Mechanical transients initiated by ramp stretch and release to P_o in frog muscle fibers

G. A. CAVAGNA, M. MAZZANTI, N. C. HEGLUND, AND G. CITTERIO
Istituto di Fisiologia Umana, Università di Milano, and Centro di Studio per la Fisiologia del Lavoro Muscolare del CNR, 20133 Milano, Italy

CAVAGNA, G. A., M. MAZZANTI, N. C. HEGLUND, AND G. CITTERIO. *Mechanical transients initiated by ramp stretch and release to P_o in frog muscle fibers.* Am. J. Physiol. 251 (Cell Physiol. 20): C571–C579, 1986.—Single fibers from the tibialis muscle of *Rana temporaria* were subjected to ramp stretches during tetanic stimulation at a sarcomere length of ~2 μm. Immediately after the stretch, or after different time delays, the active fiber was released against a constant force equal to the isometric force (P_o) exerted immediately before the stretch. Four phases were detected after release: *1*) an elastic recoil of the fiber's undamped elements, 2) a transient rapid shortening, *3*) a marked reduction in the velocity of shortening (often to 0), and *4*) an apparently steady shortening (sometimes absent). Increasing the amplitude of the stretch from ~2 to 10% of the fiber rest length led to an increase in phase 2 shortening from ~5 to 10 nm per half-sarcomere. Phase 2 shortening increased further (up to 14 nm per half-sarcomere) if a time interval of 5–10 ms was left between the end of large ramp stretches and release to P_o. After 50- to 100-ms time intervals, shortening occurred in two steps of ~5 nm per half-sarcomere each. These findings suggest that phase 2 is due to charging, during and after the stretch, of a damped element, which can then shorten against P_o in at least two steps of ~5 nm/half sarcomere each.

muscle mechanics; muscle velocity transients; muscle stretch

IT HAS BEEN SHOWN that stretching an active muscle increases its ability to perform positive work during subsequent shortening (3, 5, 8, 13, 20). The elastic energy stored during the stretch can only account for part of the greater positive work done after the stretch. This discrepancy suggests that the contractile component itself is in some way "enhanced" by stretching (8). Subsequent experiments have shown that shortening of a previously stretched muscle could take place also against a constant force equal to or even greater than its maximal isometric force (P_o). At isotonic loads below P_o, the velocity of shortening was greater after a stretch, indicating that the force-velocity curve was shifted along the velocity axis: the shift was maximal when the load was near P_o, and it decreased rapidly to zero with decreasing load (5). Further experiments suggested that the isoforce velocity enhancement has a transient character. Rapid shortening against P_o occurred only over a limited distance, and afterward muscle length either remained constant or decreased at a much slower rate (6). The described shift in the force-velocity curve after a stretch was also observed in single fibers; however, no velocity transients were described (13). Also, bundles of two to three fibers appeared to shorten linearly immediately after a quick decrease in load, from a load above P_o, again without any transient character in the load-sustaining ability (20). Velocity transients are observed, however, when the force is suddenly reduced below P_o from a state of isometric contraction (12).

The length changes initiated in a single fiber by a ramp stretch followed by sudden isotonic release to P_o have been measured in this study to determine whether, after stretching, the force-velocity curve is obeyed instantaneously or only after some velocity transients. Measuring the shortening against P_o is a convenient way to measure the "enhancement" that can be attributed to a modification induced by stretching. If the force applied after stretching were greater than the isometric force, some of the effect may not take place. If the force were smaller, the effect of the previous stretch would be augmented by the energy-delivering mechanisms that are normally elicited when the force is reduced below P_o. Also, to observe the time course of the effect of previous stretching (hitherto studied only from the kinetics of the decay of the isometric tension with the muscle held active at the stretched length) (1, 13), the load steps were applied at different intervals after the end of the stretch.

To rule out the possibility that the enhancement due to stretching could simply be due to a more favorable filament overlap attained at the stretched length, the force applied after the stretch was set equal to the maximal isometric force developed at the optimal length, and the lengthening of the fiber was confined to the plateau region of the isometric force-length curve (from sarcomere length of 1.9–2.1 μm to sarcomere length of 2.1–2.3 μm). Care was taken to distinguish between the length change due to the elastic recoil of the undamped elements during the drop of the force and the length change taking place during subsequent rapid early shortening against a constant force. For this reason, we used an apparatus capable of achieving the large stretches necessary (up to 0.5 mm) to get the maximal enhancement and also capable of relatively fast load steps to P_o (~0.3 ms).

A preliminary account of this work has been presented to the Seventh International Conference on Comparative Physiology (10).

METHODS

Principle of the Method

The fiber was held in Ringer solution with one tendon

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attached to a force transducer and the other to a moving-coil ergometer. A transistor switch allowed the ergometer to be controlled by either a signal proportional to the ergometer position (position feedback) or a signal proportional to the force developed by the fiber (force feedback). An example of experimental tracings recorded during several contractions is given in Figs. 1 and 2. Two pairs of tracings were recorded during each contraction: the first pair gives the force developed by the fiber (top tracing) and the fiber length change (bottom tracing) on a slow time scale (500 μs/point); the second pair shows a shorter time interval of the same force and length changes on a 100-fold faster time scale (5 μs/point).

Initially, the length of the fiber was maintained in the position feedback mode. Two traces (slow records in Figs. 1 and 2) of a four-trace digital oscilloscope were triggered, and the fiber was tetanically stimulated isometrically (rise in the force in the slow records of Figs. 1 and 2). The fiber force was continuously measured by a microcomputer through an analog-to-digital (A/D) converter. When a force plateau was detected, the value of P_o was recorded, and a ramp of a preset amplitude and duration was sent to the ergometer via a digital-to-analog (D/A) converter, resulting in the linear lengthening of the fiber and the rise in the force above the isometric value. Immediately after lengthening, or after a preset time interval during which the fiber was held active at the stretched length, the other two traces of the oscilloscope were triggered (fast records in Figs. 1 and 2), a signal proportional to the isometric force (P_o) was sent from the microcomputer to the ergometer via a D/A converter, and the ergometer was switched to force feedback mode. This resulted in a sudden fall of the force to P_o and an abrupt elastic recoil of the fiber (best seen in the fast records of Figs. 1 and 2), followed by a slower shortening against P_o (slow length records of Figs. 1 and 2). After a preset interval of isotonic shortening, stimulation was stopped, and the fiber was brought back to its initial length under position feedback (fall of the force almost to 0 followed by some redevelopment of tension in Figs. 1 and 2).

