Intersarcomere Dynamics of Single Muscle Fibers During Fixed-End Tetani

RICHARD L. LIEBER and RONALD J. BASKIN
From the Department of Zoology, University of California at Davis, Davis, California 95616

ABSTRACT The contraction dynamics of end and center regions of single fibers have been measured during fixed-end tetani. Experimental control and data acquisition are provided by a digital system that can acquire diffraction data as fast as every 260 μs for 300–700 ms. Tension records are simultaneously displayed on a storage oscilloscope. Resting sarcomere length variation between the end and center regions was analogous to that of Gordon et al. (1966). During the rapid rise in force (<45 ms), the end regions contract almost twice as fast as the center regions. During the slow rise in force, the velocity of contraction of the end regions was 3.8 times the velocity of stretch of the center regions. In addition, factors that affected the rate and extent of the slow rise in tension also affected the rate and extent of end shortening. In 58% of the cases studied, the amount of shortening observed in the end region was enough to explain the extent of the slow rise in tension. These data support the explanation of creep first proposed by A. V. Hill (1953) and used by Gordon et al. (1966) to justify their use of the back-extrapolation technique in measuring the isometric force-generating capability of a single fiber. These data also indicate that the laser diffraction technique may provide an effective, noninvasive method for studying sarcomere dynamics during creep and related phenomena.

INTRODUCTION

The length-tension curve of Gordon et al. (1966) is considered by many to be a classic example of a structure-function relationship. The tension traces at long sarcomere lengths showed both a rapid and slow rising phase in tension. The existence of this slow phase presented a potential problem in defining the isometric tension-generating capability of a single fiber. Gordon et al. (1966) analyzed the tetanic tension values based on the existence of the “creep” phenomenon first proposed by A. V. Hill (1953). This phenomenon was consistent with the observations of Huxley and Peachey (1961) and Carlsen et al. (1961), and with the behavior of the tension trace when a servo device was used to prevent end sarcomeres from contracting (Gordon et al., 1966, Fig. 7) and with the tension records before and after sarcomere length variation.
disruption (Gordon et al., 1966, Fig. 14). Recent physiological studies have provided further evidence for the original explanation of creep (Julian et al., 1978; Julian and Morgan, 1979a). In addition, Haskell and Carlson (1981) have detected axial displacement of the scattering material within single fibers using coherent light scattering.

The conclusions regarding creep are not unequivocal (ter Keurs et al., 1978). ter Keurs et al. were not able to detect the presence of shortening end sarcomeres. They concluded that the length-tension relation of muscle could not be considered unequivocal support for the sliding-filament theory (Huxley and Hanson, 1954; Huxley and Niedergerke, 1954), but could be explained by other theories of force generation (Iwazumi, 1979). Their conclusions have been criticized by some because their study used the laser diffraction technique to measure sarcomere length. It has been argued that this technique may be insensitive to the “relatively few” sarcomeres in the end region that can determine the magnitude of the tension generated during isometric contraction (Julian and Moss, 1980).

This laboratory has studied the physical basis of laser diffraction and its value as a tool for monitoring sarcomere length (Yeh et al., 1980; Baskin et al., 1981; Lieber et al., 1983a). The purpose of the present study was to monitor the end and center sarcomere regions during fixed-end tetani to measure directly the sarcomere length changes in different areas of the fiber.

**METHODS**

**Fiber Preparation and Mounting**

Single fibers from the frog (*Rana pipiens*) were dissected from the *m. tibialis anterior* (lateral head). The fibers were dissected in chilled (0°C) Ringer’s solution composed of (mM): 115 NaCl, 2.5 KCl, 2.15 Na₂HPO₄, 0.85 NaH₂PO₄, and 1.8 CaCl₂, adjusted to pH 7.0. The muscle tendon was held during dissection with stainless-steel weights. (Brass weights had been used, but toxic levels of copper had been shown by atomic absorption spectroscopy to leach into the dissecting solution.) After dissection, the major and minor axes of the fiber (Blinks, 1965) were measured using a Bausch & Lomb calibrated eyepiece (Bausch & Lomb Inc., Rochester, NY) in combination with a Zeiss precision calibrated ruling (Carl Zeiss, Inc., Thornwood, NY). Fibers were selected for diameters <60 μm to decrease the probability of sarcomere length error measurement using laser diffraction (Lieber et al., 1983a). All of the phenomena reported here were detected in both the right and left first orders.

