

The Effect of Shortening on the Time-Course of Active State Decay

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ABSTRACT The active state describes the force developed in a muscle when the contractile elements are neither lengthening nor shortening. Recently it was suggested that perturbations used to measure the active state also alter the time-course of the active state. The present research was undertaken to assess quantitatively the effect of two such perturbations, isotonic shortening and quick release, on the active state in frog sartorius muscle. Methods were developed which allowed the determination of active state points following periods of controlled isotonic shortening or quick release early in the contraction cycle. All experiments were carried out within the plateau region of the length-tension curve. Both isotonic shortening and quick release altered the active state decay. The active state force decreased as the extent of shortening or release was increased. For each 0.1 mm of isotonic shortening there was a 2% decrease in active state force. Quick release produced a larger decrement. From this data we conclude that the time-course of active state can be measured only in relative terms because it is altered by the motion which takes place in the contractile machine while the active state is being measured. This finding helps to resolve paradoxes in the literature relating to the time-course of the active state, calculated and experimentally determined isometric tetanic myograms, and the heat of shortening.

INTRODUCTION

The active state concept has been widely employed in muscle mechanics since it was defined by Hill (8) as the force which the contractile elements could bear if they were neither lengthening nor shortening. The time-course of the active state force has been measured by techniques in which a variety of manipulations are used, such as quick and controlled stretches and releases. These manipulations were designed to render the contractile element of the muscle motionless, if only for an instant, so that an active state value for a given moment could be determined. All methods used for determining the active state time-course were efforts to eliminate the effects of the series elastic component on the force generated by the muscle.

Recently alterations in the active state have been attributed to the manipulations used to measure it. Ritchie (13), Ritchie and Wilkie (14), Edman and Kiessling (4), and Jewell and Wilkie (11) suggested that the active state changed as a function of sarcomere length in frog sartorius muscle. Edman (3) and Edman and Kiessling (4) quantitated the change in active state associated with a change in sarcomere length in single frog semitendinosus fibers. Edman and Kiessling (5) also discovered that the active state was decreased during relaxation by the process of isotonic shortening, independent of active state changes brought about by sarcomere length alterations.

However, a number of questions remain unanswered regarding the effects of shortening: (a) Will perturbations introduced early in the twitch alter the active state? (b) Is there a difference between quick release and isotonic shortening in producing this effect? (c) What is the relationship between the extent of shortening and the active state decrease? It is the objective of these studies to answer the questions raised. A method was developed in which the effects of movement (isotonic shortening or quick release) on active state decay could be assessed, independent of changes in sarcomere length. Our results indicate that isotonic shortening and quick release during the period of twitch contraction decrease the active state force of that twitch. Quick release produces a greater decrease in active state force than does isotonic shortening.

R A T I O N A L E

Range of Muscle Lengths Over Which Active State Is Constant

To evaluate the effect of the shortening process or quick release on active state decay, the effect of a passive change in contractile element length on the active state must be eliminated or negligible. For the reasons outlined below we assume the effects of sarcomere length on the active state can be ignored when the muscle shortened or was released over a range of lengths where isometric twitches are superimposable despite a change in muscle rest length. The time-course of the isometric twitch is determined by the stress-strain properties of the series elastic element and the time-dependent force-velocity properties of the contractile component. The stress-strain properties of the series elastic component do not change appreciably as a function of muscle length from 30% below to 12% above the muscle rest length, L_0 (10). The time-dependent force-velocity properties of a muscle also remain constant if the muscle is in the plateau region of filament overlap and if the intensity and time-course of active state are constant (6). If the muscle moves out of the plateau range of filament overlap, or the active state changes, the force-velocity relationships within the muscle change. As a result the time-course of the isometric twitch is correspondingly altered. Only if the time-course of the

active state is constant over the entire plateau region of the filament overlap will isometric twitches superimpose despite a change in muscle length. Thus a region where twitches are congruent, despite a slight change in rest length, may be taken as a region where the active state remains constant. The muscle should be able to move within this region without a change in active state, unless the movement itself alters the active state.

