The Sarcomere Length-Tension Relation in Skeletal Muscle

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ABSTRACT Tension development during isometric tetani in single fibers of frog semitendinosus muscle occurs in three phases: (a) in initial fast-rise phase; (b) a slow-rise phase; and (c) a plateau, which lasts > 10 s. The slow-rise phase has previously been assumed to rise out of a progressive increase of sarcomere length dispersion along the fiber (Gordon et al. 1966. J. Physiol. [Lond.]. 184:143-169; 184:170-192). Consequently, the "true" tetanic tension has been considered to be the one existing before the onset of the slow-rise phase; this is obtained by extrapolating the slowly rising tension back to the start of the tetanus. In the study by Gordon et al. (1966. J. Physiol. [Lond.]. 184:170-192), as well as in the present study, the relation between this extrapolated tension and sarcomere length gave the familiar linear descending limb of the length-tension relation. We tested the assumption that the slow rise of tension was due to a progressive increase in sarcomere length dispersion. During the fast rise, the slow rise, and the plateau of tension, the sarcomere length dispersion at any area along the muscle was <4% of the average sarcomere length. Therefore, a progressive increase of sarcomere length dispersion during contraction appears unable to account for the slow rise of tetanic tension. A sarcomere length-tension relation was constructed from the levels of tension and sarcomere length measured during the plateau. Tension was independent of sarcomere length between 1.9 and 2.6 μm, and declined to 50% maximal at 3.4 μm. This result is difficult to reconcile with the cross-bridge model of force generation.

INTRODUCTION

Tension development during an isometric tetanus in skeletal muscle occurs in three distinct phases: tension initially rises rapidly, then climbs more slowly, and finally reaches a plateau which lasts many seconds. This time-course is observed during isometric contractions of muscles (Abbott and Aubert, 1952; Deleze, 1961), muscle fiber bundles (Sugi, 1972) and single fibers (Ramsey and Street, 1940; Edman, 1966; Gordon et al., 1966 b), as well as in muscle fiber segments held at constant length during the tetanus (Gordon et al., 1966 b).

On the basis of certain experimental observations (see Discussion) Gordon et al. (1966 b) found convincing rationale for assuming that the slow climb of tetanic tension—or in their terms, the "creep"—was solely due to the development of progressive dispersion of sarcomere lengths: "There can be little doubt
that this (i.e. the slow rise of tetanic tension) is due to the progressive
development of irregularities of striation spacing during the tetanus which is to
be expected because of the instability represented by the negative slope of this
part of the length-tension relation (Hill, 1953). . . . Although we have no direct
evidence that this is the cause of the 'creep' we do regard it as sufficiently
probable to lay more emphasis on the value of tension extrapolated back to the
beginning of the tetanus . . . than on the maximum tension reached.

The plot of extrapolated tension against sarcomere length gave the familiar
length-tension relation, which proved remarkably consistent with a principal
expectation of the cross-bridge theory: that tension was proportional to the
number of cross bridges in the zone of overlap of the thick and thin filaments.

Although a number of studies have shown that the sarcomere length disper-
sion in skeletal muscle is small (Edman, 1966; Marikhin and Myasnikova, 1970;
Kawai and Kuntz, 1973; Paolini and Roos, 1975; Paolini et al., 1976, 1977) and
that the dispersion generally does not increase by more than several percent
during the tetanus (Marikhin and Myasnikova, 1970; Cleworth and Edman,
1972; Kawai and Kuntz, 1973; Paolini and Roos, 1975; Paolini et al., 1976, 1977)
no systematic analysis of sarcomere length dispersion at long sarcomere lengths,
where the slow climb of tension is most prominent, has been attempted.

This study is specifically directed toward the question of whether an increase
of sarcomere length dispersion occurs during the tetanus, and if so, whether
the increase is sufficient to explain the slow climb of tension. As it will be shown
that the latter question is answered negatively, we will describe a sarcomere
length-tension relation derived from measurements made during the plateau
phase of the tetanus.

**METHODS**

*Preparation*

Single fibers of semitendinosus muscles of *R. Pipiens* were carefully dissected from the
dorsal head of the muscles. Extreme care was taken to avoid stretch of the regions of the
end plate and contiguous capillaries.

Criteria for acceptability of fibers were based on uniformity of sarcomere length. We
measured the variation of sarcomere length along the freshly mounted fiber at three
degrees of stretch, nominally 2.2, 2.8, and 3.4 μm. If the peak-to-peak variation
exceeded 0.2 μm in any one of these runs the fiber was discarded. Although variations
slightly >0.2 μm occasionally arose during the ensuing experiment, the peak-to-peak
variation in most fibers remained on the order of only 0.1 μm (cf. Fig. 4).

It should be emphasized that the majority of fibers dissected exhibited the type of
nonuniformity commonly found by other investigators (Huxley and Peachey, 1961;
Carlsen et al., 1961; Gordon et al., 1966a, b), i.e., relatively shorter sarcomere lengths
near the ends of stretched fibers. From these we selected for experimentation only those
fibers in which this nonuniformity was minimal or absent. Apart from long-term viability,
the selection process evoked a second distinct advantage, namely, that the specimens
could be considered effectively length-clamped over a segment (the entire fiber) whose
striation spacing was uniform.
Experimental Chamber and Solutions

After dissection, the fibers were mounted in a chamber through which the bathing solution flowed at a rate high enough (50 ml/min) to replenish the chamber with fresh solution about once per minute. A small plexiglass funnel created laminar flow parallel to the fiber, thereby ensuring that the fiber did not move in the transverse direction away from the spot illuminated by the laser during the tetani. The high flow rate along the fiber diminished the tendency for oxygen bubbles to form on the fiber surface; bubbles act as scatter sources, and thereby interfere with the analysis of sarcomere length dispersion.

The fibers were generally able to sustain tetani of well over 10 s duration. However, during our preliminary experiments in which we used conventional Ringer's solutions, the developed tetanic tension declined after 3-4 s. Therefore, we used an extracellular bathing solution which more closely approached in situ conditions, particularly with regard to pH (Reeves, 1972; Rahn et al., 1975). It contained 95 mM NaCl, 2.5 mM KCl, 1 mM MgSO4, 25 mM NaHCO3, 1.8 mM CaCl2, 1 mM Na2HPO4, 5 mM glucose, 5 IU/liter insulin, and 0.01 mM D-tubocurarine, and was bubbled vigorously with a mixture of 95% O2 and 5% CO2 both during dissection and the experiment to adjust the pH to 7.5 at a temperature of 8°C. In this bathing solution the fibers were able to generate steady, long-lasting tetani. With 5-min intervals between 5-s tetani, tension development in successive tetani appeared to be identical for at least several hours. A number of fibers were bathed in a solution identical to the one described above, except that the insulin had been omitted. We found no differences in the time-course or levels of tension development in these fibers, or in the shape of the length-tension relation.