The tendons were trimmed to a width of ~0.3 mm, and small aluminum foil clips (mass 100 μg) were applied (Ford et al., 1977). The tendon at each end of the fiber was cleared of broken fiber stubs and trimmed as close to the fiber insertion point as possible. The fiber was then transferred to a Teflon (DuPont Corp., Wilmington, DE) chamber mounted on the stage of a Zeiss IM-35 inverted microscope (Carl Zeiss, Inc.). The chamber volume was ~3 ml (Fig. 1). Chilled Ringer’s solution (12°C) circulated through the chamber (except during data acquisition) at a rate that replaced the chamber volume every 15 s. During some experiments, after several hours, a decrease in fiber force was observed. In many cases, this was reversible after addition of 5 mM D-glucose. Insulin was also added (15 IU/liter), but did not seem to affect the time course or extent of force redevelopment and so was not routinely used.
The fiber was mounted between a force transducer and a high-speed motor. The force transducer had a sensitivity of 0.51 mV/mg and a resonant frequency of 4.55 kHz. It was constructed using a Dynaguage (Dynaguage Inc., Pasadena, CA) pressure transducer with a small stainless-steel hook epoxied to the pressure-sensing diaphragm. The combined mass of the hook and epoxy was 3.6 mg. The force output of the diaphragm was converted to an analog signal and displayed on a storage oscilloscope (model 5115; Tektronix, Inc., Beaverton, OR). The motor used was the Cambridge Technology model 300S (Cambridge Technology, Cambridge, MA). A small (1.6 mg) hook was epoxied to the end of the arm supplied with the motor, yielding a total rotational inertia of 0.036 g·cm² (0.013 g·cm² from the arm and hook, 0.023 g·cm² from the motor hub). The transient response of the motor to a step change in command signal was ~450 μs (10–90%) in this configuration.

**Fiber Stimulation Protocol**

Bipolar stimulation of the fiber was accomplished by feeding a Grass model S48 stimulator (Grass Instruments, Quincy, MA) into a stimulus isolation unit (Grass model SIU 5), which fed into a KEPCO model 500M operational power supply (KEPCO Power Supplies, Flushing, NY). The output of the power supply was applied to cylindrical platinum electrodes (2.5 mm diam), which extended the length of the chamber (~3 cm). The fiber was typically stimulated supramaximally at a fusion frequency of 70 Hz, a duration of 0.5 ms, and a train duration of 400–700 ms for tetanic contractions. During an experiment, the electrodes were placed 3.76 mm apart, yielding a typical voltage gradient of ~7 V/cm. The entire motor arm, motor hook, and tension transducer were isolated from analog ground using Krylon coating (Krylon Inc, Norristown, PA) to prevent excessive current draw.
The motor and the tension transducer were mounted on micromanipulators (model M-2; Narishige, Tokyo, Japan), which permitted precise lateral and vertical translation of the fiber. In experiments where different regions of the fiber were examined, the initial fiber length \( L_0 \) was measured with the Bausch & Lomb eyepiece mounted in a dissecting microscope overlooking the chamber. The fiber was then translated a known amount as measured on the micromanipulator scales (precision 0.05 mm) and reset to the original \( L_0 \). The magnitude of translation could then be directly determined by subtraction on the micrometer scales. In observing end regions, the fiber was moved into the laser until the tendon just started to scatter the laser beam. Thus, for the purposes of this report, “end region” refers to the last 0.8 mm of the fiber. Since the intensity of the laser was approximately Gaussian, a region \( \sim 0.3 \) mm wide, 0.2 mm away from the tendon received the majority of the incident intensity. The “center” regions discussed in this report refer to the central regions of the fiber, which were at least 2.0 mm from either tendon.

After any translation, the fiber was passively stretched and released (see below) to “reorder” the fiber (Gordon et al., 1966). If this procedure is followed, tetanic tensions and sarcomere length records are reproducible for 3–6 tetani (Fig. 2). All sarcomere length traces for different regions of the fiber have thus been obtained within five tetani of each other.

After mounting, the tetanus-to-twitch ratio was determined for the fiber. Typically, this value was between 2 and 3, with a tetanic tension of 150 mg (or 0.19 N/mm\(^2\) for a fiber diameter of \( \sim 50 \mu m \)). A waiting period of at least 5 min was imposed between each tetanus. In this setting, the fibers gave reproducible contractions for \( \sim 6 \) h.

**Data Analysis**

Tension traces were photographed from the storage oscilloscope. These data were then photocopied for further analysis. Back extrapolation was performed from the 200-ms tangent to the tension trace. The intersection between this line and the rapid rise in tension was defined as the “before-creep” tension value. Back extrapolation from the 150-ms tangent was shown to yield similar results. To determine whether the amount of shortening in the end region accounted for the slow rise in tension, sarcomere length was related to tension-generating capability using a length-tension curve with \( P_0 \) at 2.25 \( \mu m \) and 0 \( P_0 \) at 3.65 \( \mu m \). This relationship was consistent with the behavior of the fibers used in this study. Tension values relative to specific sarcomere lengths were interpolated from this relationship (see the examples given in Results). The sarcomere length and tension records were examined while the end sarcomeres contracted at constant velocity. Thus, the decrease in force caused by the contracting end sarcomeres was constant throughout this period and was not taken into account for analysis of sarcomere shortening and force generation.