Isotonic-Isometric Method for Measuring Active State Decay in a Twitch

Active state decay curves are generated following a precisely determined extent of isotonic shortening by the procedure illustrated in Fig. 1, I. The

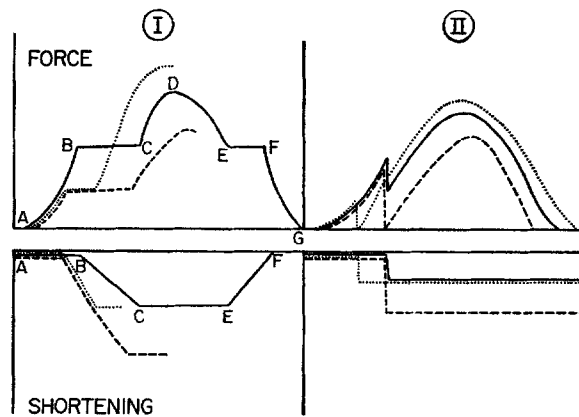


FIGURE 1. Diagram of isotonic-isometric (I) and quick-release (II) contraction. In the isotonic-isometric protocol tension development begins at *A* and proceeds until load is lifted at *B*. Shortening occurs until lever hits the stop at *C*. Isometric tension development against the shortening stop occurs from *C* to *E*. *D* is the peak of tension redevelopment. The load is lowered from *E* to *F*. The isometric phase of relaxation occurs from *F* to *G*. The dotted and dashed lines indicate smaller loads lifted through the same and a longer distance. In each case the time-course of tension redevelopment is a function of the load lifted and the shortening distance. Displacement is indicated in the lower portion of I. In the quick-release protocol the muscle is allowed to develop tension and is then released (solid line). The time of release (dotted lines) or the extent (dashed line) can be varied, altering the time-course of the subsequent force redevelopment. Displacement records are indicated in the lower portion of II.

system is set for an afterloaded, isotonic contraction. After the stimulus the muscle develops force until the afterload is lifted (*A* to *B*). Isotonic shortening ensues (*B* to *C*). The shortening phase of the contraction is terminated when the lever hits a micrometer stop positioned a few tenths of a millimeter below the lever in the base line position (*C*). Shortening stops are adjusted to restrict shortening to the range for twitch congruence. The muscle then continues to contract isometrically against the stop. The isometric phase of con-

traction reaches a peak (*D*) and isometric relaxation occurs (*D* to *E*), followed by isotonic relaxation (*E* to *F*). The peak of force redevelopment, *D*, is considered to be a point on the twitch active state decay curve since the contractile element is stationary when the rate of change for force (dP/dt where *P* is force and *t* is time) is zero (3). Different points on the active state decay curve can be obtained by altering the isotonic phase of the contraction. The load lifted during isotonic contraction or the distance of isotonic shortening can be altered as long as the constraint of remaining within the range of twitch congruence is adhered to (Fig. 1, I). Either change in the isotonic phase of contraction will result in the muscle hitting the stop at a different time. The time-course of isometric force redevelopment will consequently be altered and a different active state decay point revealed. All active state points generated by slight alterations in the isotonic phase of the contraction should lie on the same active state decay curve unless the isotonic phase of contraction alters the active state.

Quick-Release Assessment of the Active State

Jewell and Wilkie (11) determined that the active state decay assessed by quick releases of 0.5 mm at varying times during the twitch was different from the decay curve obtained by releases of 2.0 mm. The discrepancy between curves could be explained if the final contractile element lengths resulting from different releases were associated with inherent differences in active state at those contractile element lengths. Another explanation is that the active state was decreased in proportion to the extent of release. The present experiments were designed to test this latter possibility.

If all releases are kept within the length range of twitch congruence, different amounts of release should give differing points on the same active state curve, unless the release causes the active state to change. Releases ranging from 0.1 to 1.0 mm cause the muscle to have varying amounts of tension in the contractile elements following the release (Fig. 1, II). According to the three-component mechanical model of muscle and the Hill formulation of the active state, varying levels of postrelease tension in the contractile component will not cause differences in the active state decay measurements. As long as the release is great enough to discharge tension in the contractile component below the level of active state tension, the contractile element redevelops tension following the quick release until tension is equal to active state tension. At that instant, the isometric redevelopment reaches its peak, the contractile element ceases motion, and the active state decay point is revealed. If the active state is independent of the perturbation used to measure it, varying the extent and time of release will produce a series of isometric redevelopment peaks which fall on one active state decay curve. Conversely, if a number of

active state decay curves are produced which depend on the extent of release (providing the extent of release falls within the range of twitch congruence) then the perturbation alters the active state decay.

METHODS

Experimental Preparation

Sartorius muscles of *Rana pipiens* (<1 mm thick) were dissected in a chamber of oxygenated frog Ringer solution (NaCl, 120 mM; KCl, 2.5 mM; CaCl₂, 1.8 mM; glucose, 10 mM; NaH₂PO₄, 0.5 mM; Na₂HPO₄, 1.5 mM; tubocurarine chloride, 10 mg/liter, pH 7.4). A cylindrical clip manufactured to fit the force transducer used in these experiments was crimped directly onto the distal tendon of the muscle. A piece of 0.28 inch stainless steel tubing which was used to link the muscle to the displacement lever was tied to a piece of pelvic bone by 4-0 silk suture. Body rest length was measured (rest lengths for these muscles varied from 2.85 to 3.10 cm, averaging 3.0 cm) and the muscle was then removed and mounted in the muscle chamber. The length was adjusted to body rest length and the muscle was allowed to equilibrate in oxygenated Ringer solution at 4°C for 8 hr before stimulation was begun.