Stimulation Procedures

The fibers were stimulated by pulses from a Grass stimulator (type S-4, Grass Instrument Co., Quincy, Mass.), using a Grass stimulus isolator (type SIU-5). Salt bridges were used for the stimulus electrodes to ensure that electrolytic bubble formation did not take place in the neighborhood of the fiber. One salt bridge had a 12 × 0.5-mm slit-shaped opening; the other had a 20 × 0.5-mm slit-shaped opening. Both were positioned parallel and close to the muscle. The salt bridge with the short opening was used as cathode at short fiber lengths, whereas the other was used as cathode at long fiber lengths. With the use of twice-threshold stimuli, synchronous activation of the fiber, as shown by uniformity of the contractile pattern along the fiber (see Results), could be obtained at all sarcomere lengths.

Optical Analysis of the Sarcomere Length Distribution

We used laser diffraction methods in this study. This technique gives comprehensive, readily obtainable information about sarcomere length and sarcomere length distribution. Details of the apparatus are more fully described elsewhere (Pollack and Krueger, 1976; Pollack et al., 1977; Iwazumi and Pollack). In short, the fiber was illuminated by a laser beam, which in most of these experiments was compressed to a diameter of ~180 μm (cf. Fig. 1). The cross-striated muscle acts as a grating and therefore diffracts the incident laser light into a zeroth order band and multiple higher order band pairs. The spacing between bands is uniquely related to the striation spacing in the fiber.

In order to perform very high resolution diffractometry, measurements must be in the far field, or Fraunhofer region. In this region, however, the spacing between orders

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Iwazumi, T., and G. H. Pollack. On-line measurement of sarcomere length from diffraction patterns in cardiac and skeletal muscle. Submitted for publication.
is very large, and is not conveniently measured with the miniature sensors we had at our disposal. Therefore, we used a microscope objective (Fig. 1) so that a Fraunhofer diffraction pattern could be obtained in a region closer to the specimen. If the objective lens were an ideal Fourier transformer, the relationship between the sarcomere length, \( SL \), and the distance between the zeroth and first order maxima, \( d \), would be given by the simple inverse relation:

\[
SL = \frac{K\lambda}{d},
\]

where \( K \) is a constant, and \( \lambda \) is the wavelength of the laser light (0.6328 \( \mu \)m). This inverse relation results directly from the similarity theorem of the Fourier transform (Goodman, 1968). Although the objective lens used (\( \times 40 \), Carl Zeiss, Inc., New York) was very well corrected for aberration, it nevertheless caused a slight deviation (<1%) from the true spacing because of the relatively large diffraction angles involved. This error was corrected electronically after direct calibration of the sarcomere length computing circuitry using glass diffraction gratings.

The Fraunhofer diffraction pattern formed on the rear focal plane of the objective was projected onto three sensors. First, a video system was used for monitoring the gross features of the diffraction pattern. Second, the diffraction pattern was projected upon a translucent screen, and the image on the screen was filmed at two frames per second (exposure duration 10 ms, Tri-X film, Eastman Kodak Co., Rochester, N.Y.) with a camera (C-4K, Grass Instrument Co.). This allowed us to determine the sarcomere length from densitometric measurements of the photographed pattern. Third, the pattern was compressed along the length of the bands with a cylindrical lens and projected upon a 128-element photodiode array (RL-128A, Reticon Corp., Sunnyvale, Calif., with an element size of 25 \( \times \) 490 \( \mu \)m and a spacing between the elements of 50 \( \mu \)m). The array was scanned five times per millisecond.

Each scan generated a profile of intensity vs. distance, \( d \), along the photodiode array (Fig. 1). The area under the profile of the first order peak was calculated by integrating the intensity distribution with respect to \( d \), starting from the zeroth order side. The values of \( d \) at which the integral reached 25, 50, and 75% of the total area were computed, and their respective sarcomere lengths were calculated from Eq. 1. The sarcomere length corresponding to the 50th percentile of the intensity distribution under the first order was taken as the median sarcomere length. The resolution of this measurement was limited by noise which was \( \sim 5 \) nm peak-to-peak. The difference

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**Figure 1.** Diagram of the optical apparatus. The diffraction pattern is projected onto three sensors: a television monitoring system; a translucent screen from which the diffraction bands are filmed; and a photodiode array after the diffraction pattern has been compressed by a cylindrical lens. The median sarcomere length is computed from the median of the first order intensity distribution; the local dispersion of sarcomere lengths is computed from the width of the intensity distribution. The image of the muscle is visible through the oculars of the microscope (not shown) at all times. Optical parameters: laser beam diameter and angular divergence at muscle - 180 \( \mu \)m and \( \sim 5 \) mrad with beam compressor; 800 \( \mu \)m and 1.1 mrad without compressor. Objective: Zeiss water immersion, numerical aperture 0.75, used at specimen-to-lens distance of 1.6 mm, the normal distance for microscopy. A tungsten light source and condenser could be inserted into the optical path to allow microscopy with Köhler illumination.
Computation of median sarcomere length and dispersion

Intensity

Position

Median dispersion

Compressed diffraction pattern

128 element photodiode array

cylindrical lens

beam splitters

Projection lens

Focussed diffraction pattern

40x objective

to motion generator

to tension transducer

to tension transducer

to tension transducer

translatable chamber-muscle assembly

He-Ne laser

FIGURE 1
between the sarcomere lengths corresponding to the 25th and 75th percentiles, or the
"half-width," was defined as the local sarcomere length dispersion (cf. Fig. 1). All
computations were performed on-line electronically, with a time resolution of 200 μs.

The spread of light intensity under the principal maximum of the first order of the
diffraction pattern arises not only from sarcomere length dispersion. With the optical
system described above, the spread of light intensity is also increased by limited beam
diameter and by scatter from the muscle and from particles in the solution and on the
surfaces of optical elements. The contribution due to the finite number of striations (90-
50) included in the beam width (180 μm) amounts to between 1.1 and 2.0% (Born and
Wolf, 1965). The contribution due to scatter is larger; its magnitude depends upon the
experimental conditions, particularly the thickness of the specimen. Thus, the apparent
sarcomere length dispersion, taken as the half-width of the light intensity distribution
under the first order peak, is always larger than the real sarcomere length dispersion.
Nevertheless, this technique was convenient for determining the time-course of changes
of local sarcomere length dispersion.

In some experiments we also measured the direct Fraunhofer diffraction pattern, i.e.,
the one obtained without the use of lens elements or laser beam compressor. Analysis of
photographs of these patterns gave values of local dispersion less affected by scatter
introduced by the optical system or by limited beam diameter. These direct diffraction
measurements were therefore used to obtain more accurate estimates of the absolute
value of local dispersion.