**Data Acquisition System**

The fiber was transilluminated by a low-power helium-neon laser (beam diameter 0.8 mm; model 05-LHR-151; Melles Griot, Irvine, CA). The diffraction pattern obtained from the muscle was collected by a Zeiss 40x, 0.75 N.A. water-immersion lens and projected through the Zeiss cine-tube onto a linear photodiode array (Fig. 1). The primary image plane (the diffraction pattern) was focused onto the array using a phase-contrast focusing telescope. In addition, the laser image of the illuminated region could be observed through the oculars to monitor the fiber striation pattern and fiber “cleaness” during an experiment. Although this was not proper
Koehler illumination because of the coherent light source, deterioration of the fiber and nonuniformities developing during experiments were easily observed.

The digital data acquisition system is fully described by Lieber et al. (1983b) and is only summarized here. The photodiode array used was the Reticon model 128S (EG&G Reticon, Sunnyvale, CA), chosen for its large aperture (2.5 mm) and wide dynamic range (10,000:1). Approximately 80% of a first-order line was imaged onto the array. Movement of the zero order caused by fluid surface ripple could lead to erroneous sarcomere length measurements. Absolute positions of the zero and first orders were measured during a rapid passive stretch (Fig. 3A). Note the parallel movement of both peaks, which implies that refraction at the fluid surface was responsible for the movement. Identical results were obtained for left and right first orders. To eliminate refraction artifacts, the zero order was filtered in front of the array, while the first order remained unfiltered. The intensity of both peaks was then in the dynamic range of the photodiode array, and dynamic peak separation measurement (yielding sarcomere length) was possible. Sarcomere length was determined from peak centroid spacing using the plane grating equation for normal incident angle. Splitting of the diffraction orders (Morgan, 1978) was never observed during force generation or during the slow tension rise, but was occasionally seen during relaxation. An example of the effect of filtering the zero order on sarcomere length determination is shown in Fig. 3B for a rapid passive stretch of 4%.

The analog output of the photodiode array was fed into a video speed analog-to-digital converter (model TDC1007J; TRW, Redondo Beach, CA). The digital output of the ADC was buffered and sent via ribbon cable to the LSI-11 minicomputer (Digital Equipment Corp., Maynard, MA), where it was stored in memory via a custom Direct Memory Access interface (Lieber et al., 1983b). This system can perform data acquisition as fast as 492 kHz, which enables storage of a photodiode array sweep in 260 μs (128 elements × 2.03 μs/element). For the tetanic contractions described here, data were typically stored at a rate of 1.0 ms/scan. The memory

![Figure 2. Repeatability of contraction in a given area of the fiber. Three separate sarcomere length traces from tetanic contractions are shown along with a tension record (inset). Calibration bars for tension record: horizontal = 100 ms, vertical = 50 mg.](image-url)
capacity of the computer allows acquisition of up to 380 sweeps of data per experiment. In some experiments, data acquisition was delayed for 300 ms after stimulation began to allow recording of events late in contraction.

Experimental control was performed by the data acquisition system. Before the experiment, the user enters the appropriate experimental parameters (e.g., number

![Graph A](image1)

**Figure 3.** (A) Movement of the zero- and first-order lines during a rapid passive stretch. The movement of the first (trace A) and zero (trace B) orders is shown. Absolute peak position is represented after rapid passive stretch. After the stretch, the movement of the orders is almost identical, which suggests that refraction at the solution surface is probably responsible for the movement. (B) Dynamic measurement of peak spacing. The position of both peak centroids was determined by the analysis program and the separation was calculated. Thus, movement artifacts such as those seen in A do not result in sarcomere length variations.
of sweeps to be taken, magnitude of rapid length change, and appropriate delay values). The experimental sequence then proceeds as follows: (a) the computer triggers the stimulator to deliver a train of pulses; (b) the computer waits a delay value (if any) and then begins data acquisition; (c) the computer acquires a given number of sweeps and then performs a rapid length change (if any); (d) the computer acquires a given number of sweeps and terminates data acquisition; (e) the computer displays the acquired data.

Delay between segments b and c, and c and d of the data acquisition program due to program execution was never greater than 60 μs and was typically 30 μs. Thus, corrections for these delays were not incorporated into the data analysis. The acquired data could be stored on floppy disk for later analysis via an interactive FORTRAN program (Lieber and Lubell, 1983). The entire experimental system is illustrated in Fig. 1.

