2.4        | 2.8             | 0.62       |                |                 |
| 4QQ    | 0.4–1.4        | 1.9             | 0.85       | 6              | 2.85            |
|        | 1.4–2.4        | 2.3             | 0.76       |                |                 |
| 100    | 0.1–1.1        | 3.7             | 0.54       | 9              | 2.8             |
|        | 0.5–1.5        | 7.9             | 0.31       |                |                 |
| 200    | 0.2–1.2        | 1.6             | 0.74       | 5              | 2.85            |
| 2NN    | 0.1–1.1        | 3               | 0.40       | 4              | 3.14            |
Tx1/2 was 3.6 ± 0.8 ms SEM. These results are similar to those observed on horizontally mounted whole sartorius muscles (amplitude of 0.43 ± 0.02 ms (SEM)) and a T1/2 of 0.97 ± 0.02 ms SEM during the first 0.5 s of the tetanus plateau at ψ = 30°. The decay amplitude for the small bundles of fibers would be expected to be larger than that for whole muscle due to the diminished illuminated volume of muscle and the concomitant increase in spatial coherence at the detector. Considering the difference in sarcomere lengths (2.3–2.5 μm for sartorius and 2.8–3.1 μm for semitendinosus) the values of T1/2 are quite comparable (T1/2 increases with sarcomere length).

The near identity of the intensity autocorrelation functions obtained on small bundles of semitendinosus fibers (as few as two fibers) with those obtained from whole sartorius muscle leads to the conclusion that the observed intensity fluctuations are not derived from processes that depend on the number of muscle fibers or on interactions among them, but rather they must arise from processes occurring within single muscle fibers during contraction.

**DISCUSSION**

*Model of Relative Axial Motions of Muscle Elements during Tetanus Plateau*

The g(2)(τ) measurements made during the plateau of isometric tetani indicate that myofibrillar sarcomeres move significant distances axially, but not radially, during the times comparable to T1/2 and they also undergo, independently, fluctuations in their intrinsic optical properties: Thus there are two independent components to the field autocorrelation function. One component, C₁(ψ, τ) depends primarily if not exclusively on the direction of the myofibrillar sarcomere motion with respect to ψ. This component is due to the axial displacement velocities of the myofibrillar sarcomeres. The second component, C₂(τ), of the field autocorrelation function arises only from changes in the optical polarizability within the displaced myofibrillar sarcomeres themselves. We assume a model, therefore, having two independent processes with correlation functions C₁(ψ, τ) and C₂(τ) each of which alone would produce a Gaussian scattered field with zero mean. The resultant nonnormalized intensity autocorrelation function, G²(τ), for such a model is:

\[
G^{(2)}(τ) = B + A C₁(ψ, τ) \cdot C₂(τ).
\]  

For vertically oriented tetanized muscle, the radial velocities in the direction of ω are so small that C₁(ψ, τ) is essentially constant over the range of τ, for which C₁(τ) decays to zero. Consequently, G(2)(τ) is determined primarily by C₂(τ), which is independent of ψ (Fig. 6). Axial velocities will have a zero component along ω and will make no contribution to C₁(ψ, τ). For horizontally oriented muscle, however, axial velocities determine C₁(ψ, τ) and its decay rate is comparable to that of C₂(τ) as well as proportional to sin(ψ).
our model, the functional form of $C^a_\omega(\psi, \tau)$ is assumed to be the same as that observed for $C^a_\omega(\tau)$ for vertically orientated muscle, namely that given by Eq. 7 with $s = 0.7$. Using this model, the zero-angle intercept and the slope of the $q$ dependence of $1/T_{1/2}$ (Fig. 6) were empirically determined from the observed decay times of $C^a_\omega(\tau)$ and $C^a_\omega(\tau \sin \psi)$, respectively. In the model these times are determined solely by the parameter $t_2$ in Eq. 7 for a fixed value of $s$. Calculated values for $G^{(2)}(r)$ given by the model were obtained using values for $T_{1/2}$ of 1.3 ms for $C^a_\omega(\tau)$ and 0.9 ms for $C^a_\omega(\psi = 90^\circ, \tau)$. $G^{(2)}(\tau)$ for the model should have the same characteristic functional form for all $\psi$, since the data showed that this functional form did not vary significantly with $\psi$. Actually the functional form of the $G^{(2)}(\tau)$ assumed for the model varies slightly for angles between 10 and 60$^\circ$, but the variations are less than 1% and hence the agreement with experimental results is good (Fig. 6).