The accuracy of the sarcomere length determination by the photodiode array
technique was assessed by two methods. First, gratings of three spatial frequencies (Haas
and O'Hara, 1966) spanning the relevant range were substituted for the muscle before
each experiment. The resultant calibration error never exceeded 1%. Second, in two
experiments with the fiber in place, we compared the median sarcomere length
measured electronically with that measured from photographs of the striation pattern.
Comparisons were made at a series of contiguous regions along the fiber, at several
degrees of stretch. The correspondence between the two measurements were almost
always within 1%. However, in the myotendinous regions (the final 20-30 striations)
where the fiber tapers sharply and the diffraction signal becomes weak, the sarcomere
length computed electronically tended to be several per cent higher than the values
measured from the photographs. We are aware that when the first order intensity is low
the photodiode array exhibits increased distortion due to crosstalk between diodes; this
tends to shift the first order peak toward the zeroth order, resulting in a higher than
actual value of sarcomere length. Such errors, however, are limited to the terminal 50-
100-μm of the fiber. <0.5% of fiber length at each end.

The accuracy of the photodiode array has also been demonstrated in dynamic
situations. The details of the time-course of sarcomere shortening measured with the
photodiode array corresponded closely with details measured simultaneously with a
Schottky barrier photodiode position sensor and with kymographic records of the
diffraction pattern (Pollack et al., 1977).

**Mechanical Measurements**

One tendon of the fiber was held close to the fiber insertion with a small clamp, which
was connected to a servomotor system. In these experiments the servo system was
effectively unused; i.e., the fiber length was kept constant during contraction. The other
tendon was clamped in similar fashion and connected to a conventional strain gauge
transducer (UC-2, Statham Instruments, Inc., Oxnard, Calif.). The transducer was
attached to a micrometer, which was used to adjust the static fiber length. The force
transducer had a sensitivity of 1 V/g and a resonant frequency of 150 Hz. In some
experiments, we used a high frequency force transducer (AE803, Aksjeselskapet Mikro-
Electronikk, Horten, Norway). Its resonant frequency, including the clip, was 1.4 kHz,
and its sensitivity was 10 V/g.

Tension levels were obtained in two ways. First, the tension level at the start of the
slow rise of tension was obtained by extrapolation of the tangent at 400 ms back to its
intersection with the extrapolated fast phase (see Fig. 9 B), a procedure comparable to
the one chosen by Gordon et al. (1966 b). Second, tension measured during the plateau
of tension was obtained directly.

**Experimental Protocol**

After dissection, the fibers were observed for a period of 1 hr. If visible damage had not
developed during this period the fiber was mounted. It was then inspected microscopi-
cally, and often remounted several times to minimize any skewing of the striation pattern
or kinks in the fiber. The fiber was then examined at three degrees of stretch to
determine whether or not the selection criterion had been satisfied (see above).
Acceptable fibers were returned to slack length, and fiber dimensions were recorded
midway along the fiber. Although we did not record cross-sectional dimensions continu-
ously along the fiber, we estimate from routine stereo microscopic tendon-to-tendon
scans that the variation of cross-sectional area was on the order of 10-15%, similar to the
value reported by Edman (1975).

Initially, fibers were subjected to a series of twitch contractions, and the sarcomere
length changes were observed at about 10 different locations along the fiber. Some fibers
with excellent resting sarcomere length uniformity exhibited substantially nonuniform
contraction. Such fibers generally proved unable to sustain long tetani and were
discarded.

Subsequently, a series of tetanic contractions was initiated. Fused tetani lasting 3–8 s
were elicited by stimulus trains at a frequency of 25 Hz. The interval between tetani was
5 min or longer. Muscle length was varied from 100 to 170% of slack length in a random
sequence, and optical and mechanical measurements were carried out. In these experi-
ments resting sarcomere length was always recorded at the same spot along the fiber.
This was achieved by adjusting the position of the sampled area under microscopic
observation during each change of muscle length such that the position of a natural
marker along the fiber, such as a remnant of connective tissue, always appeared at the
edge of the optical field. The criterion for the choice of region was that it exhibited a
minimum amount of translation during contraction at all lengths; one or two such
regions could be found in most fibers. Thus, the length-tension relation for each
specimen refers to a specific region along the fiber.

At muscle lengths of 100, 130, and 160% slack length, variation of the median
sarcomere length along the fiber was evaluated before contraction. The measurement
then was repeated at these respective lengths during the plateau phase of tetani lasting
8 s.

**Intact Muscles**

To compare the properties of single fibers with those of intact muscles a number of
experiments were repeated on the long extensor muscle of the fourth toe of frogs. The
muscles were rapidly dissected after decapitation of the animal and perfusion of the
blood vessels with the bathing solution to rinse away blood cells, which act as light
scattering sources and thereby increase the width of the diffraction bands. Subsequently,
small fiber bundles running from the toe muscle to the neighboring anterior tarsal
muscle were carefully dissected. The muscles were mounted in the same way as the single
fibers and the experimental protocol was similar. Careful examination of the relation
between stimulus intensity and tetanic tension showed that at all lengths all fibers were activated. Results obtained with six toe muscles are presented.

RESULTS

The Time-Course of Tension Development

Fig. 2 shows the time-course of tension development in a single fiber at various degrees of stretch. Tension rose rapidly at first for all sarcomere lengths. The rapid rise was followed by a slow-rise phase which lasted longer at the longer sarcomere lengths. A plateau of tension was always reached by 3 s after the start of contraction. However, a slight overshoot of tension following the slow rise was often noticed before the tension settled at the plateau level. Using longer

![Figure 2](image)

**Figure 2.** Time-course of isometric tension development at various sarcomere lengths. Fiber diameter, 90 μm; slack length, 16 mm. Sarcomere lengths shown at left were those measured at rest. Sarcomere lengths during the plateau of tetanus were 2.09, 2.77, 3.15, and 3.55 μm (top to bottom). Tension development shows a fast initial rise, a slow rise, and a plateau. A slight overshoot of tension was often noted in contractions at sarcomere lengths beyond 2.7 μm. The slow rise is most prominent at the longer sarcomere lengths.

lasting tetani than those illustrated in Fig. 2, we found that the tension remained at the plateau level for ~10 s, and then gradually decayed with a time constant on the order of 20 s. The maximal tensions developed by the fibers varied between 2.5 and 3.5 kg/cm².

In the intact muscles the time-course of tension development was indistinguishable from that in the single fibers.

The length dependence of the slow-rise phase of tension development in the single fibers is explored quantitatively in Fig. 3. Here we have plotted the slope of the slow-rise phase, normalized by the magnitude of the tension reached before the onset of the slow rise. Although the slow-rise is most dominant at the longest sarcomere lengths, it remains in evidence at sarcomere lengths as low as 2.3 μm.

Fig. 3 also shows the results of several experiments carried out at temperatures bracketing the 8°C used for the majority of fibers. Elevation of temperature increased the slope and decreased the duration of the slow rise phase, whereas
diminution of temperature had the opposite effect. The temperature dependence is qualitatively similar to that reported by Mittenthal and Carlson (1971) in frog sartorius muscle.