RESULTS

Resting Sarcomere Length Differences

Absolute resting sarcomere length difference between end and center regions averaged 109 ± 58 nm (n = 38; range = 31–287 nm; Table 1). This corresponds to a relative difference of 4.18 ± 1.9% (n = 38; range = 1.9–8.7%; Table 1). These values were positively correlated with sarcomere length and best fit by a line (r = 0.726). Thus, during passive stretch, the magnitude of the difference between the end and center sarcomeres increased. Sarcomere variation along the center region was <1%. This is in agreement with previously reported data for frog skeletal muscle (Huxley and Peachey, 1961; Carlsen et al., 1961).

Sarcomere Length Differences During Stimulation

The time course of contraction of the end and center regions of a single fiber with an initial center sarcomere length of 2.54 μm is shown in Fig. 4. In the center region (Fig. 4, trace A) there is a rapid (0.65 SL/s) stretch for the first 20 ms, followed by a transient phase, followed by a slow stretch at a velocity of 0.012 SL/s. The end region (Fig. 4, trace B) shows a rapid (1.6 SL/s) contraction for the first 10 ms, followed by a transient phase, followed by a moderate (0.34 SL/s) contraction for the last 240 ms of contraction. During this steady shortening, a slow rise in tension is observed (Fig. 4, inset). Rapid stretching of the center region was observed in 20% of the fibers studied and the mechanism was not investigated. Averaged data from 25 regions of 15 fibers indicate that during the fast rise in tension (the first 45 ms) both regions usually show rapid contraction, and on the average, the end regions contracted faster (0.782 SL/s; n = 26) than the center regions (0.396 SL/s; n = 24; Table I). This was not due to differential electric field density along the stimulating electrodes. During the remainder of the stimulus (the next 300–725 ms), the end regions shortened while the center regions stretched. Data from 14 different regions of 8 fibers indicate that the average
velocity of contraction in the end regions during the creep phase was 3.8 times the velocity of stretch of the center regions (−0.19 SL/s vs. 0.05 SL/s; Table I). The average center stretching velocity was $\sim 0.9\% V_{\text{max}}$ using the data of Edman (1979), assuming a $Q_{10}$ of 2.67.

**Figure 4.** Sarcomere dynamics of the center (trace A) and end regions (trace B) of a single fiber. Fiber length = 5.47 mm. Center region = 2.03 mm from right tendon. End region = 0.32 mm from right tendon. Temperature = 12°C. Fiber 6112. Calibration bars for tension record: horizontal = 100 ms, vertical = 50 mg.
Sarcomere Lengths in Relation to Tetanic Tension

The data from 12 regions of 6 fibers were analyzed to determine whether the amount of shortening in the end regions was sufficient to explain the tension rise observed during the creep phase of contraction. The data are presented in tabular form in Table II. In 58% of the cases, the amount of shortening in the end regions was enough or more than enough to account for the tension rise occurring during the slow phase. This was determined as described in Materials and Methods. Data from a single fiber are shown in Fig. 5. After the rapid rise in tension, the end sarcomere length is 2.520 μm (Fig. 5, trace B). This corresponds to a tension level of 0.804 $P_0$. After 250 ms the tension has increased to 0.887 $P_0$ while the sarcomere length has decreased to 2.403 μm (Fig. 5, trace B and inset). The predicted length change given the relative tension change is −117 nm and the actual change is also −117 nm, which is enough to explain the extent of the slow rise. For the data shown in Fig. 4, the corresponding values are as follows. After the rapid rise in tension (50 ms) the end sarcomere length is 2.389 μm (Fig. 4, trace B). This corresponds to a tension level of 0.901 $P_0$. After 250 ms of stimulation the tension has increased to 1.000 $P_0$, while the sarcomere length has decreased to 2.236 μm (Fig. 5, trace B and inset). The predicted length change is −139 nm, while the actual length change is −153 nm, which is enough to explain the extent of the slow rise. During this period, the center region (Fig. 4, trace A) is stretching at +0.03 SL/s. Data from 12 regions of 6 fibers are summarized in Table II. When the measured sarcomere length change was greater than or within 5 nm of the predicted length change (5

| Fiber | T before creep* | T after creep* | SL before creep† | SL after creep‡ | Actual SL change§ | Predicted SL change¶ |
|-------|----------------|---------------|-----------------|-----------------|-------------------|-------------------|
| 5316  | 0.810          | 0.960         | 2.510           | 2.292           | 218               | 210               |
| 5806  | 0.840          | 0.929         | 2.472           | 2.439           | 33                | 123               |
| 5818  | 0.890          | 0.959         | 2.402           | 2.259           | 143               | 95                |
| 5819  | 0.907          | 0.964         | 2.380           | 2.315           | 70§               | 74                |
| 5820  | 0.917          | 0.959         | 2.366           | 2.316           | 50                | 58                |
| 6020  | 0.707          | 0.778         | 2.660           | 2.595           | 65                | 99                |
| 6026  | 0.817          | 1.000         | 2.506           | 2.461           | 45                | 256               |
| 6105  | 0.804          | 0.887         | 2.520           | 2.403           | 117§              | 117               |
| 6112  | 0.901          | 1.000         | 2.389           | 2.236           | 153§              | 139               |
| 6207  | 0.889          | 0.972         | 2.406           | 2.385           | 21                | 117               |
| 6208  | 0.864          | 0.903         | 2.440           | 2.386           | 54§               | 54                |
| 6318  | 0.794          | 0.851         | 2.540           | 2.490           | 50§               | 53                |