Mechanics Apparatus

The equipment used for mechanical studies was modified slightly from that described by McCrorey et al. (12). The capacitance transducer used to measure tension (15) was built into the bottom of the muscle chamber. The stiff cantilever spring capacitor plate had a stainless steel hook soldered to its outer face which extended up into the muscle chamber through a small channel drilled into the base of the chamber. The channel afforded a clearance of 0.008 inch on all sides of the hook. This arrangement created a frictionless connection between the muscle clip and the force transducer. Liquid did not leak into the transducer. The natural frequency of the force transducer was 500 Hz when the chamber was filled with fluid. The derivatives of force and shortening were measured by active electronic differentiators. A solenoid-activated quick-release lever, armed with a spring on a separate movable stand, could be positioned to support the displacement lever for quick-release studies. The solenoid was activated by a Tektronix Pulse Generator (Tektronix, Inc., Beaverton, Ore.) triggered by a Waveform Generator. When the armed quick-release lever was activated, it was pulled out of the way of the displacement lever at a speed which in no way limited displacement changes observed in the sartorius muscle at 4°C.

Stimulation of the Muscle

Massive platinum electrodes, 50 × 6 mm, were positioned 0.75 cm on either side of the muscle for stimulation. The electrodes were mounted on Lucite strips in order to concentrate the current flow across the face of the electrodes. Pulses were delivered to the electrodes by two Grass S₄ stimulators (Grass Instrument Co., Quincy, Mass.) connected in a modulator-stimulator configuration. The output from the "stimulator" S₄ was amplified by a complementary symmetry stimulus amplifier, designed to give up to a 3 amp current output across the 20 ohm resistance of the

bath. An adequate stimulus was determined to be 0.20 msec at 1 amp. During the experiments, the stimulus employed was 0.5 msec at 1 amp. Tetanic stimuli were administered for 750 msec at a rate of 35 Hz.

Experimental Protocol

After an 8 hr equilibration, twitch stimulation of the muscle at a rate of 0.33/min was carried out for 1 hr to obtain a constant isometric myogram. This slow rate of stimulation was continued throughout the course of the experiment. Under these conditions the problem of fatigue was minimized. If twitch tension fell by more than 5% the experiment was terminated. The area of the length-tension curve where isometric twitches superimposed was then determined by changing muscle length in 0.1 mm increments and comparing the twitch traces.

Active State Decay Determined by Isotonic-Isometric Method

The procedure for determining an individual active state decay value was described in the Rationale section. To obtain various points for the decay curve: (a) the load was varied and the distance shortened held constant; (b) the distance shortened was varied and the load was held constant; and (c) the distance shortened and the load were varied. Care was taken in these and all subsequent experiments to see that the shortening perturbations used were within the previously determined range of lengths at which twitches were superimposable. All interactions used to generate the active state decay points for any particular experiment were employed in random order.

Active State Decay Determined by the Quick-Release Method

The method used was essentially that developed by Ritchie (13). The extent of release and the time of release were varied. The family of curves of redeveloped tension were used to evaluate active state decay as a function of the extent of release. In a given experiment, an active state curve was determined by setting the extent of release while the time at which the release occurred was varied from early in the twitch to the peak of contraction. From the family of redeveloped tension curves which resulted the active state decay for a given extent of release was determined by joining the peaks of the redeveloped tension traces. Although some of the releases were not to zero tension, as long as force redevelopment occurred the data could be used in the analysis. After an active state decay curve was determined, the extent of release was changed and the protocol repeated to develop another active state decay curve. For a given extent of release in a muscle the time at which the release occurred was randomized. Between muscles the extent of the release was randomized.

Comparison of the Isotonic-Isometric and Quick-Release Methods for Determining the Active State Decay

A valid comparison of the isotonic-isometric and quick-release methods requires that those perturbations occur at the same time during the twitch and that the length of the contractile element (and that of the series elastic element) be the same immediately following either shortening or release. Comparisons were made within these

constraints in the following manner. The muscle was subjected to a small quick release early in the twitch which always left some tension in the muscle at the time isometric force redevelopment resumed. For the isotonic-isometric experiment the afterload was set to precisely the residual force remaining in the muscle after the release. The afterload stop was adjusted to control the isotonic phase of shortening so that the isometric phase of tension development following the isotonic shortening began at precisely the same time during the twitch as tension redevelopment in the quick-release portion of the experiment. At this point in time, the series elastic elements and the contractile elements in both experiments are at the same lengths. The series elastic element lengths are the same because the external tension at this point in time is the same in both experiments. Contractile element length is the difference between total length and series elastic element length. Since external muscle length is fixed and the series elastic element length is fixed, contractile element length is fixed and identical in the two situations. From this point, the two curves of isometric tension development can be compared.