In other experiments, the fiber was released, without previous stretching, from a state of isometric contraction to a force of ~0.9 the isometric value. These experiments were similar to those described above except that no ramp was sent to the ergometer; releases were made from fiber lengths equal to the initial and final lengths reached during stretching in the experiments described above.

The slow length records were used to describe all of the early isotonic shortening. The fast records were used to measure the starting length of the early isotonic shortening (not clearly seen on the slow length record).

Apparatus

Trough. The fiber was mounted in a trough made of four walls of Plexiglas and a glass bottom surrounded by thick walls of stainless steel, except for one end, which was all Plexiglas and served as a support for the force transducer. One end of the fiber was attached to a 100-μm-diameter glass hook that protruded from the force transducer through a hole in the end of the trough. The other end of the fiber was attached to another glass hook fixed to a 1-mm-diameter needle, which passed through a hole in the other end of the trough. The holes in either end of the trough were greased to prevent Ringer from escaping. Two platinum electrodes (25 mm long and 2.5 mm wide) slid into vertical grooves in the ends of the trough, flanking the fiber. The distance between the electrodes was ~3 mm.

At the beginning of each experiment, the trough with the fiber was mounted in a channel in a stainless steel water jacket, and the needle on the free end of the fiber was attached with shellac to a stainless steel tube protruding from the ergometer. The distance from the trough to the ergometer could be changed, for rough presetting of the fiber length, by sliding the trough back and forth in the channel. The temperature of the trough could be lowered to the desired value by circulating fluid from a constant temperature bath (Churchill thermocirculator) through the water jacket. The temperature of the Ringer in proximity to the fiber was measured before each experiment by means of a thin thermocouple.

Ergometer. The ergometer was constructed from a moving-coil device (Ling 101) rigidly fixed to a concrete base. The maximal displacement of the coil was ±1.25 mm, but in the present experiments it was limited to 0.5 mm. Current was passed through the 3-Ω coil by a 60-W (12 V, 5 A) power operational amplifier (Torque Systems PA-111). The step response of the ergometer was improved by reducing the mass of the moving parts to 2.9 g; this allowed the fiber length change taking place during elastic recoil to be completed in 0.2–0.4 ms.

Position sensor. A position sensor was constructed from an infrared light-emitting diode attached to the moving coil of the ergometer, and a fixed lateral effect photodiode. The photodiode (United Detector Technology LSC/5D) had a rise time (10–90%) of 0.5 μs; the output of the photodiode was amplified ~800 times (Analog Devices 606). Calibration was made by measuring the coil displacement with a dial gauge; sensitivity was adjusted so that the output was ~1 μm/mV.

Force transducer. The force transducer and its driving circuit were constructed as described by Huxley and Lombardi (16) and Cambridge and Haines (4). A sensitivity of ~150 mV/mN and a resonant frequency of ~30 kHz were obtained. The drift of the force base line was practically eliminated by enclosing the whole system (ergometer, trough, water jacket, and force transducer) in a Plexiglas box through which air was slowly recirculated and dried.

Fibers. The fibers were dissected from the caput mediale (1st experiments) and from the caput laterale (later experiments) of musculus tibialis anterior of the frog (*Rana temporaria*) using a Zeiss stereomicroscope (DV4, with dark field illumination). Clips were attached to the tendons as described by Ford et al. (15); the tendon left between clip and fiber was the minimum possible: always <0.5 mm, sometimes ~0.2 mm. The length of the fiber was measured by means of a micrometer eyepiece fitted to a microscope (Zeiss, OPMi I, total magnification ×16) above the trough. At the end of the experiment, the trough and fiber were placed below another microscope

(×32 objective and ×25 micrometer eyepiece) to measure the width and thickness of the fiber, to calculate the cross-sectional area (15) and the average sarcomere length. The composition of the Ringer solution was (in mM); 115.5 NaCl, 2.0 KCl, 1.8 $CaCl_2$, 2.0 Na phosphate buffer, pH 7.0. The optimal length of the fiber (force plateau region) was determined before each experiment by plotting the isometric twitch force-length diagram; in the last experiments, the average sarcomere length of the muscle at rest was also measured by laser diffraction.

Stimulation. The stimulation consisted of alternating polarity square pulses (500-μs width), with an amplitude ~1.5 times threshold, and the frequency necessary to have a fused tetanus (20–25 Hz) at the temperature of the fiber (1.9–3.0°C). Voltage, duration, frequency, and number of pulses could be preset on a stimulator (F. Haer Pulsar 6bp, modified) driving a custom power stage.

The command signals driving the ergometer were provided by two D/A converters. The first D/A converter, operated from the computer keyboard, made it possible to shorten or lengthen the fiber in steps of ~2 μm; this was used to determine the isometric force-length diagram before each experiment or to give a resting length offset to the fiber. The second D/A converter was used to output the ramp stretching the fiber during the experiment. The velocity of stretching was 7 mm/s, corresponding to 1.0–1.4 fiber lengths/s.

Feedback loop. The ergometer was controlled by an operational amplifier that summed a command signal, a position or force feedback signal, and a velocity signal. The computer selected either position or force feedback by operating a CMOS transistor switch (Analog Devices AD7512DI, 300-ns switching time). In position control mode, the command signal was the sum of the outputs of the two D/A converters described above, a feedback signal from the photodiode position sensor, and velocity feedback from the differentiated photodiode signal. In the force control mode, the command signal was the sum of a D/A converter output (which was proportional to the isometric force developed by the fiber), the feedback from the force transducer, and velocity feedback from either the differentiated force signal or the differentiated position signal (the latter was preferred).