For comparison we plotted on the same axes the normalized slopes of the slow rise phase measured (by us) from the records published by Gordon et al. (1966 a, b). These records were obtained from segment length clamped fibers. Although there existed some uncertainty in the measurement of slope at 400 ms after the start of the tetanus, and the number of relevant published records was limited, Fig. 3 shows that the characteristics of the slow rise phase apparently did not differ appreciably in the two sets of experiments. Nor did they differ 

substantially from the results obtained by Mittenthal and Carlson (1971), although a different analytical method was used in the latter study.

**Sarcomere Length Distribution at Rest**

Fig. 4 shows the variation along a representative fiber of the median sarcomere length, as measured by the computational circuitry. These records were obtained by translating the chamber containing the specimen across the optical path (cf. Fig. 1) and recording median sarcomere length vs. the position of the chamber on an X-Y recorder. The striation pattern along the fiber was always distinct right up to the tendinous insertions, so that good quality diffraction patterns could be obtained along the entire fiber length. The root mean square variation of median sarcomere length along each fiber was determined from the X-Y records. The pooled values for six fibers at three degrees of stretch and one fiber at two degrees of stretch \( n = 20 \text{ runs} \) gave a mean value of 0.03 ±
0.01 μm SD, or approximately a 1% root mean square (rms) variation of sarcomere length from tendon to tendon. With highly stretched fibers the sarcomere lengths were sometimes shorter near the tendons than in the remainder of the fiber, but these differences were usually less than 0.1 μm. The peak-to-peak variation of sarcomere length along any fiber never exceeded 0.28 μm. The local dispersion, as measured by the half-width of the first order diffraction band, also varied on average by ~1% along each fiber. Although our

fibers were selected specifically on the basis of the uniformity of their striation patterns, comparably uniform fibers, particularly at unstretched lengths, have been reported by others (Cleworth and Edman, 1969; Marikhin and Myasnikova, 1970; Cleworth and Edman, 1972; Kawai and Kuntz, 1973; Paolini et al., 1976).

Sarcomere Length Changes during Tetanus

Fig. 5 shows the time-course of sarcomere length changes that occurred during isometric tetani in a representative fiber. At all initial lengths the sarcomeres shortened during the first 50 ms of tetanus by 0.15 μm or less. In some of the fibers there was a slight amount of lengthening instead of shortening. (Either

![Graph showing sarcomere length changes during tetanus.](image-url)
could be found in a given fiber, depending upon which region was selected for scrutiny.) After the initial length change the sarcomere length varied ≤0.1 μm during the subsequent 4 s of contraction. In one fiber no sarcomere length changes were detectable during seven successive tetani which started at sarcomere length ranging from 2.2 to 3.4 μm; in this muscle, therefore, tension

**FIGURE 5.** Sarcomere length changes, tension development, and local dispersion. A–D show records of isometric tetani starting from various sarcomere lengths. The upper pair of traces in each record shows tension development on a time scale (T) of 50 and 500 ms/div., respectively. The lower pair of traces shows the sarcomere length (SL) changes during these tetani on the same time scales. Stimulus pulses (stim) are shown at bottom. Panels C' and D' show records of the intensity profile of the first order of the diffraction pattern at rest and during tetani for the contractions (contr.) shown in C and D. Each record consists of an exposure of the film to 5,000 consecutive scans of the intensity profile. The exposure period during the tetanus is indicated by the white arrowed bar above the lower sarcomere length trace. C' shows slight shortening of the sarcomeres in the illuminated area with a small increase of the local dispersion. D' shows slight lengthening of the sarcomeres and a decrease of the local dispersion. The second order is visible in panel D'.
development in the region under observation was truly isometric. Sarcomere length changes at various regions along the muscle were highly reproducible in successive contractions.

Time-Course of Local Dispersion

We used three methods to study local dispersion. Firstly, the intensity distribution of the first order of the diffraction pattern incident on the photodiode array was recorded. Fig. 5 C' and D' show the distributions photographed during rest and during the plateau phase of tetani starting from 2.74 and 3.29 µm, respectively. The figure illustrates the main feature of local dispersion changes during tetani, which is that only very small changes of dispersion occur between rest and the plateau phase of the tetani. The records were made by using 1-s exposures of the film to scans of the intensity profile of the first order peak displayed on the oscilloscope. The superimposition of a large number of scans during this exposure (5,000 scans per second) still resulted in a sharp delineation of the intensity profile both during rest and during tetanus. The film sensitivity was sufficient that a single trace could have been detected. It is evident from the figure that there is a near-total absence of change of sarcomere length dispersion during the tetani.

Secondly, the dispersion was computed on-line from the half-width of the first order intensity distribution. A representative example of the time-course of local dispersion during contraction measured this way is shown in Fig. 6. Generally there was some fluctuation during the rapid-rise phase of tetanus (during which time there was usually a small amount of sarcomere shortening or lengthening); beyond this time, however, there was little change of local dispersion, as already shown in Fig. 5 C' and D'.

A small ambiguity existed in this second method. To optimize the signal-to-noise ratio, we always adjusted conditions such that the photodiode array was near saturation when the muscle was at rest. When, occasionally, the intensity of the first order increased during contraction (Fig. 5 C'), the first order became truncated, and the computed half-width suffered slight artifactual increase. Up to the last few experiments we were unaware of this difficulty. We are now fairly certain that the artifactual broadening could never have exceeded 0.1 µm. However, we felt that this potential error was of enough significance that we elected not to do detailed analysis of these data.

The third and independent measurement of dispersion was performed by analysis of photographic records of the direct diffraction pattern (cf. Methods). Fig. 7 illustrates that only minor changes of the diffraction pattern took place during the course of tetani lasting several seconds. During the plateau, fine structure often became prominent in the first orders, and during relaxation a broadening of the first orders was often noted (cf. Fig. 7 A) which was similar to that reported by others (Cleworth and Edman, 1972; Borejdo and Mason, 1976).

Table I shows the results of densitometric analysis of photographic records such as those illustrated in Fig. 7 (but for a different fiber). As with the electronic technique, the local dispersion was taken as the half-width (see Methods) of the first order intensity profile. The results show that local dispersion most often increased modestly from rest to plateau, but sometimes
showed no change, or decreased. Results were comparable in the other fibers. We could identify no trend toward more or less of an increase of dispersion at any particular sarcomere length, although such a trend might have become evident with more data.