Estimation of Series Elasticity in the Sartorius Preparation

The force-extension curves of the series elastic component of a sartorius muscle was determined by the method of Jewell and Wilkie (10), using the same release lever system employed for quick-release active state determinations.

RESULTS

Change in Active State as a Function of Muscle Rest Length

Preliminary experiments revealed that force development and the time-course of force development in isometric twitches varied only slightly over the length range of $L_0 \pm 2\%$. This length range was explored in subsequent experiments to find a length range for congruence of isometric twitches. Congruence was defined as no discernible difference in two oscilloscope traces over the entire twitch. The error involved in such an assessment was equal to the width of the oscilloscope trace or 0.003 N at the sensitivities employed. In 48 of 50 muscles studied, a range of lengths on the length-tension curve was located where isometric twitches superimposed. The range of length alterations over which congruence occurred varied from 0.7 to 1.3 mm. The amount a muscle subsequently was allowed to shorten or was released was limited to this range so that a change in active state decay would not be due to a change in muscle length.

Series Elasticity and Internal Contractile Element Length at Varying Isometric Forces

While external muscle length could always be held within the length range for twitch superimposition, internal muscle length at the peak of the contraction was a function of the force developed during the contraction and the compliance of the series elastic elements in the preparation. Because the muscle

was susceptible to fatigue, it was impractical to check the series compliance of each preparation to see if twitches that varied in force output were still within the length range of congruence when considering internal contractile element length. From measurements of series compliance in six representative sartorius muscles, it was determined that if peak force achieved in the contraction varied between 0.15 and 0.29 N, the maximum difference in the extension of the series elastic element averaged 0.30 ± 0.03 mm (Table I). For a 0.15 N difference in peak force (the maximum difference in peak force measured) 95% of the contractile element length differences would be no greater than 0.37 mm (whereas the length range of twitch congruence was no less than 0.7 mm for any preparation). Thus under the most extreme circumstances changes in sarcomere length during the experiment fall within the range of congruence.

TABLE I
THE FORCE EXTENSION RELATIONSHIP OF THE SERIES ELASTIC
ELEMENTS DETERMINED BY QUICK RELEASE

	Force, N							
	0.03	0.05	0.07	0.10	0.15	0.20	0.25	0.30
Extension of the series elastic element, mm	0.22	0.30	0.36	0.43	0.53	0.65	X	0.85
	0.25	0.28	0.44	0.54	0.70	0.85	0.92	1.01
	0.20	0.25	0.33	0.40	0.48	0.60	X	0.82
	0.31	0.37	0.42	0.46	0.54	0.68	0.76	0.87
	0.40	0.51	0.60	0.65	0.70	0.78	0.84	0.95
	0.08	0.14	0.22	0.30	0.38	0.46	0.52	0.63

The Active State Decay Curves Determined by the Isotonic-Isometric Method

A typical isotonic-isometric record from which points on the muscle's active state decay curve were determined is illustrated in Fig. 2. The points of interest are the three peaks of isometric contraction, considered to be points on the muscle's active state decay curve. The loads lifted were limited to those which would allow the muscle to have an isometric force redevelopment phase within the contraction cycle; if the load were too heavy, the contraction would be totally isotonic. Shortening was restricted to the length range of twitch congruence.

The isotonic-isometric method was employed to generate active state points while holding either load or distance shortened constant. The first variation of the method investigated was that of lifting a constant load through a variable distance. The data points in Fig. 3, open circles, were generated when a 0.02 N load was lifted distances varying between 0.21 (highest active state point on curve) and 1.3 mm (lowest active state point on curve). A line connecting peak of force redevelopment following each of these procedures plotted

against time is the graph of the apparent time-course of active state decay. It can be seen that the active state decayed very quickly when evaluated by this means. Each increment of distance shortened is associated with a decrease in active state force.

When distance of external shortening was held constant and load was varied to generate different points on an active state decay curve, the slope of the section of the curve measured was considerably smaller (Fig. 3, closed circles).