The position feedback could be adjusted once and then left unaltered throughout a series of experiments. The force feedback, on the contrary, was more critical and had to be adjusted during each experiment. Increasing the gain of the undifferentiated feedback led to oscillations of the ergometer; decreasing the gain resulted in an increase in the step duration and a drift in the force applied to the fiber during the period of isotonic contraction. A compromise was sought by trial and error.

Fiber force and length changes were recorded on a four-channel digital oscilloscope (Nicolet 4094); two channels had a sampling rate of 500 μs/point for slow records; the other two channels had a sampling rate of 5 μs/point for fast records. The four records taken for each tetanus were stored on disk (Nicolet XF-44). A microcomputer (Cromemco Z-2) controlled the timing and most other aspects of the experiments, including the oscilloscope memory and data acquisition, triggering the stimulator and oscilloscope channels, determining and recording the value of the isometric force plateau, controlling the feedback and sending the appropriate length or force levels to the ergometer, reading the data from the oscilloscope memory, and doing the required measurements and calculations on the data.

The length values, required to measure the amplitude of the isotonic shortening, were read directly from the cursor position on the oscilloscope tracing. The cursor was positioned on the fast tracing at the end of the elastic recoil to calculate the beginning of the early isotonic shortening. Because the end of the elastic recoil is disturbed by oscillations (Figs. 1 and 2), the length was measured by backward extrapolation of the tracing. The amplitude of the early isotonic shortening was calculated from the reading of the fast length tracing at the end of the elastic recoil minus the reading of the slow length tracing at the end of the rapid shortening (see inset of Fig. 1).

Critical Evaluation of Experimental Tracings

The measure of sarcomeres shortening during phase 2 (ordinates of Figs. 3 and 5) is subjected to two possible sources of error: *1*) a reading error made in measuring the starting length of phase 2 through the vibrations on the fast length tracings (see Figs. 1 and 2), and *2*) a spurious shortening due not to the sarcomeres but to stress-relaxation of viscoelastic structures outside the sarcomeres (so called end compliance: tendons, connections, and force transducer). These two causes of error are discussed below.

The interrupted lines drawn through the fast length and force tracings in Figs. 1 and 2 indicate the procedure followed in determining the beginning of phase 2 shortening after the recoil of the undamped elastic elements taking place during phase 1. It can be seen that the determination of the initial length is based on the backward extrapolation of both the length and force fast tracings. This procedure gave consistent results. In fact, successive readings on the same pair of tracings were always very similar (often identical), and the data points of the mirror experiment of Fig. 1 (numbered squares in Fig. 3) show little scatter, indicating that both the reading error and the physiological variability were small. The reproducibility of the results, however, does not rule out the possibility of a systematic error. Because the velocity of phase 2 shortening is often maximal just at the beginning of the isotonic shortening (disturbed by the oscillations), it is likely that the backward extrapolation (based mainly on the subsequent less-steep part of the tracing with smaller oscillations) would yield a smaller isotonic shortening and, as a consequence, a larger elastic recoil (phase 1). Even in the worst case, however, the extrapolated length could not be increased by >1 nm/half sarcomere (hs) and still be consistent with the subsequent trend of the fast length tracing.

Stress-relaxation of the structures outside the sarcomeres does not seem to be large enough to modify the conclusions reached in the present study for the following reason. The overall compliance (sarcomeres + end compliance) has been measured in this study from the force

and the length changes taking place during the recoil of the undamped elastic elements (phase 1). Assuming a linear relationship between force and length of the elastic elements, the compliance is usually expressed as the amount of shortening (y_o) necessary to make the force fall from P_o to zero. The average value of y_o measured in this work was ~10 nm/hs, i.e., 6 nm/hs greater than the currently accepted value for the undamped recoil of the sarcomeres (15). In the four experiments illustrated in Fig. 4, the overall compliance changed appreciably (possibly due to different amounts of tendons): from the upper to the bottom row, y_o was 8.2, 8.6, 11.5, and 13.2 nm/hs, respectively. This means that the end compliance ranged from 4.2 to 9.2 nm/hs. A 100% change in the end compliance should involve a corresponding change in the stress-relaxation resulting from it. Yet the tracings in Fig. 4 and the corresponding data points in Fig. 5 show that the amplitude of the isotonic velocity transients is very similar in the four fibers studied. This suggests that the records are affected mainly by length changes taking place within the sarcomeres and that stress-relaxation of the tendons (i.e., of the largest component of the end compliance) has a negligible effect.

RESULTS

Figures 1 and 2 give the results of one experiment. In general, it can be seen that when the force exerted by the active fiber at the end of a stretch is suddenly reduced to a value equal to the isometric force developed before the stretch (P_o), the muscle shortens in four phases. Phase 1 is an elastic recoil occurring simultaneously with the fall in the force. Phase 2 is a rapid isotonic shortening. Phase 3 is an inflection in the length-time curve as a result of an extreme reduction, or even a reversal, in the velocity of shortening. Phase 4 is a slow isotonic shortening, which is of longer duration and much lower velocity than phase 2.

If the force applied after a stretch is greater than P_o, the velocity of shortening during phase 4 may decrease to zero or even become negative, although some shortening during phase 2 always occurs. If the force applied after a stretch is smaller than P_o, the velocity of shortening during phase 4 increases, and the distinction between phase 2 and phase 4 becomes less sharp (i.e., phase 3 becomes less distinguishable).

The rapid early shortening (phase 2) is studied as a function of the amplitude of the stretch and of the time interval during which the fiber is held active at the stretched length. In some experiments, the muscle was also released from a state of isometric contraction by applying a force of 0.8–0.9 P_o (lower right tracings of Fig. 2).