The results obtained with the direct diffraction technique were comparable to those obtained with the electronic technique, except that the absolute values of dispersion both at rest and during contraction were considerably larger in the latter than in the former. With the electronic technique, the diffracted light passed through several lenses, each of which tended to scatter light and broaden the diffraction bands. The photographic (direct diffraction) technique was lensless. The contribution of the optical system to the spread of light intensity under the principal maximum of the first order could be obtained by replacing the specimen by a uniform grating and measuring the half-width of the first

![Figure 6. Tension development (upper trace), sarcomere length (s.l., middle trace), and local dispersion (l. disp.) of sarcomere length (lower trace) during isometric tetani at three initial sarcomere lengths in a fiber whose diameter was 85 μm. The tension trace in the upper panel shows the stimulus pulses superimposed. The absolute values of local dispersion shown here are overestimates of the true local sarcomere length dispersion since broadening of the orders is caused by the optical system, itself. The changes of dispersion during contraction are small.](image)
order. Using a 3.3-μm grating, we found that half-width was ~0.2 μm with the electronic technique, but only 0.03 μm with the direct diffraction technique. Evidently the photographic technique provides a more faithful representation of absolute values of dispersion (while sacrificing time resolution). Notwithstanding these differences in absolute values, both techniques showed only minor changes of local dispersion during contraction.
Global Dispersion

By rapidly translating the stage during the plateau phase of 8-s tetani, we were able to measure the variation of local median sarcomere length along the fiber axis. A representative result is shown in Fig. 8. The root mean square variation about the mean was 2.0% at 2.70 μm, 0.9% at 3.15 μm, and 1.2% at 3.32 μm. The rms variation always increased from rest to the plateau phase of contraction, but in no instance did the latter value ever exceed 4%.

| Table 1 |
| CHANGES OF LOCAL DISPERSION FROM REST TO CONTRACTION |

| Halfwidth of first order |
|--------------------------|
| Resting sarcomere length | At rest | During tetanic plateau |
| μm | (3 s after onset) | μm |
| 3.12 | 0.08 | 0.08 |
| 2.22 | 0.07 | 0.08 |
| 3.38 | 0.08 | 0.09 |
| 2.25 | 0.09 | 0.06 |
| 3.61 | 0.11 | 0.09 |
| 2.84 | 0.06 | 0.09 |
| 2.66 | 0.08 | 0.08 |
| 2.34 | 0.09 | 0.10 |

In several instances we extended the tetanus duration to 13 s and were able to scan from the knee tendon to the pelvic tendon and part way back again before the tetanic plateau ended. The results of the forward and backward scans were virtually superimposable, reconfirming (cf. Figs. 5 and 6) the absence of substantial time variation of local sarcomere length during the tetanic plateau.

Ideally, one might like to supplement these results with a map of sarcomere behavior along the fiber at all instants throughout contraction. This could be

**Figure 7.** Tension development and the changes of the direct diffraction pattern during contraction. Records from two initial sarcomere lengths are shown. The upper graph depicts the tension development (reconstructed from the original tension record) during a 4-s isometric tetanus. The series of photographs shows the direct diffraction pattern taken at 0.5-s intervals during the tetanus at the moments indicated by the arrows. Only the zeroth and one of the two first orders is shown in each frame. The diffraction pattern in A shows an increase of the local dispersion of sarcomere length (half-width of first order) during the tetanus from 0.05 μm at rest to 0.07 μm during the tetanus. No measurable change took place during the tetanic plateau. The sarcomeres lengthened from 2.70 μm at rest to 2.80 μm during the tetanus. The diffraction patterns in B show no change in the value of the local sarcomere length dispersion, 0.08 μm, during contraction and no measurable change of the median sarcomere length. Despite the small change of the median sarcomere length and the local dispersion of sarcomere lengths, both tension records show a phase of slow tension development. A broadening of the first order was often noted during relaxation, as evident in A. The faint spots on the meridional axis are reflections of the incident laser beam and are not part of the diffraction pattern.
achieved by extremely rapid repetitive scanning along a tetanized fiber. From such a map one would like to test whether initially shorter sarcomeres shortened further and stretched the ones which were initially longer. However, the variations in initial sarcomere length were so small that to proceed testing such a hypothesis, one would need to insist that all other factors, with the exception of sarcomere length which might influence the local strength of contraction—level of activation, time-course of activation, myofilament density, and cross-

![Graph 1: Variation of median sarcomere length along the fiber during the plateau of tension](image1)

**Figure 8.** Variation of the median sarcomere length along the fiber during the plateau of tension. Each record was made by translating the microscope stage in the axial direction during the plateau of a tetanus of 8s duration, and recording the computed median sarcomere length on a chart recorder. Because the rate of translation may not have been steady, there is not necessarily a direct correspondence between points on the abscissa and position along the muscle, as there was in Fig. 4. The sarcomere lengths noted on the records were the mean values along the fiber at rest. The arrows correspond to these values. The sharp downward deflections on the right side of the records are artifacts caused by bubbles on the fiber surface.

sectional area—must be extraordinarily uniform along the fiber, certainly within 1%. The variation of cross-sectional area alone, was 10–15%. Thus, we did not deem it fruitful to proceed along these lines.

In summary, these results show that during tetani at sarcomere lengths between 2.0 and 3.6 μm, only small changes in sarcomere length dispersion occurred in the fiber both locally and globally. Despite the absence of large increases in dispersion, the tension development at sarcomere lengths above 2.3 μm always showed clearly the presence of a slow-rise phase.

**The Sarcomere Length-Tension Relation**

Fig. 9 shows the relation between length and tension. In Fig. 9 B we plot the relation between tension and the sarcomere length as obtained by extrapolation
FIGURE 9. The relation between tension and sarcomere length. (A) Results obtained from tension measurements during the plateau of tension and the sarcomere length at the moment of tension measurement in seven single fibers. Each symbol indicates the results from one fiber. The curve in the lower right hand corner gives the passive tension of single fibers. The curve through the data points is drawn by eye. Most points were obtained in contractions in which there was a small amount of initial shortening or lengthening (usually <0.1 μm); however in one fiber (□) 7 of the 13 data points were obtained in contractions in which there were no detectable changes of sarcomere length. (B) Results of a measurement of tension by extrapolation according to the procedure shown in the inset, and sarcomere length at that time. The drawn line is the relation obtained by Gordon et al. (1966 b).
of the slowly rising tension back to the start of the tetanus. The sarcomere length values were those existing at the time the extrapolated tension was reached. Previous studies using servo control of the length of a segment of fiber (Gordon et al., 1966 b), and data from isometric tetani of whole muscles (Close, 1972) show a similar correspondence between sarcomere length and extrapolated tension. The relation, therefore, does not seem to depend critically upon the experimental procedure.

However, the relation does depend to some extent upon the tension extrapolation procedure, because tension increase during the slow phase often has no clearly straight portion that can be confidently fitted with a line of extrapolation. An exponential fit was, in fact, preferred by Mittenthal and Carlson (1971). The values in Fig. 9 B were obtained by extrapolating the 400-ms tangent. If one extrapolates the tangent immediately before the start of the plateau (cf. Fig. 2), the tension may exceed the values in Fig. 9 B by as much as 30% of maximal tension.