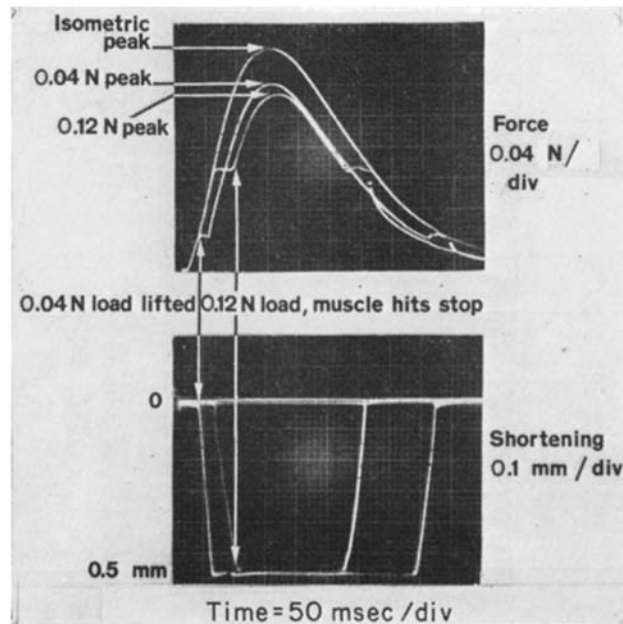


FIGURE 2. A record of an experiment which illustrates the isotonic-isometric method for studying the active state. In the upper panel there is a recording of an isometric twitch and isotonic-isometric records in which 0.04 and 0.12 N are lifted through 0.5 mm. The time at which the peak of twitch occurs in the cases is determined from the point at which the derivative of force with respect to time is zero (not shown). The lower panel is a recording of displacement against time.

In several experiments, the two variations of the isotonic-isometric method were run in one muscle. In all experiments holding the distance of external shortening constant while varying load produced an active state decay curve which was less steep than when load was held constant and distance of shortening varied. The curves intersected where the constant load was lifted through a distance equal to the constant "distance of shortening" (Fig. 3). For each muscle where at least five data points per decay curve were obtained, the data were subjected to linear regression analysis to develop an equation for active state decay against time (Table II). In the muscles examined, the slope of the linear regression of active state force on time was greater when a constant

load was lifted varying distances than when varying loads were lifted a constant distance. Thus the nature of the perturbation had altered the apparent rate of active state decay.

To understand the phenomenon more fully, active state curves were determined for a given muscle in which distance shortened was held at a constant value while the loads were varied. The distance shortened was then changed to a new constant value and another set of active state points was generated by varying the afterload. Several active state decay curves were determined in

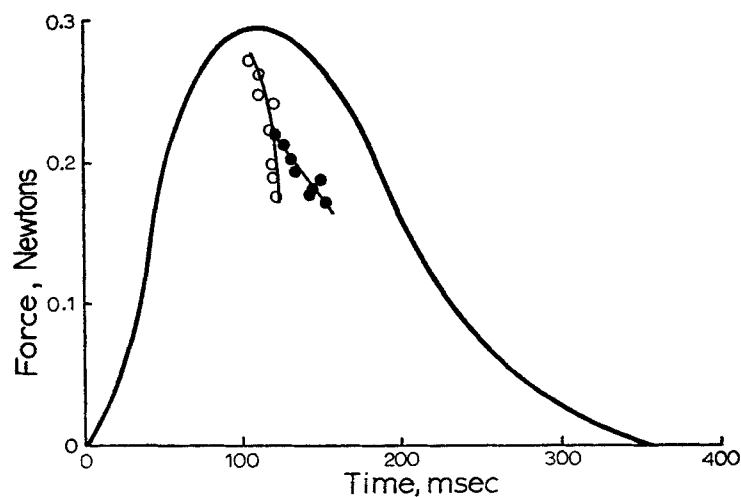


FIGURE 3. The active state decay curve derived by means of the isotonic-isometric method when the load or the distance shortened is held constant. The open circles were obtained when the load was constant at 0.02 N and the distance shortened varied between 0.21 and 1.3 mm. The closed circles were obtained by holding the distance shortened constant at 0.7 mm and varying the loads lifted between 0.02 and 0.15 N. The curves intersect where the 0.02 N load was lifted through a distance of 0.7 mm. A curve of the isometric twitch tension, recorded over the range of twitch congruence, is presented for comparison.

this way for each muscle. An example of the results obtained in this experiment is presented in Fig. 4. Here the distance shortened was set at 0.28, 0.70, and 1.10 mm while loads were varied to obtain each decay curve. The maximum distance shortened is well within the range of twitch congruence for this muscle. Different decay curves, each associated with a different amount of external shortening, are generated. Visual inspection of any set of decay curves revealed that the more external shortening allowed during the perturbation phase of the isotonic-isometric contraction, the less the active state force at any later instant on the decay phase examined. Furthermore, the greater the distance shortened, the faster any active state curve appeared to decay.