Effect of Amplitude of Stretch

Previous studies have shown that the enhancement in the energy delivered by a muscle during shortening after a prestretch increases, within limits, with the velocity and the amplitude of the stretch (7). In this investigation, the fibers were stretched with ramps of velocity great enough to obtain the maximal enhancement; the effect of stretch amplitudes ranging from ~1.3 to 10% resting length (L_o) was studied. Results similar to those described below have been obtained in six fibers (Fig. 3).

In the tracings of Fig. 1, the amplitude of the stretch was ~2 (left tracing), 5 (middle tracing), and 10% L_o (right tracing). The slow force tracings show that during

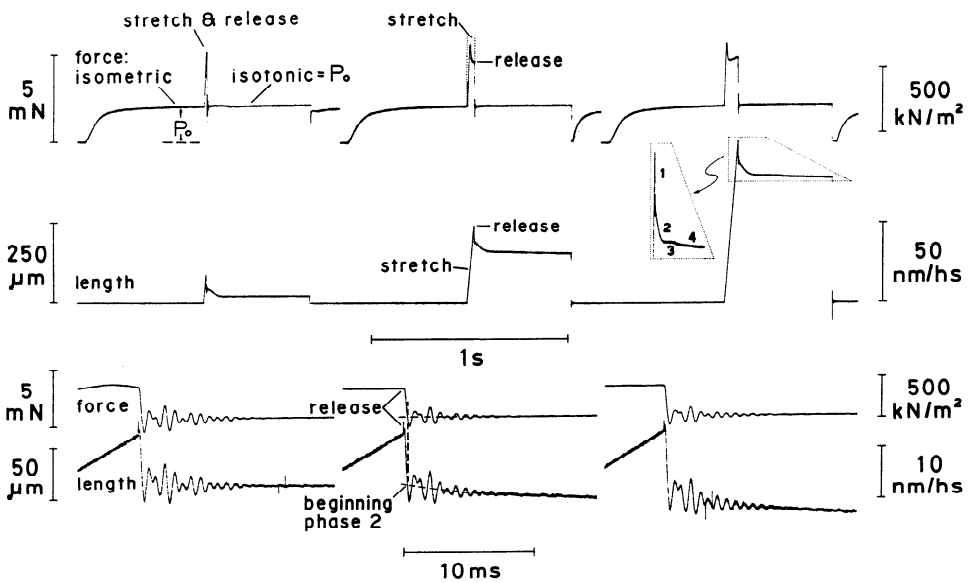

FIG. 1. Records from digital oscilloscope. The 3 sets of tracings show effect of ramp stretches of increasing amplitude (~0.1, 0.25, and 0.5 mm at 7 mm/s) on velocity transients taking place when force is suddenly reduced (release), immediately after stretching, to isometric value developed before stretching (P_o). Two *upper traces* show force (above) and length changes (below) on a slower time scale. Two *lower traces* show a shorter time interval (just before and after elastic recoil) of the same force and length changes on a 100-fold faster time scale. Subsequent phases of shortening (1–4, see text) are better seen in vertically expanded *inset* near slower length tracings. Extrapolating procedure followed to determine initial length, at beginning of phase 2 shortening, is indicated by *interrupted lines* through the "fast" force and length tracings. Experiment of 15 Oct., 1984: length of fiber, 5 mm (caput laterale); cross section, 7,350 μm^2; initial sarcomere length (before stretch), 2.03 μm; temperature, +1.9°C.