* Tension values relative to $P_0$.
† Sarcomere length values in micrometers.
‡ Change in sarcomere length values in nanometers.
§ Enough end shortening to explain tension rise.
nm = system resolution), the change was considered sufficient to account for the slow tension rise.

Passive stretch-release cycles have been shown to decrease the rate of the slow creep phase, presumably by decreasing the resting dispersion along the fiber (Gordon et al., 1966). The time course of tension and sarcomere length shortening was measured before and after three passive stretch-release cycles of +10%/-20%. The results are shown in Fig. 6. Before the first tetanus, 15 consecutive twitches were given to “disorder” the fiber. Then, the dotted sarcomere length trace in Fig. 6, trace B, and upper tension trace in Fig. 6 were obtained. The sarcomere length change is enough to account for the slow tension rise. A series of three stretch-release cycles was then imposed and the solid sarcomere length trace in Fig. 6, trace A, and the lower tension trace in Fig. 6 were obtained. Note the diminished magnitude of the slow phase as well as the decrease in the amount of end shortening after the stretch-release cycles.

The size of the end region, as well as the existence of a transition zone (Morgan et al., 1982), was studied by moving along the fiber in small increments. The results are shown in Fig. 7. Fig. 7A presents a schematic view of the locations along the fiber of the four regions studied. A relatively smooth transition from the stretching central sarcomeres to the contracting end sarcomeres is seen in Fig. 7B, traces A–D. Averaged data from nine fibers indicate that the transition from contracting to stretching sarcomeres

---

**Figure 5.** Sarcomere dynamics of the center (trace A) and end regions (trace B) of a single fiber. Fiber length = 7.41 mm. Center region = 2.58 mm from left tendon. End region = 0.33 mm from left tendon. Temperature = 12°C. Fiber 6105. Calibration bars for tension record: horizontal = 100 ms, vertical = 50 mg. Note that the amount of end shortening is enough to account for the slow rise in tension. See text and Table II for details.
occurs ~0.7 mm from the end. However, since the data were not taken continuously along the fiber, this value is approximate.

**Effect of Rapid Length Change on Sarcomere Length Changes**

If a quick stretch (magnitude <0.5%) was imposed during the creep phase of contraction, the velocity of sarcomere shortening in the end region decreased by ~70% relative to the prestretch value (n = 6; Table I). The average prestretch contraction velocity for six regions of four fibers was ~0.32 SL/s, while the poststretch value was ~0.06 SL/s. Fig. 8 shows the sarcomere length dynamics in the end region of a single fiber given rapid stretches 50 ms (Fig. 8, trace A, tension inset) and 150 ms (Fig. 8, trace B, tension record not shown) after initial stimulation. After stretch, the velocity of contraction in the end region decreases significantly. The tension traces show a slight force enhancement and "leveling." In the control tetanus, the creep tension approaches the stretch tension late in contraction (Fig. 9). The sarcomere length trace in the center region is shown in Fig. 9. During a control tetanus (Fig. 9, trace B), the sarcomere length slowly stretches to a final value. If a quick stretch is imposed, the sarcomere length is rapidly increased to about the ending level of the control contraction, after which it slowly stretches for

![FIGURE 6. Effect of passive stretch-release cycles on tension record and sarcomere dynamics. The fiber was given 15 twitches and the dotted sarcomere length (B) and upper tension traces were obtained. Then, three to four passive stretch-release cycles were imposed, and the solid sarcomere length trace (A) and lower tension traces were obtained. Notice that a decrease in the extent of the slow rise is paralleled by less shortening in the end region. Fiber length = 5.30 mm. End region = 0.28 mm from right tendon. Calibration bars for tension record: horizontal = 100 ms, vertical = 50 mg. Temperature = 12°C. Fiber 5316.](image)
the remainder of the stimulation (Fig. 9, trace A). We found that a rapid release did not affect the contraction or the stretch velocity of the end or center sarcomere populations.

![DIAGRAM](https://example.com/diagram.png)

**FIGURE 7.** (A) Scale drawing of the different locations along the fiber from which the records shown in B were obtained. The number in parentheses represents the distance in millimeters from the left tendon. (B) Sarcomere dynamics from the different regions shown in A. Note that there is a relatively smooth transition from stretching to contracting regions. Temperature = 12°C. Fiber 5810.