TABLE II
 LINEAR REGRESSION ANALYSES OF ACTIVE STATE CURVES GENERATED BY THE TWO VARIATIONS OF
 ISOTONIC-ISOMETRIC METHOD

Muscle No.	Type contraction	N	\bar{X} msec	\bar{Y} N	Slope* $N/\text{msec} \times 10^{-4}$	F ratio for slope	Peak T_{10} force N	Time to peak tension msec
210	(a) 0.01 N, (0.2-0.8) mm	7	144	0.225	-4.41 (-2.06 to -6.76)	12.4†	0.282	134
	(b) 0.07 mm, (0.01-0.2) N	7	150	0.225	-1.27 (-0.88 to -2.08)	10.62†	0.282	134
115	(a) 0.02 N, 0.06 N, (0.2-8) mm	8	160	0.245	-2.64 (-1.86 to -3.43)	51	0.299	145
	(b) 0.28 mm, (0.04-0.26) N	7	155	0.284	-0.78 (-0.39 to -1.18)	14.6§	0.299	145
121	(a) 0.02 N, (0.2-1.1) mm	10	116	0.245	-6.37 (-3.04 to -9.72)	15.5§	0.304	116
	(b) 1.1 mm, (0.02-0.14) N	10	136	0.206	-1.76 (-1.37 to -2.16)	43	0.304	116
112	(a) 0.06 N, (0.15-0.7) mm	5	143	0.206	-6.17 (-5.00 to -7.35)	99	0.382	128
	(b) 0.4 mm, (0.02-0.18) N	6	163	0.206	-1.18 (-0.11 to -0.12)	325	0.382	128
126	(a) 0.08 N, (0.1-0.9) mm	6	148	0.189	-2.65 (-1.67 to -3.63)	27.4§	0.241	126
	(b) 0.7 mm, (0.01-0.17) N	8	152	0.196	-0.20 (-0.34 to 0.0)	75.9	0.241	126

\bar{Y} = active state force, N.

\bar{X} = time, msec.

N = number of points in line.

(a) = load constant, distance of shortening varied between numbers in parentheses. (b) = distance shortening constant, load varied between numbers in parentheses.

* = the numbers in the parentheses under slope indicate the 95% confidence limits of slope.

† = $P < 0.05$.

§ = $P < 0.01$.

|| = $P < 0.001$.

A summary of the data from seven muscle preparations in which at least five points were obtained for each distance shortened while the loads varied is presented in Table III. The force at peak tension and time to peak tension were 0.297 ± 0.032 N and 128 ± 13 msec. From the regression line calculated for each distance shortened with varying loads by means of least square regression analysis, an active state force at the time of peak twitch tension and a slope of decay were calculated. The active state force at this time, i.e., the computed Y at T_w (twitch) peak (Table III), is less than the peak twitch and decreases with each increment of isotonic shortening. From linear regression analysis of the computed active state force at the peak of twitch and the distance shortened it was determined that the active state decreases 0.067 N/mm

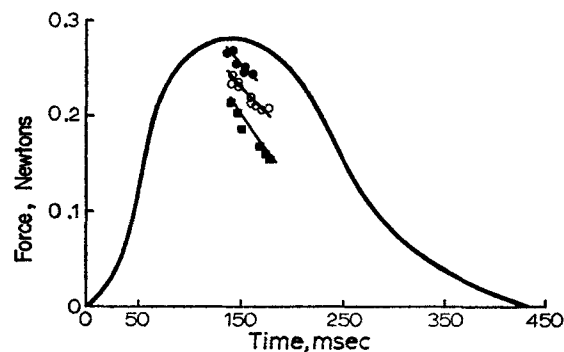


FIGURE 4. A set of active state decay curves which were generated when the distance shortened was held constant at one of three values and loads varied. The experimental parameters for distance shortened and load are ●—● 0.21 mm, 0.02–0.20 N; ○—○ 0.70 mm, 0.02–0.16 N; ■—■ 1.10 mm, 0.02–0.13 N. A curve of the isometric twitch tension, recorded over the range of twitch congruence, is presented for comparison.

of isotonic shortening ($P < 0.001$). It should be noted that there is a variability in peak force and in contraction time. To minimize this variability muscles were normalized in terms of per cent peak twitch force and per cent contraction time. The values of active state force at 100 and 110% time to peak tension were read from the active state curve determined for a given distance shortened for 10 muscles. Normalized active state force values for the two time periods were plotted against the distance of external shortening allowed during the generation of that active state curve (Fig. 5). Mean active state force at 100% time to peak tension (TPT) was 88.5% peak twitch force while mean active state force at 110% TPT was 81% peak twitch force. The idea reflected in this difference in force values is that active state force falls between 100 and 110% TPT. For these time periods, the regression of active state values on distance shortened was fitted by a line whose slope was different from zero ($P < 0.001$). The variability in the data is still large, despite the attempts to