In the preceding paragraphs we showed that the increase in dispersion of sarcomere length along the fiber during tetanus was small. Consequently, we thought it reasonable to wait until transient changes of tension were complete and also plot steady-state tension vs. sarcomere length at that time. Fig. 9 A shows the relation between sarcomere length and plateau tension. Tension was independent of sarcomere length between 1.9 and 2.6 μm, declined to 50% maximal at 3.4 μm, and declined further at longer sarcomere lengths.

Passive tension developed beyond a sarcomere length of 3.3 μm and rose exponentially with increasing sarcomere length. The passive tension at 3.65 μm was ~10% of the maximally developed active tension. The contribution of passive tension in an actively contracting fiber at sarcomere lengths below 3.4 μm, therefore, was minor or negligible, as others have found (Edman, 1966; Gordon et al., 1966 a,b).

The Sarcomere Length-Tension Relation of Intact Muscle

We tested whether the time-course of tension development and the changes of sarcomere length dispersion in intact muscle permitted a relation to be constructed between plateau tension and sarcomere length for whole muscles, as we did for single fibers. We chose the toe muscle of the frog for this study because of the small number of fibers contained in the muscle, and because of their precise alignment, which properties give rise to crisp diffraction patterns.

The time-course of tension development in these muscles was similar to that observed in single fibers at comparable sarcomere lengths. The rms variation of the median sarcomere length along or across the resting muscles was 0.10 μm and 0.08 μm, respectively, for the six muscles studied, slightly larger than in the single fibers. The former value increased on average by 0.2 μm during contraction. These results on sarcomere length dispersion in the muscle at rest and during contraction are similar to those reported by Paolini and Roos (1975).

Fig. 10 shows the length-tension relation obtained from the intact muscles. Fig. 10 B shows that the relation between extrapolated tension and sarcomere length corresponds closely to the relation found in single fibers and to the relation described in previous studies of whole muscles (Close, 1972). Fig. 10 A
shows the relation between the tension during the plateau phase and the sarcomere length at that time. This relation is similar to the one obtained from the single fibers. The passive tension, however, rises much more steeply and becomes evident at shorter sarcomere lengths than it does in single fibers.

**Figure 10.** The sarcomere length-tension relation in the toe muscle of the frog. (A) Tension was plotted vs. sarcomere length during the plateau (see inset). The curve drawn through the points is the same one as drawn in Fig. 9 A. The data were obtained from six muscles. Passive tension develops at sarcomere lengths above 2.8 μm and reaches 100% of the maximally developed active tension at a sarcomere length of about 3.2 μm (△). (B) The results of measurement of tension by extrapolation according to the procedure shown in the inset, and the sarcomere length at that time. The drawn curve is the curve obtained by Gordon et al. (1966 b).
DISCUSSION

Comparison with Previous Results

It is evident from the time-course of tetanic tension that either of two values of tension may be selected to plot on a length-tension diagram. One value is obtained by extrapolating back to the end of the fast-rise phase of tension development, whereas the other is obtained from the plateau levels of tension. Both types of length-tension diagram have been constructed by previous workers, and notwithstanding some differences in methodology, our results are in good agreement with these.

Gordon et al. (1966 b) used the extrapolated values of tension to construct the length-tension relation. To maintain constant striation spacing during contraction, they imposed a length clamp over a segment along the fiber. The clamp obviated the tendency for the segment to be stretched during contraction by the shorter sarcomeres near the ends of the fiber. Our approach to this problem was to select only those fibers which did not show such nonuniformities of the initial sarcomere length. Consequently, but for the small compliance of the tendons and clips, the entire length of the fiber was clamped in our experiments. This amounted to roughly twice the length of the segments clamped by Gordon et al. In one of our experiments (see Results), fortuitously, a de facto clamp was observed locally as well.

As best we could tell, the results of the two experiments were similar. The tensions reached by the end of the fast-rise phase were comparable, as the length-tension relations constructed using extrapolated tensions were similar (Fig. 9 B). This similarity extended to our experiments with whole toe muscles as well (Fig. 10 B). Furthermore, assuming the few illustrative examples provided by Gordon et al. (1966 a, b) were representative, the rates of rise of tension during the slow-rise phase of tension development were also similar in the two sets of experiments (cf. Fig. 3).

Our data are not directly comparable with those of Edman (1966) or Ramsey and Street (1940). In these experiments length-tension relations were constructed from contractions which were of insufficient duration for plateaus to have been attained at all sarcomere lengths. (In the latter study, although it is stated in Methods that plateaus had been attained, this is not substantiated in the Results). The length-tension relations obtained in these two experiments fall intermediately between the ones we obtained by plotting extrapolated tensions and plateau tensions.

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Our results are in excellent agreement with those of Carlsen et al. (1961) who used plateau tensions obtained from tetani lasting 3-4 s to construct the length-tension relation. The length-tension relation remained relatively flat well beyond rest length. The sarcomere length at which a tension fell to 50% maximal was ~3.4 μm, as found here (Figs. 9 A and 10 A).

One area in which our results depart from those of others is at sarcomere lengths where overlap of thick and thin filaments presumably just ends, i.e., ~3.6 μm. Although our study was not designed specifically to explore tension development in this sarcomere length region, the few data points we have suggest that tension development falls to zero around 3.7 μm. Both Ramsey and
Street (1940) and Carlsen et al. (1961) show some tension development at sarcomere lengths of 3.6–3.7 μm and above. We agree with the interpretation of Gordon et al. (1966 a) that the shorter sarcomeres near the tendons in these preparations may have generated much of the tension, which was then sustained largely by the stretched parallel elastic elements of the longer sarcomeres in the central regions of these fibers; the apparent active tensions well beyond overlap may, therefore, be artifactual. The few records we have around 3.6 μm indicate that the central region was not stretched beyond overlap during contraction, thereby explaining the relatively lower tensions we found.

Finally, it is noteworthy that there is at least a tentative correspondence between our results and those obtained from experiments with skinned fibers. The plateau tension-sarcomere length relation obtained using maximally activated, skinned toad muscle fibers was relatively flat from 2.0 to 2.8 μm, and dropped to half-maximal at 3.3–3.4 μm (Endo, 1972). In that study the striation pattern was monitored only while the fiber was at rest; therefore, the possibility that some inhomogeneity may have developed during contraction cannot be ruled out, so the study warrants repetition. On the other hand, it is unlikely that a substantial change of average sarcomere length along the fiber could have occurred upon contraction, so the validity of Endo’s results in terms of plateau tension vs. average sarcomere length probably is not compromised.

In another study with skinned fibers Schoenberg and Podolsky (1972) reported a length-tension relation resembling the one published by Gordon et al. (1966 b). Frog semitendinosus fibers were used. These results are also open to criticism in that the fibers failed to relax completely after contraction. The records show substantial “residual tension,” a phenomenon studied subsequently by the same group (Thames et al., 1974), and attributed largely to cross-bridge tension. It may therefore have been more appropriate to plot the sum of residual tension and developed tension against sarcomere length, in which case the length-tension relation would have been more closely reconciled with that reported by Endo (1972).