**DISCUSSION**

A question exists as to whether the slow creep phase of tension development is a manifestation of the interaction between sarcomeres as has been suggested by Hill (1953), Huxley and Peachey (1961), and Gordon et al. (1966), or is actually a fundamental property of the sarcomere itself (ter Keurs et al., 1978).
Gordon et al. (1966) presented evidence consistent with the proposal that the slow creep phase of tension development is due to interaction between sarcomeres that have "typical" force-velocity (Katz, 1939) and length-tension (Gordon et al., 1966) characteristics. Their data were consistent with the earlier observations of Huxley and Peachey (1961), who documented the existence of the shorter end sarcomeres. These data, along with those obtained by Gordon et al., provided justification for the use of the "back-extrapolated" tension values as the true tension-generating capability of the fiber.

Figure 8. Effect of rapid stretch on end sarcomere contraction dynamics. Inset: a rapid stretch (upper trace of inset shows motor arm movement; magnitude = 35 μm, 0.51% of L₀) is imposed 50 ms after contraction begins. The tension trace shows a rapid rise and then decays to a relatively constant value. Calibration bars for tension record: horizontal = 100 ms, vertical = 50 mg. Sarcomere length traces shown for a stretch 50 ms (trace A) and 150 ms (trace B) after contraction begins. Note that the velocity of contraction after stretch decreases. Fiber length = 4.92 mm. End region = 0.39 mm from left tendon. Temperature = 12°C. Fiber 6123.

Ter Keurs et al. (1978) have criticized this method of data analysis because of their inability to detect end sarcomeres that shorten a sufficient amount to explain the magnitude of the slow tension rise during fixed-end tetani. As a result, they chose to plot the peak tension reached later in the fixed-end tetanus as the true tension-generating capability of the fiber. These data led to a length-tension curve considerably different from that of Gordon et al. (Ter Keurs et al., 1978, Fig. 9).

We have performed similar experiments using three fiber selection criteria: (a) fibers have a major diameter <60 μm; (b) fibers exhibit a tentanusto-
twitch ratio of 2–3; and (c) fibers generate reproducible tetani over a period of hours. The first criterion is based on experiments that determined the effect of fiber diameter on sarcomere length measurements in single fibers (Lieber et al., 1983a). We found that significant errors in sarcomere length could be obtained at fiber diameters >75 μm. These errors may be due to thick grating effects such as Bragg diffraction (Rüdel and Zite-Ferenczy, 1979a, b; Baskin et al., 1981).

All of the data obtained in the present study could be measured from either the left or right first-order line. This indicates that the sarcomere lengths measured were not biased by the sampling problem associated with Bragg reflections. In addition, the zero-order line was filtered to allow dynamic measurement of zero-order position (Fig. 3). Other investigators have measured the first-order relative to a fixed zero-order position.

The Creep Phenomenon

The results presented here represent the first direct measurement of the dynamics of "creeping" sarcomeres proposed three decades ago by A. V. Hill (Figs. 4 and 5). The sarcomere dynamics are consistent with force-velocity data for frog muscle in that the velocity of contraction of the end sarcomeres is considerably different from the velocity of stretch of the center sarcomeres (4:1 ratio). This asymmetry of intersarcomere velocities is paral-
led by the discontinuity in the force-velocity curve for whole fibers (Edman, 1979).

Our data are also consistent with a sarcomere length-tension relationship where, beyond \( \sim 2 \mu m \), longer sarcomeres develop less tension than shorter sarcomeres. The exact form of this relationship was not studied. End sarcomeres thus stretch center sarcomeres because of the resting sarcomere length variation. This was tested by changing the "uniformity" of the fiber, as shown in Fig. 6. We found, as did Gordon et al. (1966), that the rate of creep decreased after passive stretch-release cycles. In addition, direct measurement of the end sarcomeres showed a decreased contraction velocity, consistent with the tension record (Fig. 6).

Our data show that the length-tension curve and the force-velocity curve are able to account for mechanical interaction between sarcomeres. These relationships have been elucidated in whole muscles and single fibers and extrapolated to describe the properties of the contracting sarcomere. Using these properties, we have been able to account for the contraction dynamics observed in isometric tetani. This provides more direct evidence that the length-tension and force-velocity relationships obtained in single fibers do describe the contraction characteristics of sarcomeres.

The initial contraction velocity of the end sarcomeres has been shown to be almost twice that of the center sarcomeres. This could be due to inherent maximum contraction velocity differences along the fiber, or a sarcomere length dependence on \( V_{\text{max}} \). Edman (1979) has shown that \( V_0 \) as measured by the slack test shows very little variation from sarcomere length 1.65 to 2.7 \( \mu m \). However, for a given finite load, the velocity will be greater for shorter sarcomeres. Thus, these data may be a manifestation of the initial sarcomere length distribution. Alternatively, since muscle fibers of the fast-twitch type possess a greater density of sarcoplasmic reticulum than do slow-twitch fibers (Close, 1972), regional variation in sarcoplasmic reticulum may explain velocity differences between end and center sarcomeres.