TABLE III
REGRESSION OF FORCE ON TIME FOR LIFTING VARYING LOADS THROUGH CONSTANT DISTANCES

Muscle No.	Contraction	N	\bar{X}	\bar{Y}	Computed* Y at T_w peak	Slope†	F ratio	Force	Time to peak tension
126	0.28 mm, 0.02-0.20 N	6	148	0.225	0.236 (0.233-0.245)	-0.49 (-0.29 to -0.69)	44§	0.241	126
	0.70 mm, 0.01-0.17 N	9	155	0.196	0.219 (0.193-0.244)	-0.78 (-0.59 to -0.98)	108§	0.241	126
	1.10 mm, 0.05-0.15 N	9	162	0.167	0.207 (0.166-0.248)	-0.88 (-0.49 to -1.27)	31.4§	0.241	126
1211	0.28 mm, 0.02-0.23 N	10	121	0.274	0.291 (0.271-0.311)	-1.27 (-0.49 to -2.06)	8.8	0.311	108
	0.70 mm, 0.02-0.20 N	10	129	0.255	0.281 (0.250-0.313)	-1.27 (-1.08 to -1.47)	80.7§	0.311	108
	1.10 mm, 0.02-0.135 N	10	136	0.206	0.236 (0.201-0.271)	-1.67 (-1.08 to -2.25)	42.6§	0.311	108
210	0.28 mm, 0.01-0.25 N	6	147	0.255	0.263 (0.250-0.275)	-0.59 (-0.12 to -0.98)	9.93	0.282	134
	0.70 mm, 0.01-0.29 N	7	150	0.255	0.246 (0.217-0.275)	-1.27 (-0.49 to -2.06)	10.6	0.282	134
	1.10 mm, 0.04-0.14 N	9	156	0.186	0.219 (0.195-0.242)	-1.47 (-1.40 to -1.56)	14.8	0.282	134
115	0.28 mm, 0.04-0.25 N	7	155	0.284	0.296 (0.276-0.335)	-0.78 (-0.39 to -1.18)	14.6	0.314	145
	0.70 mm, 0.05-0.20 N	11	168	0.255	0.272 (0.261-0.284)	-1.47 (-1.18 to -1.76)	264§	0.314	145
	1.10 mm, 0.01-0.16 N	7	175	0.216	0.234 (0.216-0.253)	-1.57 (-1.40 to -1.76)	219§	0.314	145
129	0.28 mm, 0.02-0.25 N	5	152	0.274	0.288 (0.253-0.323)	-1.18 (-1.08 to -1.27)	14.9	0.305	140
	0.70 mm, 0.01-0.18 N	9	158	0.245	0.268 (0.205-0.330)	-1.27 (-0.88 to -1.67)	63.9§	0.305	140
	1.10 mm, 0.01-0.13 N	5	162	0.196	0.218 (0.160-0.277)	-1.86 (-0.69 to -3.04)	1532§	0.305	140
128	0.28 mm, 0.02-0.26 N	10	126	0.304	0.310 (0.292-0.327)	-0.98 (-0.59 to -1.37)	42.1§	0.340	116
	0.70 mm, 0.02-0.21 N	9	166	0.270	0.278 (0.221-0.335)	-0.78 (-0.69 to -0.88)	127.4§	0.340	116
	0.28 mm, 0.02-0.24 N	8	135	0.265	0.270 (0.259-0.282)	-0.78 (-0.59 to -0.98)	70.6§	0.284	128
0.70 mm, 0.02-0.18 N	10	144	0.245	0.255 (0.235-0.274)	-0.57 (-0.20 to -0.98)	11.8¶	0.284	128	

Contraction = distance shortened, range of loads.

\bar{X} = active state time, msec.

\bar{Y} = number of data points used in computing regression line.

* The numbers in the parentheses under "Computed Y at T_w peak" indicate the 95% confidence limits of peak.

† The numbers in the parentheses under Slope indicate the 95% confidence limits of slope.

§ $P < 0.001$.

|| $P < 0.05$.

¶ $P < 0.01$.

normalize it. Nevertheless, there is a regression of active state force on distance of external shortening allowed during the perturbations. The extent that the active state is diminished at 100 and 110% time to peak tension is 20%/mm external shortening allowed. In these muscles of very uniform rest length, this diminution amounted to 20% per 3.3% change in muscle length.