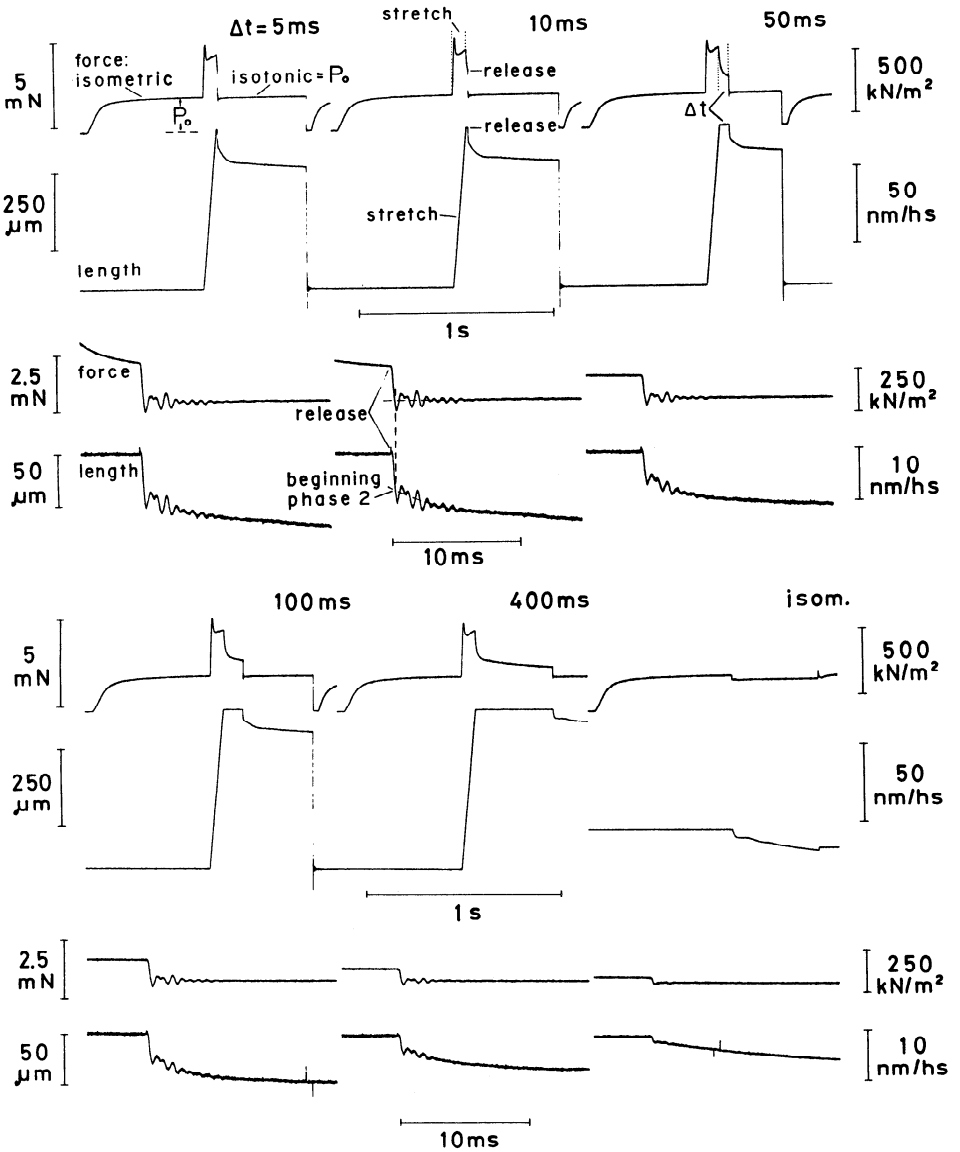

FIG. 2. As Fig. 1, but with the same amplitude of ramp stretch (0.5 mm at 7 mm/s) and indicated time intervals between end of stretch and release, to show time course of effect of previous stretching. Set of tracings in *bottom right corner* shows a release from a state of isometric contraction, at initial length, against a force 11% smaller than isometric one. Same experiment as Fig. 1.

stretching, after an initial sharp rise, the force "gives" and then increases again, with the result that the force attained at the end of the stretch is roughly independent of the amplitude of the stretch (13). The slow length tracings show that the amplitude of the early isotonic shortening increases progressively with the amplitude of the preceding stretch (Fig. 3). The interrupted line in Fig. 3 indicates the average amplitude of the early isotonic shortening taking place after release from isometric contractions at the initial length.

Effect of a Time Interval Between Stretch and Release

The tracings in Fig. 2 (same fiber as Fig. 1) were obtained with stretches of 10% L_o amplitude, followed by different time intervals between the end of the stretch and the release against P_o. The slow length tracings of all the experiments are expanded in Fig. 4 to show more clearly the shortening patterns taking place during phase 2 after the different time intervals. It can be seen that the amplitude of the early isotonic shortening attains a maximum (11–14 nm/hs) after a time interval of 5–10 ms. If the time interval is increased further, from 50 to 100 ms, an inflection of progressively greater duration divides the early isotonic shortening into two steps of ~5 nm/hs each. The first step is faster than the second. A step of ~5 nm/hs is recorded also from a state of isometric contraction (interrupted tracings in Fig. 4).

The isotonic shortening taking place during phase 2 was measured, for all the fibers shown in Fig. 4, as the amplitude of the greatest clearly recognizable step seen in the tracings and was plotted in Fig. 5 as a function of the time interval between the end of the stretch and the release. It can be seen that for delays of 50–200 ms, phase 2 shortening takes place in steps of either 5 or 10 nm/hs. For delays >200 ms, only the amplitude of the first step after release has been plotted in Fig. 5, even if the second step of phase 2 is often still evident; the amplitude of the second step has not been plotted because it is not possible to distinguish its end from the subsequent continuous shortening taking place during phase 4. The total amount of shortening during phase 2 after intervals >200 ms is therefore greater than that shown in Fig. 5. The

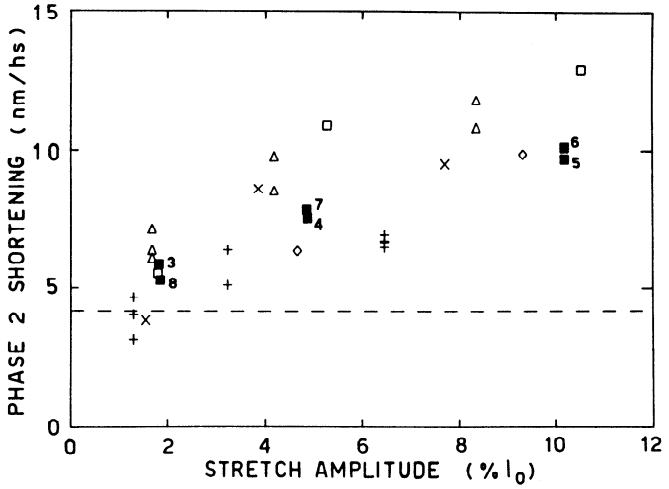

FIG. 3. Effect of stretch amplitude [*abscissa*, % of resting length (L_o)] on amount of early isotonic shortening (*ordinate*, nanometers per half-sarcomere). *Solid squares* refer to experiment shown in Fig. 1; numbers near *symbols* give order of successive stimulations. During tetanuses 1 and 2, fiber was released from a state of isometric contraction. Other *symbols* refer to different experiments as follows: *crosses*, expt. of 31 Jan., 1984 [fiber length, 7.7 mm (caput mediale); cross section, 9,540 μm^2; sarcomere length, 2.03 μm; temperature, 2.0°C]; ×'s, expt. of 4 May, 1984 [fiber length, 6.5 mm (caput mediale); cross section, 11,940 μm^2; sarcomere length, 1.95 μm; temperature, 2.65°C]; *diamonds*, expt. of 16 May, 1984 [fiber length, 5.4 mm (caput mediale); cross section, 10,890 μm^2; sarcomere length, 2.03 μm; temperature, 2.7°C]; *triangles*, expt. of 3 Oct., 1984 [fiber length, 6.0 mm (caput mediale); cross section 13,200 μm^2, sarcomere length, 2.03 μm; temperature, 2.3°C]; *open squares*, expt. of 30 Oct., 1984 [fiber length, 4.7 mm (caput laterale); cross section, 7,010 μm^2; sarcomere length, 2.08 μm; temperature, 2.0°C]. *Interrupted line* indicates average phase 2 shortening after release from a state of isometric contraction at resting length.

interrupted line in Fig. 5 indicates the average phase 2 shortening taking place after release from an isometric contraction at the initial length.