In our preparation, the amount of shortening by the end sarcomeres was sufficient to account for the slow tension rise in 58% of the cases studied (\( n = 12 \); Table II and Fig. 5). We have seen cases in which most of the shortening occurs at only one end of the fiber. Since we did not routinely examine both ends of the preparation, our results may reflect the statistical probability of observing the shortening end. In addition, our beam was characterized by a Gaussian distribution with a standard deviation of 0.15 mm. Thus, \( \sim 80\% \) of the incident intensity is concentrated in the central 0.30 mm of the beam. We are thus only able to project the majority of the intensity within \( \sim 0.3 \) mm of the end of the fiber before scattering occurs. Shortening sarcomeres at the extreme ends of the fiber cannot, therefore, contribute a significant portion of the diffraction signal measured. This problem can be overcome by optically altering the profile of the incident beam.

**The Effect of Quick Stretch**

A small (<0.5%) stretch was shown to decrease the velocity of end contraction by 68%. At the same time the velocity of center stretching decreased (Fig.
A similar qualitative observation has been made by Julian and Morgan (1979b). The tension trace shows a leveling when the stretch is imposed early in the creep phase. A simple explanation for this phenomenon is that the end sarcomeres are less stiff than the center sarcomeres so that the quick stretch extends the ends more than the center. The sarcomere length difference between the end and center would then be decreased, resulting in a decreased rate of creep. This could act in vivo as a "reset" to increase the stability of the contracting population during gait, for example, when active stretching of the anterior tibialis and quadriceps occurs. Unfortunately, we failed to detect a significant difference in the stiffness between the end and center regions as measured by quick stretch. Julian and Morgan (1979b) have reported the opposite effect—that most of the length change is taken up by the center regions to cause further destabilization. Their experiments did, however, use stretches of greater magnitude and lower velocities.

Our fibers showed an end-to-center sarcomere length variation comparable to that of Gordon et al. (1966). Thus, our preparation seems more analogous to that of Huxley and Peachey (1961) and Gordon et al., than to that of ter Keurs et al. (1978), who selected for sarcomere length uniformity along the fiber.

Potential problems of interpretation when very uniform fibers are used have been suggested by Morgan et al. (1982). In addition, we have confirmed two hypotheses suggested by their model: (a) we have directly measured the dynamics of the contracting end sarcomeres and stretching central sarcomeres (Figs. 4 and 5); (b) we have observed a "transition zone" in which sarcomeres progress from stretching to contracting (Fig. 7).

We were unable to correlate the extent of end shortening with resting end-center sarcomere length variation (Morgan et al., 1982, Figs. 4 and 5). We did find a slight positive correlation between the initial sarcomere length nonuniformity (end/center ratio) and the rate of end shortening ($r = 0.532$).

Laser Diffraction Studies of Creep

An important aim of this study was to establish that phenomena such as creep, which have been considered difficult to observe dynamically, are observable using the laser diffraction technique. Many references have been made to the "relatively few" sarcomeres in the end regions of fibers that shorten at the expense of stretching center sarcomeres. Julian and Moss (1980) showed that in skinned fibers, the length-tension relation of Gordon et al. (1966) was most accurately reproduced when the shortest sarcomere of a region was plotted against tension developed. According to their analysis procedure, this represents at least 50 sarcomeres in series.

With photomicrography, their optical section was $\sim 1 \mu$m. Thus, a single "layer" of myofibrils is being photographed. With laser diffraction, a weighted average of the sarcomere lengths is obtained. This value is weighted based on the degree of order in a region. If the shortest sarcomeres in a region are ordered vertically throughout the fiber, but there are fewer than 50 sarcomeres along the fiber, laser diffraction might be the best way to
study their dynamics. Thus, although it is not applicable to every case, the technique of laser diffraction should be considered useful in studying such phenomena.

Further studies are required to determine the actual numbers of sarcomeres that belong to the different populations. A combination of microscopic and diffraction information might be the best method. Such a system is in use, described by Iwazumi and Pollack (1979).

We would like to thank Drs. David L. Morgan and Lincoln E. Ford for their helpful comments. In addition, we acknowledge the skillful fiber dissection of Martha E. Corcoran.

R. L. Lieber was a National Institutes of Health predoctoral fellow.

Received for publication 20 December 1982 and in revised form 23 March 1983.

REFERENCES

Baskin, R. J., R. L. Lieber, T. Oba, and Y. Yeh. 1981. Intensity of light diffraction from striated muscle as a function of incident angle. Biophys. J. 36:759–773.