The theoretical values of the axial motions of the myofibrillar sarcomeres obtained from the model agree with the experimental data within the accuracy of measurement. The velocity distribution is nearly Gaussian for the model and has a mean magnitude along the fiber axis of approximately 20 nm/ms. Throughout the plateau of a tetanus each myofibrillar sarcomere is constantly executing appreciable random, fluctuating axial displacements only. Random fluctuations in the length and hence tension developed by each sarcomere must be the underlying cause of fluctuations in the displacement of other sarcomeres in the same myofibril. These random fluctuations in sarcomere position and length would sum and could conceivably produce the large axial velocities observed. If the myofibrillar sarcomere volume is invariant under length changes then the resultant radial velocities would be more than an order of magnitude less than the observed axial velocities and not observable in the presence of the dominant intrinsic scattering process, $C_A(\tau)$.

The large decay amplitude of $g^{(3)}(\tau)$ observed on vertically oriented muscles is attributed to either (a) fluctuations in the scattering power of individual myofibrillar sarcomeres or (b) local fluctuations in the optical path length through the muscle. The scattering power of a sarcomere will vary with variations in shape or refractive index of its A or I band. The optical path length through a particular region of the muscle will change if a myofibrillar sarcomere in the region were displaced sufficiently to locate an A-band where an I-band had been or vice versa. Because of the difference in index of refraction between A and I bands the phase shift associated with this change would be approximately $\lambda_s/20$. The random axial velocities of myofibrillar sarcomeres should cause fluctuations in optical path length for about 1% of the sarcomeres in the path every millisecond. The total path length through the scattering volume might change significantly during a decay time. Both of these possibilities require further study.
Model of Radial Velocity Field During Twitches

During the rising phase of twitch and tetanus all sarcomeres initially shorten synchronously with a nonzero mean velocity (Cleworth and Edman 1972, Kawai and Kuntz 1973, Larson et al., 1968). If myofibrillar sarcomere volume is constant during shortening, the radial speed, \( v_r \), for sarcomeres will increase with increasing radius, \( r \), from the center of the scattering volume of muscle (taken to be cylindrical) as: 

\[
V_r = \left( \frac{r}{2} \right) L^{-1} \left( \frac{dL}{dt} \right)
\]

where, \( L \) is the muscle length; \( L^{-1} \left( \frac{dL}{dt} \right) \) is the shortening velocity in units of muscle length per unit time. Using this expression for \( V_r \) and the result of Nossal (1971) it can be shown that the probability distribution of \( V_r \), \( P(V_r) \), is semicircular with a mean at zero and the corresponding radial speed phase component, \( C_\theta \left( \psi - \frac{\sin \psi}{2} \right) \) has the form

\[
\frac{J_1(\eta \sin \psi/2)}{\eta \sin \psi/2} J_1(\eta) \]

\( J_1 \) is the first-order Bessel function and \( \eta \) is a constant determined by the range of radial velocities. We assume as was done for the model of axial velocities that \( G^{(2)}(\tau) \) consists of a constant term plus the product of \( C_\theta \left( \psi - \frac{\sin \psi}{2} \right) \) and a term, \( C_\tau(\tau) \), which is equal to \( G^{(0)}(\tau) \) as \( \psi \to 0 \). The data can then be fit by \( C_\tau(\tau) \) and a \( C_\theta(\psi = 60^\circ, \tau) \) that have values for \( T_{1/2} \) of 21.2 \( \mu s \) and 13.1 \( \mu s \), respectively. These values for the parameters of the model imply maximum radial speeds of 3 mm/s which for the 1-mm radius of the scattering volume, gives a shortening speed of about 6 \( L_o/s \) at 18-20°C.

Comparison with other Studies

Sarcomere Diffraction Pattern

Reports have appeared on changes observed in the sarcomere diffraction pattern as a result of contraction (Cleworth and Edman, 1969, 1972; Larson et al., 1968; Kawai, 1971; Kawai and Kuntz, 1973). Larson et al. (1968) reported that there were 10- to 20-Hz fluctuations in mean sarcomere length for large volume samples of frog's sartorius muscle during the plateau of a tetanus. Fluctuations in mean sarcomere length must be a cooperative, that is a long-range spatially correlated phenomenon. The decrease in the decay amplitude of \( g^{(n)}(\tau) \) with an increase in scattering volume reported here (see above) definitely indicates that these fluctuations are not cooperative. Moreover, the "dither" (Larson et al., 1968) of the mean sarcomere length would cause the angular position of the sarcomere diffraction bands to fluctuate with the same period (100–200 ms). We found no component of the intensity fluctuations with a \( T_{1/2} \) of 100–200 ms. The intensity fluctuations reported here are not due to fluctuations in the mean sarcomere length. The mean length remains constant while the lengths of individual myofibrillar sarcomeres fluctuate.