Effect of a Step Stretch

In some experiments, a step stretch (0.8–1.4% L_o, completed in ~1 ms) was given instead of a ramp stretch. One of these experiments is shown in Fig. 6. The amplitude of the early isotonic shortening is negligible when the fiber is released immediately after the step stretch and attains a maximum after a time interval of 30 ms. In the experiment shown in Fig. 6, the amplitude of the stretch (1.1% L_o) was just below that required to effect "give" in the force. In spite of the high force attained, however, the amplitude of the early isotonic shortening (5–7 nm/hs) is much less than after ramp stretches, which were prolonged well beyond give. A similar amplitude of the early isotonic shortening was also measured after the larger step stretches.

Oscillations in the length are particularly evident when release takes place 5–10 ms after the step stretch. These oscillations are similar to those shown by Armstrong et al. (2) and may be due to the mechanism described by Pringle (18) and Ruegg et al. (19).

DISCUSSION

Relation to Earlier Observations

The present results indicate that after stretching a contracting muscle, positive work is done during *1*) the recoil of the muscle's undamped elastic elements (phase 1 in Fig. 1), which is not discussed in this paper because of the unknown elastic recoil of structures outside the sarcomeres (tendons, connections, and force transducer); *2*) a period of rapid isotonic shortening (phase 2 in Fig. 1, which will be discussed below); and *3*) a period of much slower isotonic shortening (phase 4 in Fig. 1, which is only incidentally discussed here). In spite of these clear-cut divisions, it is not easy to isolate experimentally the three mechanisms. It is likely that most of the existing experimental evidence of muscle enhancement due to a prestretch results from a combination of all three. For example: shortening during phase 2 would be underestimated and shortening during phase 1 would be overestimated if the time resolution of the apparatus were too small; in fact shortening at the beginning of phase 2 takes place with a velocity so high that it can easily be considered to be the end of phase 1. This possibility of error is clearly shown by the difference between the position of the arrow and the apparent beginning of phase 2 shortening in some of the slow length tracings of Fig. 4. For the same reason, most force-shortening curves used in the past to describe the apparent elastic behavior of muscle include shortening during both phases 1 and 2 (e.g., 5, 7). In addition, the described shift in the force-velocity of shortening relationship due to stretch may include shortening during phases 2 and 4 (5, 13, 20).

Some investigators have not obtained the same results. Edman et al. (13) found no shift of the force-velocity relation below sarcomere length of 2.3 μm, but they probably measured only shortening during phase 4 (which is enhanced at high sarcomere lengths) and not shortening during phase 2; length-time tracings were not reported. Sugi and Tsuchiya (20) found no velocity transients after a quick decrease in the load applied during continuous isotonic lengthening. This led them to conclude that even rapidly lengthening fibers can start to shorten with a constant velocity immediately after a step decrease in load, in contrast to the response of isometrically contracting fibers to a quick decrease in load (12). It is possible that in the experiments of Sugi and Tsuchiya the amplitude and the velocity of the isotonic stretches in the fiber bundles were not sufficient to cause a clearly detectable phase 2 shortening.

Results consistent with those described in this paper have been obtained in similar experiments on whole muscle: a transient isotonic shortening against P_o equivalent to ~4 nm/hs has been measured in frog sartorius after a 7% L_o stretch [Fig. 1 of Cavagna et al. (6)], and, more recently, an increase in the work done against P_o after stretches of increasing amplitude has been described in experiments on frog semitendinosus (9).

Conclusions That Can Be Drawn from the Experimental Results

Assuming that the shortening measured during phase 2 is due to shortening of the sarcomeres (see discussion of this point in METHODS) and is proportional to the length change of each half-sarcomere, i.e., nonuniformity