Blinks, J. R. 1965. Influence of osmotic strength on cross-section and volume of isolated single muscle fibers. J. Physiol. (Lond.). 177:42–57.

Carlsen, F., G. G. Knappeis, and F. Buchthal. 1961. Ultrastructure of the resting and contracted striated muscle fiber at different degrees of stretch. J. Biophys. Biochem. Cytol. 11:95–117.

Close, R. 1972. Dynamic properties of mammalian skeletal muscles. Physiol. Rev. 52:129–197.

Edman, K. A. P. 1979. The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. J. Physiol. (Lond.). 246:255–275.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. J. Physiol. (Lond.). 269:441–515.

Gordon, A. M., A. F. Huxley, and F. J. Julian. 1966. Tension development in highly stretched muscle fibers. J. Physiol. (Lond.). 184:143–169.

Haskell, R. C., and F. D. Carlson. 1981. Quasi-elastic light-scattering studies of single skeletal muscle fibers. Biophys. J. 33:39–62.

Hill, A. V. 1953. The mechanics of active muscle. Proc. R. Soc. Lond. B Biol. Sci. 141:104–117.

Huxley, A. F., and R. Niedergerke. 1954. Structural changes in muscle during contraction. Interference microscopy of living muscle fibers. Nature (Lond.). 173:971–973.

Huxley, A. F., and L. D. Peachey. 1961. The maximum length for contraction in vertebrate striated muscle. J. Physiol. (Lond.). 156:150–165.

Huxley, H. E., and J. Hanson. 1954. Changes in the cross-striations of muscle during contraction and stretch, and their structural interpretation. Nature (Lond.). 173:973–976.

Iwazumi, T. 1979. A new field theory of muscle contraction. In Cross-Bridge Mechanism in Muscle Contraction. H. Sugi and G. H. Pollack, editors. University Park Press, Baltimore, MD. 611–632.

Iwazumi, T., and G. H. Pollack. 1979. On-line measurement of sarcomere length from diffraction patterns in muscle. IEEE Trans. Biomed. Eng. 26:86–93.

Julian, F. J., and D. L. Morgan. 1979a. Intersarcomere dynamics during fixed end tetanic contractions of frog muscle fibers. J. Physiol. (Lond.) 293:365–378.

Julian, F. J., and D. L. Morgan. 1979b. The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. J. Physiol. (Lond.). 293:379–392.

Julian, F. J., and R. L. Moss. 1980. Sarcomere length-tension relations of frog skinned muscle fibers at lengths above the optimum. J. Physiol. (Lond.). 304:529–539.
Julian, F. J., M. R. Sollins, and R. L. Moss. 1978. Sarcomere length nonuniformity in relation to tetanic responses of stretched skeletal muscle fibers. Proc. R. Soc. Lond. B Biol. Sci. 200:109–116.

Katz, B. 1939. The relation between force and speed in muscular contraction. J. Physiol. (Lond.). 96:45–64.

Lieber, R. L., and B. A. Lubell. 1983. Real-time data acquisition of diffraction spectra from single skeletal muscle fibers. Proc. Fall 1982 DECUSS symp. 9:219–225.

Lieber, R. L., R. J. Baskin, and Y. Yeh. 1983a. The effect of beam and fiber diameter on sarcomere length measurement in single fibers. Biophys. J. In press.

Lieber, R. L., K. P. Roos, B. A. Lubell, J. W. Cline, and R. J. Baskin. 1983b. High speed digital data acquisition of sarcomere lengths from isolated skeletal and cardiac muscle cells. IEEE Trans. Biomed. Eng. 30:50–57.

Morgan, D. L. 1978. Predictions of some effects on light diffraction patterns of muscles produced by areas with different sarcomere lengths. Biophys. J. 21:88a. (Abstr.)

Morgan, D. L., S. Mochon, and F. J. Julian. 1982. A quantitative model of intersarcomere dynamics during fixed-end contractions of single frog muscle fibers. Biophys. J. 39:189–196.

Rüdel, R., and F. Zite-Ferenczy. 1979a. Interpretation of light diffraction by cross-striated muscle as Bragg reflexion of light by the lattice of contractile proteins. J. Physiol. (Lond.). 290:317–320.

Rüdel, R., and F. Zite-Ferenczy. 1979b. Do laser diffraction studies on striated muscle indicate stepwise sarcomere shortening? Nature (Lond.). 278:573–575.

ter Keurs, H. E. D. J., T. Iwazumi, and G. H. Pollack. 1978. The sarcomere length-tension relation in skeletal muscle. J. Gen. Physiol. 72:565–592.

Yeh, Y., R. J. Baskin, R. L. Lieber, and K. P. Roos. 1980. Theory of light diffraction by single skeletal muscle fibers. Biophys. J. 29:509–522.