In summary, there is an excellent correspondence between our results and those of previous studies with intact fibers, and a good, though tentative, correspondence between our results and those obtained with maximally activated skinned fibers.

Dispersion and the Slow Rise of Tension

Given two well-documented length-tension relations—steady state and extrapolated—the question arises as to which one best describes the primary contractile mechanism. To approach this question one must first consider the rationale for plotting extrapolated tensions.

Gordon et al. (1966 b) argued that the slow rise of tension was likely to arise out of “progressive development of irregularities of striation spacing,” and consequently, that the tensions extrapolated back to the time before the onset of such irregularities were the appropriate values to consider. In our fibers there was only a minor increase, and occasionally a decrease of dispersion of sarcomere length during the slow phase of tension development, a finding consistent with those of Kawai and Kuntz (1973) and Paolini et al. (1976, 1977).
Yet the characteristics of tension development in our fibers (Figs. 3 and 9 B) were similar to those reported by Gordon et al. (1966 b).

If the slow rise of tension were mediated by a population of sarcomeres 'creeping up' the linear descending limb of the length-tension curve and stretching a weaker population, as implied by Gordon et al. (1966 b), one should be able to document the existence of two progressively diverging populations. Although our records generally showed some increase of inhomogeneity of sarcomere length during the slow rise, they gave no indication of any separation into widely divergent populations (Figs. 5, 7, and 8).

The expected separation of the populations should have been large enough to be detected. Consider a contraction in a fiber whose initial sarcomere length is \( \sim 3.0 \, \mu m \). The tension developed during the rapid-rise phase is \( \sim 45\% \) of \( P_0 \), i.e., \( 45\% \) of the plateau tension at 2.2 \( \mu m \). The tension then climbs and settles at \( \sim 80\% \) of \( P_0 \). From Fig. 9 B, to account for this extra tension would require shortening up the descending limb by \( \sim 0.5 \, \mu m \). Shortening of this magnitude was never observed. In regions of our fibers in which shortening was found, it was typically on the order of 0.1 \( \mu m \) and often less (Figs. 5 through 8); in no instance did shortening in any region of any fiber exceed 0.25 \( \mu m \). This includes regions right up to the tendons (Fig. 8). Thus, even if the hypothesis that sarcomeres could 'creep up' the descending limb is tentatively accepted (see below), the observed magnitude of sarcomere shortening would have been inadequate to account for the observed magnitude of the tension increase.

To estimate the required amount of stretch of the weaker population is less straightforward. This could vary according to the mechanism assumed, i.e., passive or active. From the data of Sugi (1972), Edman et al. (1976) and Edman et al. (in press), we estimate the minimum amount of stretch needed to account for the slow increase of tension to be 0.1-0.2 \( \mu m \), although the exact value is uncertain. One can safely say, however, that at least some degree of stretch of the weaker population should have had to occur as a consequence of the shortening of the stronger population.

Therefore, in the example presented above it follows that the sarcomeres should gradually have diverged from the 3.0-\( \mu m \) initial length into two distinct clusters whose lengths were centered about the values 2.5 \( \mu m \) and somewhat beyond 3.0 \( \mu m \). A cluster which had shortened to 2.8 \( \mu m \), for example, would not be allowed by the hypothesis inasmuch as it could not sustain a high enough tension. Thus, a 'forbidden zone' is created between the two predicted clusters. Such a zone is expected for any contraction in which the initial length is between 2.2 and 3.6 \( \mu m \). Fig. 8 shows that by the time the plateau of tension had been attained the majority of sarcomeres had lengths in the forbidden zones. This rules out the possibility that the slow rise of tension could be generated by a population of sarcomeres which, for one reason or another, was transparent to our technique (Julian et al., 1978); the sarcomeres which were detected should not have had lengths in the forbidden zone.

Another kind of irregularity of striation spacing which could conceivably mediate the slow rise of tension is unbalanced overlap of thick and thin filaments within sarcomeres. Displacement of thick filaments from their central position within the sarcomere would increase the overlap in one half-sarcomere, possibly
increasing the tension. However, at sarcomere lengths of 3 μm and above, to achieve the required increase of overlap in one half-sarcomere would necessitate a decrease of overlap in the other half-sarcomere to the extent that the thick and thin filaments would no longer overlap. One might then postulate that the tension could still be supported by some connecting link between the end of the thick filament and either the Z disk or the thin filaments, but such an element would need to be stiffer than the parallel elastic element of the fiber (Iwazumi, 1970).

Theoretical arguments aside, unbalanced overlap of thick and thin filaments could not have occurred consistently during contraction, for an inhomogeneity of this type would have substantially diminished the intensity of the diffracted orders. Intensity diminution was observed often but not consistently (Fig. 5), whereas the slow rise of tension proved to be a highly consistent phenomenon.

On the basis of these considerations, we conclude that it is unlikely that progressive development of irregularities of striation spacing among or within sarcomeres causes the slow rise of tension. Consequently, we question the justification used by Gordon et al. (1966 b) in plotting extrapolated tensions.

It is relevant to point out here that a distinct slow-rise phase of tension development appears not only on the descending limb of the length-tension relation, but also on the ascending limb (Gordon et al., 1966 b, Figs. 4 and 6). If one asserts that the descending limb is unstable because of its negative slope, it follows that the ascending limb ought to be stable, because it has a positive slope. Thus, progressive dispersion of sarcomere lengths is not expected to occur on the ascending limb; yet the slow rise of tension is clearly in evidence there.

Stability

The observation that little or no dispersion occurs on contraction at any of the sarcomere lengths studied implies that the descending limb of the length-tension relation is not unstable as Hill (1953) suggested, but is highly stable. The reason may stem from the fact that the conventional length-tension curve is nothing more than a link between a series of 'static' operating points, each derived from a separate contraction. The instability argument is valid only if this curve is applicable in a dynamic situation, i.e., during a contraction. It is often assumed that some dynamic length change may be imposed upon a fully activated fiber such that it is made to 'creep' along its length-tension curve, but the evidence shows that this is clearly not so. Lengthening causes an elevation of tension (Abbott and Aubert, 1952; Deleze, 1961; Sugi, 1972; Edman et al., 1976), whereas shortening has the opposite effect (Abbott and Aubert, 1952; Deleze, 1961; Edman, 1964; Edman, 1975).

One might therefore conceive of a 'dynamic' length-tension curve which always has a positive slope. This curve is centered around an operating point on the 'static' length-tension curve. In terms of stability, the slope of the static relation is irrelevant. The positive slope of the dynamic relation ensures that a gradual increase of dispersion does not occur during contraction, thereby conferring stability upon the muscle.

The arguments concerning stability imply that a fiber which is perfectly uniform along its length ought to suffer no increase in inhomogeneity during
contraction. Most of the fiber regions we studied did show some increase in dispersion, though this was always minor. It seems reasonable to attribute this increase of dispersion at least in part to variations along the fiber of cross-sectional area or level of activation. One might expect the strong regions or the ones activated most rapidly to shorten and stretch the regions less well endowed. Unless the preparation is grossly nonuniform, however, the intrinsic stability of the contractile mechanism would limit the dispersion to a modest amount, as observed.