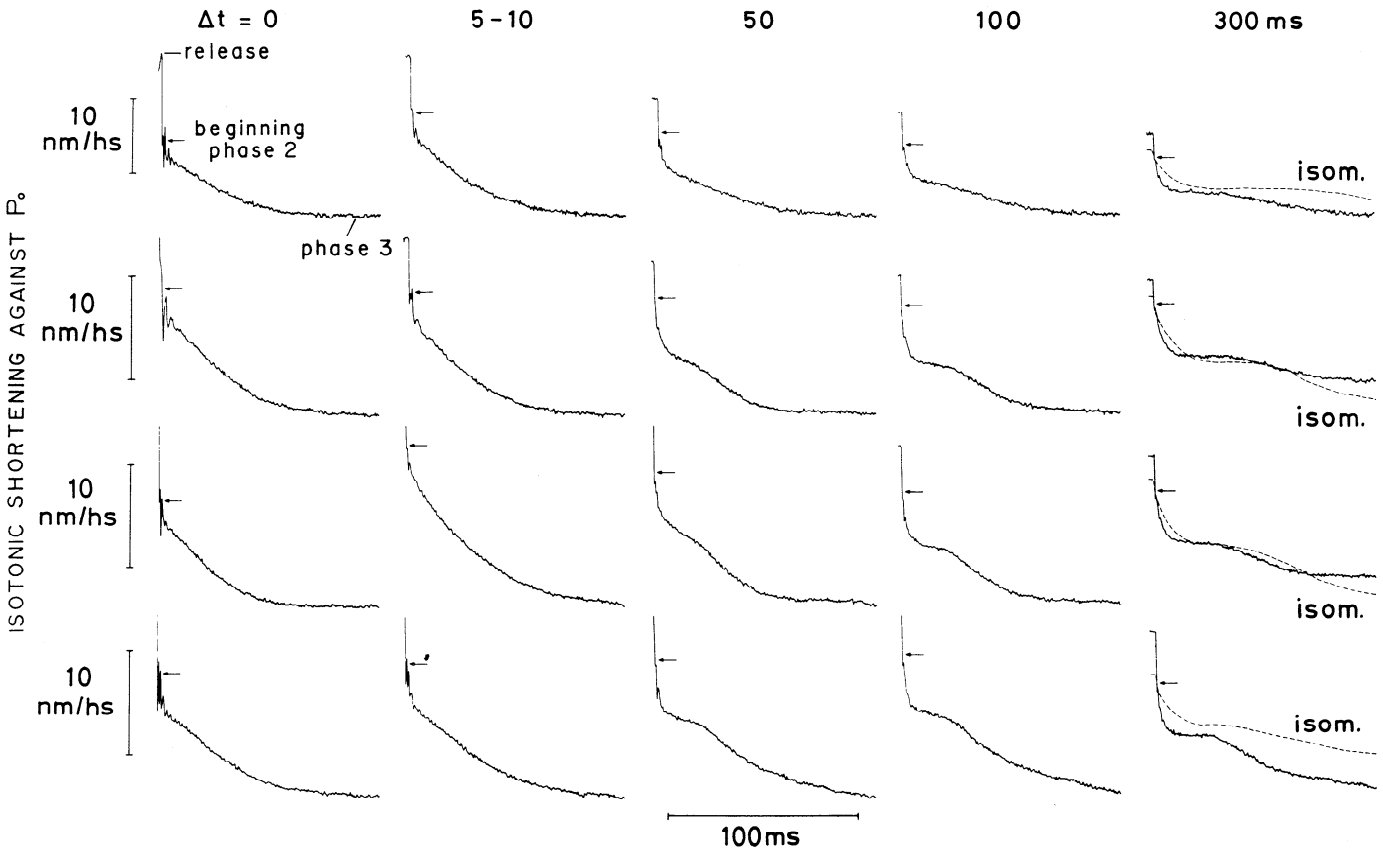

FIG. 4. Expanded length-time tracings to show (in greater detail than in Fig. 2) changes in phase 2 shortening that occur after indicated time intervals between end of stretch and release against P_o. *Arrows* indicate end of elastic recoil. *Interrupted tracings* refer to releases from a state of isometric contraction and are matched at end of elastic recoil with those obtained after a 300-ms time interval. From *above*: expt. of 15 Oct., 1984 (same as Figs. 1 and 2); expt. of 11 April, 1984 [fiber length, 6.5 mm (caput mediale); cross section, 12,500 μm^2; sarcomere length, 2.04 μm; temperature, 2.6°C]; expt. of 18 April, 1984 [fiber length, 6.4 mm (caput mediale); cross section, 15,600 μm^2; sarcomere length, 2.07 μm; temperature, 2.1°C]; expt. of 3 May, 1984 [fiber length, 6.7 mm (caput mediale); cross section, 9,800 μm^2; sarcomere length, 2.15 μm; temperature, 2.6°C].

of sarcomere length is negligible at lengths below 2.3 μm (11), the present results show the following. *1*) An energy storage and recovery mechanism exists in active muscle that is distinct from storage and release of mechanical energy by the undamped elastic elements. *2*) This mechanism seems to involve a damped structure within the sarcomeres that is charged during and after stretching. This charging occurs mainly during large ramp stretches and mainly after small step stretches. The release of energy after a ramp stretch attains a value that is about two times greater than that attained after a step stretch, in spite of the fact that the force reached with both procedures is about equal. *3*) The mechanical energy released by this mechanism increases with stretch amplitude up to filament sliding distances (e.g., 100 nm/hs) that exceed the range over which a cross bridge is thought to remain attached to actin during stretching [11–12 nm according to Flitney and Hirst (14)]. *4*) Shortening of the charged damped element against P_o seems to occur in at least two steps of ~5 nm/hs each.

Interpretation of the Experimental Results

Phase 2. To explain the tension transients that follow an abrupt length change in an isometrically contracting fiber, Huxley and Simmons (17) and Ford et al. (15) assumed the existence of a damped system within the cross bridges, which would shorten quickly after a step shortening and lengthen more slowly after a step stretch. The present data suggest that this last process is reversible and that it can accommodate a displacement greater than one would infer from experiments made using small step stretches.

If one assumes that the damped element responsible for phase 2 shortening resides within the cross bridges, then it is necessary to admit that during large fast ramp stretches the cross bridges can remain tense while they move relative to the thin filament over the large sliding distances mentioned above.

This extent of movement and the time required for it seem to be necessary to attain the maximal storage of mechanical energy by the damped element: in fact, when the same forces are attained during a step stretch, the amount of energy stored is much less. At the end of an "instantaneous" step stretch, only the undamped element of the cross bridges is charged, and the energy available for subsequent transfer to the damped element

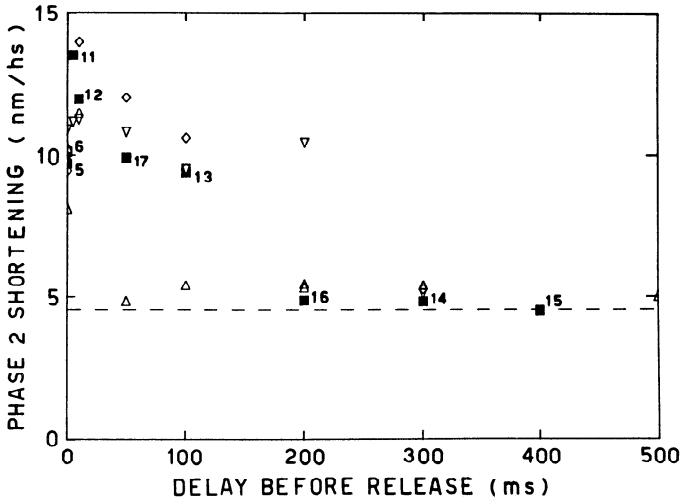

FIG. 5. Effect of a time interval between end of a ramp stretch and release against P_o (*abscissa*, ms) on amount of early isotonic shortening (*ordinate*, nanometers per half-sarcomere). *Solid squares* refer to experiment shown in Figs. 1 and 2; numbers near *symbols* indicate order of successive stimulations. During tetanuses 9 and 10, fiber was released from a state of isometric contraction. Other *symbols* refer to experiments shown in Fig. 4 as follows: *inverted triangles*, expt. of 11 April, 1984; *diamonds*, expt. of 18 April, 1984; *triangles*, expt. of 3 May, 1984. *Interrupted line* indicates average phase 2 shortening after release from a state of isometric contraction at resting length.