Randomly fluctuating sarcomeres might show a greater dispersion in length than nonfluctuating resting sarcomeres. Observations supporting this possibility have been observed by Kawai, 1971 and Kawai and Kuntz, 1973 who
attributed a broadening of the first-order diffraction bands to a 10- to 20-nm increase in the dispersion of sarcomere length. Random myofibrillar sarcomere length fluctuations could produce an increased broadening of the diffraction bands. If the sarcomere length fluctuations are independent or correlated over small groups only, there would be observable axial velocities with no significant radial velocities.

The $g^{(2)}(r)$ measurements made during relaxation showed that intensity fluctuations were rapid at first, then diminished and continued for a second or more after tension vanished. The observations of Huxley and Simmons (1970), Cleworth and Edman (1969, 1972) and Kawai and Kuntz (1973) indicated a continuing contraction of most sarcomeres during the first half of tension decline followed, after tension vanishes, by a readjustment of the sarcomeres to their resting configuration in agreement with the results reported here.

Whatever the underlying cause of the scattered light intensity fluctuations, it is clear that random structural fluctuations occur during the plateau of a tetanus and hence measurements of mean value characteristics of a microscopic quantity should not be regarded as evidence for the absence of structural fluctuations on the microscopic scale.

**LABILE HEAT** The decay time of labile heat production for frog's sartorius at 15°C is 0.15 s (Fraser and Carlson 1973). The decay rate of $g^{(2)}(r)$ decreases during a tetanus to a constant value with a decay time of 0.17 s. The similarity of these two transients opens the possibility that the labile heat rate is coupled to structural fluctuations of the myofibrillar sarcomeres.

**QUICK-RELEASE TRANSIENTS** Efforts to determine cross-bridge dynamics of contraction from macroscopic mechanical responses to imposed transients have been made by Huxley and Simmons (1972) and Podolsky and Nolan (1972). Their measurements of tension and length transients show an initial rapid recovery on the 1- to 2-ms time scale in semitendinosus muscle at 4°C, followed by slower transient phases. The structural changes that cause intensity fluctuations have characteristic rates in the millisecond range, and significant intensity changes do not occur for times shorter than 0.5 ms during an isometric tetanus at 20°C. The quick-release experiments were done at 4°C, and our measurements were made at 20°C so a direct comparison of these experiments is not now possible. It seems likely that the process or processes that limit the initial rate of recovery from quick transients and the processes that limit the rate of fluctuation of scattered light intensity are the same. This conjecture has been developed by Carlson (1975).

**X-RAY DIFFRACTION** The frog's sartorius muscle has been used in the X-ray diffraction studies of Huxley et al. (1965), Huxley and Brown (1967), and Haselgrove (1970, 1972). The changes in the average intensity of the equatorial X-ray reflections observed, indicate that the relative mass of material associated with the actin filaments and (presumed to be the myosin heads) is greater in contracting muscle than in a resting muscle, and greater still in a
rigor muscle. Furthermore, during contraction the off-meridional reflections of the myosin layer lines are only about 30% as intense as they are in relaxed muscle. No characteristic rigor layer lines have been detected in isometrically contracting muscle. These results have been interpreted to mean that many of the myosin heads move out toward the actin filament during contraction, but that azimuthal and radial disorientation among them prevents the ordered rigor reflections from appearing.

Our finding of fluctuating axial movements of the myofibrillar sarcomeres must in turn mean tension fluctuations, and hence a variation in the overlap of myosin and actin filaments. Such axial motions of the filaments would cause fluctuations in the orientations of actin-bound cross bridges and in interfibril spacings that would cause the myosin layer line intensity to decrease without the appearance of rigor-like axial reflections. Further, the filament super lattice proposed by Huxley and Brown (1967) or other interfibril spatial correlations would become disordered by random axial displacements of adjacent thick filaments and produce a loss of interference between different thick filaments and thereby diminish the intensity of the myosin layer line. This effect would be observed even if the degree of order of each thick filament remained unchanged (Arnott, 1973). In these two ways the structural disorder induced by the axial motions of the filaments that is coupled to myofibrillar sarcomere movements could account for changes in myosin layer line intensity.

Furthermore, the decrease in intensity of the myosin layer lines persists for 0.5-5.5 s at 4°C. after the last stimulus and loss of all tension according to Huxley (1972). As already noted here intensity fluctuations in frog's sartorius muscle at 20°C persist for at least 2 s after the last stimulus. This parallelism between the X-ray diffraction results and those reported here raise the possibility of a common structural basis.

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