Length Clamping and the Slow Rise of Tension

The idea that the slow rise of tension might be the manifestation of a progressive dispersion of sarcomere lengths appears to have evolved out of the segment length clamp experiments (Gordon et al., 1966 a, b). These experiments were prompted by the earlier observation of Huxley and Peachey (1961); that the striation spacing along stretched single fibers was generally nonuniform—the spacing in the regions near the tendons was consistently smaller than in the central region. When such fibers were tetanized with the tendons clamped, the shorter sarcomeres shortened further and stretched the longer ones. In the length clamp experiments, a uniform segment in the central region of the fiber was maintained at constant length throughout the tetanus, thereby eliminating (or at least reducing) the progressive sarcomere-length dispersion. Imposition of the segment length clamp also diminished the rate of the slow rise of tension. The correlation of diminished dispersion with diminished rate of slow rise of tension, taken together with certain other considerations (see Gordon et al. 1966 b), prompted the suggestion that progressive dispersion might cause the slow rise of tension, an hypothesis which is evidently in conflict with the conclusion reached in preceding paragraphs.

If our conclusion is correct, another explanation must account for the reduction of the rate of the slow rise of tension observed when the segment length clamp was imposed. We suggest the following explanation. In fixed-end tetani the central segment lengthened during the slow rise of tension; in length-clamped tetani it remained isometric. Sarcomeres which are lengthening generate more tension than when they remain isometric (Abbott and Aubert, 1952; Deleze, 1961; Sugi, 1972). Thus the rate of rise of tension in the central segment should be reduced when the length clamp is imposed, as observed.

The implication is that fibers exhibiting the highest degree of inhomogeneity might suffer the most dramatic diminution of the rate of rise of tension when the clamp is imposed. In our experiments, in which the fibers behaved in a relatively uniform manner, imposition of a length clamp over a region would probably have had little effect on the time-course of tension. If the clamp were imposed on a region in which a de facto clamp was already present (see Results), the effect on the slow rise of tension would have been zero. On the contrary, in the work of Julian et al. (1978), where the sarcomeric behavior during contraction was documented to be grossly nonuniform, imposition of the clamp reduced the rate of the slow rise of tension tenfold.
Although segment length clamping can reduce the rate of slow rise of tension, there is no evidence that it can eliminate it. Julian et al. (1978) claimed that length-clamping a uniform, relatively short, fiber segment resulted in a slow-rise phase which was "absent or just visible." To quantitate these observations we measured the rates of slow rise of tension from photographic prints of their published records. Fig. 3 shows that relative to the respective temperatures and sarcomere lengths these rates did not differ significantly from those in the experiments of Gordon et al. (1966 b), or from those measured in our experiments. Evidently a slow rise of tension exists whether the sarcomeres remain naturally isometric or are constrained to remain isometric by length-clamping.

The Cause of the Slow rise of Tension

The exploration of possible mechanisms underlying the slow rise of tension has largely been limited to studies in which sarcomere length nonuniformity had been implicated. However, two other studies bear on the question. In a theoretical approach, Mittenthal (1975) applied a modified version of the early Huxley (1957) model to a uniform fiber. Although the model could account for the characteristics of the rise of tension at full overlap, it failed at partial overlap, where the slow rise is most prominent. This result tends to rule out the possibility that the slow rise of tension at long sarcomere lengths is merely a direct manifestation of the kinetics of the cross-bridge mechanism.

Another possibility which appears to be ruled out is that the slow rise is caused by a slow increase of the concentration of intracellular calcium. Single fibers bathed in Zn++ show a greatly enhanced aequorin response, suggesting that the level of intracellular calcium is increased; yet Zn++ has little or no effect on the slow rise of tension at long sarcomere lengths (Lopez et al., 1977,2).

It appears that the mechanism underlying the slow rise of tension is unknown.

Interpretation of the Length-Tension Relations

In the preceding paragraphs we argued that it is improbable that the slow rise of tension is due to progressive dispersion of striation spacing. On the other hand, it remains possible that the slow rise is still due to some secondary process, distinct from the primary contractile process. If so, there is a continuing rationale for considering the length-tension relation obtained by extrapolation to reflect the primary contractile process. The alternative approach is to assume there is a single contractile process, but its time-course shows a fast and a slow phase before the steady state is achieved. In this approach the length-tension relation obtained using the plateau tensions best describes the primary contractile process, though the extrapolated length-tension relation could still have significance.

2 Lopez, J. R., L. A. Wanek, and S. R. Taylor. Calcium activation in muscle and length-dependent changes in contractility. Submitted for publication.
It is worthwhile examining the implications of the two approaches to determine what constraints each might impose on present views of molecular contractile mechanisms, and perhaps also to determine which of the two is most likely to be correct. In the first approach it is assumed that full activation of the myofilaments is achieved by the end of the fast rise of tension. The length-tension relation derived from tensions obtained at this time characterizes cross-bridge tension mechanisms per se. The additional tension results from a potentiating mechanism, which increases cross-bridge force over and above the full-activation levels.

This approach preserves the excellent correlation between overlap and tension which is demanded by the cross-bridge theory in its simplest form. On the other hand, inasmuch as the theory must also account for the higher plateau tensions, additional constraints need be imposed upon the theory. First, it must be assumed that the state of “full activation” is not the one in which maximum force is generated; a mechanism must be identified by which the cross-bridges are capable of generating supramaximal force. Second, the potentiation must vary with sarcomere length. The ratio of plateau tension to extrapolated tension is 1 at sarcomere lengths near 2.0 μm, but increases at longer sarcomere lengths. At 3.4 μm the potentiated tension is about five times the tension at “full activation.”

The alternative approach assumes that the activation process consists of a fast phase followed by a slow one, and that full activation is achieved when the tension plateau is reached. In this approach the extrapolated length-tension relation characterizes the length dependence of the fast phase of tension development, whereas the one obtained with steady-state tensions characterizes the fully activated cross-bridge mechanism. This approach leads immediately to the difficulty that tension is not directly proportional to the degree of overlap of thick and thin filaments. Again, additional constraints must be placed on the basic mechanism. Agreement between data and theory can be preserved if, for example, it is assumed that the cross-bridges within 0.3 μm of the center of the thick filament do not contribute to tension generation (this gives the flat region of the length-tension relation), and if the probability of cross-bridge attachment or the strength of each bridge increases with distance from the center of the thick filament (this accounts for the large tensions with little overlap).

Neither approach provides a straightforward interpretation of the length-tension data. Although it is evident from the above discussion that the cross-bridge theory can be accommodated in either of the two approaches, it is clear that the length-tension data no longer offer unqualified support for the cross-bridge theory.

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