is therefore limited to the energy that is stored in the undamped element during stretching. In our experiments the maximal shortening during phase 2 occurred 5–10 ms after the end of large stretching ramps (10% of L_o, lasting 70 ms). Possibly this lag time is necessary to complete charging the damped element at the expense of the undamped element within the strained cross bridges. However, as mentioned above, most of the charging process appears to take place during, not after, the stretching ramp.

Huxley and Simmons (17) proposed that shortening of the damped element in the cross bridge takes place, when the force is reduced below P_o, in "a small number of steps ... from one to the next of a series of stable positions with progressively lower potential energy." Transfer from one position to the next would be faster the greater the difference between potential energy of the position and tension of the undamped element of the bridge. This proposal is compatible with the shape of some of the tracings in Fig. 4. In fact, of the two steps taking place after 50- to 100-ms time intervals, the first is faster than the second (as expected), and the step taking place 300 ms after the end of stretching is faster than that from a state of isometric contraction due to the greater relative fall in the force (from ~1.3 P_o to P_o in the first case and from P_o to 0.90–0.95 P_o in the second case).

The above interpretation of the tracings of Fig. 4, however, is based on the assumption that the length changes of the sarcomeres are proportional to the length changes of the individual cross bridges, which would be true only if the bridges were all in the same state. For example, the transient response recorded immediately after a ramp stretch, or after a 5- to 10-ms delay, does not show any sign of stepwise shortening, suggesting a continuous movement of the cross bridges or, more likely, an asynchronous stepwise shortening of a nonuniform population of cross bridges.

Phase 4. The apparent similarity between the isotonic velocity transients recorded after release from an isometric contraction and after stretching may make one think that the mechanisms responsible for the four phases (inset of Fig. 1) are the same in both cases. Indeed this seems to be true for phase 1, and, if the above interpretation is correct, it may also be true for phase 2. Here the similarity ends, however. We have no reasons to think that the mechanism responsible for the steady isotonic shortening observed after stretching (phase 4 in Fig. 1) is an enhanced version of the mechanism responsible for the steady isotonic shortening observed after release from an isometric contraction. It is worth remembering, in this respect, that shortening during phase 4 increases, after stretching, with the average sarcomere length, i.e., with the possibility of sarcomere length nonuniformity. Furthermore, after the longest "end of stretch-release" intervals, the separation between phase 4 and the second slower step of phase 2 becomes difficult to make (Fig. 4). It is likely that, after these intervals, most of the slower shortening is due to a residual of phase 2 rather than to phase 4. This must be kept in

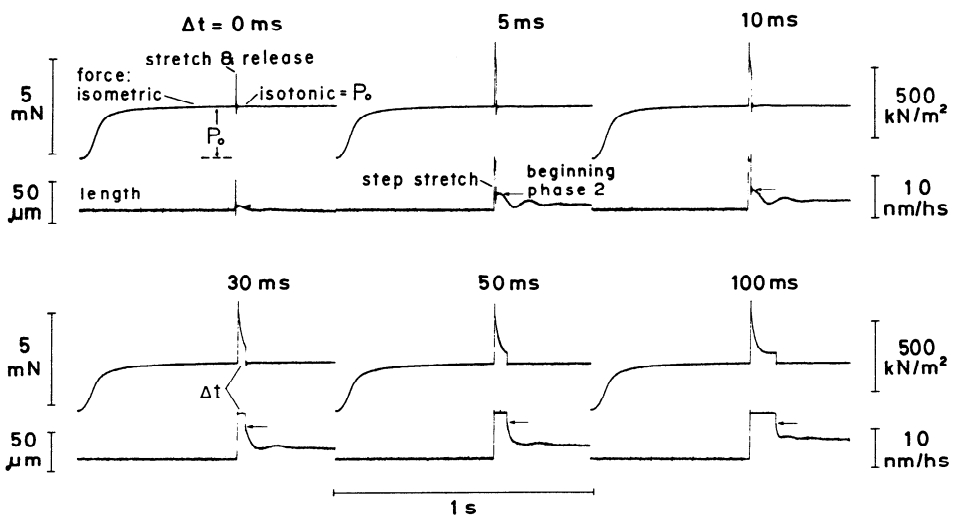

FIG. 6. Effect of a time interval between end of a step stretch and release against P_o. End of elastic recoil is indicated by *arrows*. Amplitude of early isotonic shortening (vertical distance between *arrow* and first minimum of shortening curve) attains a maximum after a time interval of ~30 ms (instead of 5–10 ms as in Fig. 2). Expt. of 19 Nov., 1984: fiber length, 5.3 mm (caput laterale); cross section, 7,570 μm^2; sarcomere length, 2.15 μm; temperature, 2.0°C.

mind when considering the data plotted in Fig. 5; as stated in RESULTS, these data refer only to the amplitude of the greatest clearly measurable step of phase 2. After long intervals, the total amount of shortening taking place during phase 2 is greater than that plotted in Fig. 5.

This work was supported in part by the Ministry of Education of Italy.

Current address of N. C. Heglund: Harvard University Biomechanics Laboratory and The Museum of Comparative Zoology, Oxford Street, Cambridge, MA 02138.

Received 30 September 1985; accepted in final form 14 May 1986.

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