The slope of the regression of active state force on time (Table III) is an index of the rate at which the active state decays, as measured by the isotonic-isometric technique. The linear regression of these slopes as a function of the distance shortened is significantly different from zero ($P < 0.01$) with a slope

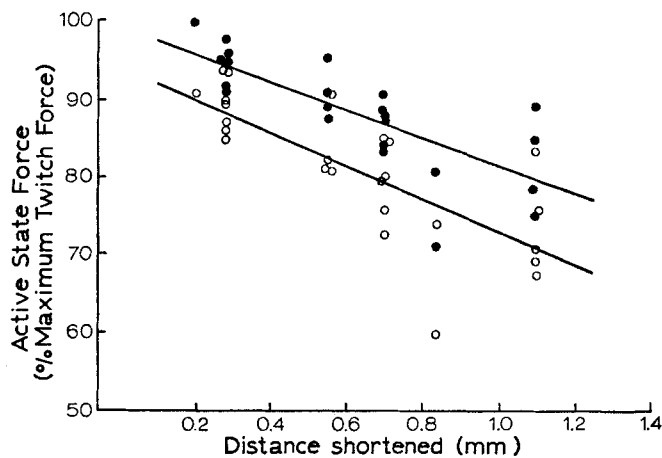


FIGURE 5. The relation between the distance shortened during isotonic release and the active state force. The closed and open circles represent measurements made at 100 and 110% time to peak tension and were determined from the regression equations of active state decay for each muscle and distance shortened. For 100% time to peak tension $Y = 100.6 - 18.5X$, $P < 0.001$; at 110% $Y = 94.9 - 22.5X$, $P < 0.001$ (Y = active state force expressed as per cent maximum twitch force, X = distance shortened in millimeters).

of -0.74 . Thus as the extent of isotonic shortening was increased, the rate of active state decay appeared to be increased as well.

The Extent of Quick Release as a Determinant of Active State Decay

Active state decay as measured by quick release should not give different decay curves for different amounts of release (within the range of twitch congruence) unless the release alters the rate of decay (see Rationale). An active state decay curve was generated by releasing the muscle a given distance at varying times. The distance of release was then changed (still within the range of twitch congruence) and the procedure repeated. It was found that the greater the distance of release, the faster the active state appeared to decay (Fig. 6). Similar results were found in five experiments.

Direct Comparison of Quick-Release and Isotonic-Isometric Methods of Determining Active State Points

In order to compare the effects of isotonic-isometric contraction and quick release on the active state, direct comparison of the force redevelopment following the two perturbations were made (Fig. 7). In two superimposed

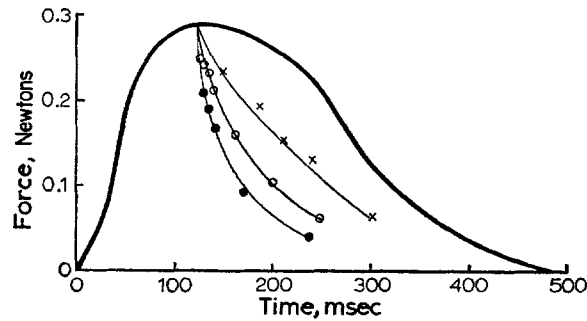


FIGURE 6. Active state decay curve determined by quick release at varying times during the contraction. The three active state decay curves were determined by releases of 0.3 mm (X—X), 0.7 mm (O—O), and 1.1 mm (●—●). A curve of isometric twitch tension recorded over the range of twitch congruence is presented for comparison.

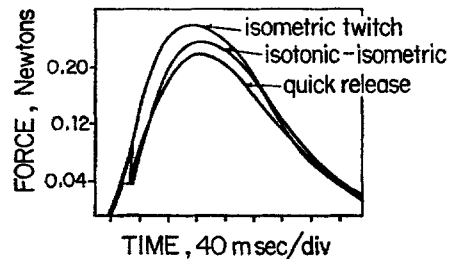


FIGURE 7. Tracings of an oscilloscope recording of tension redevelopment following quick release and isotonic release. The experiment involved a release of 0.4 mm as compared to isotonic shortening of 0.6 mm with 0.04 N afterload. Although the tracing indicates that tension redevelopment following the release starts earlier than the isotonic experiments (drawn as such for clarity), in actual experiments tension redevelopment started at the same point in both cases.

contractions the tension redevelopment, beginning at the same level of tension and the same time, are followed after a quick-release and an isotonic-isometric intervention. To equalize the contractions in terms of the series elastic component length, force, and time when the tension redevelopment began, the distance of shortening in the isotonic-isometric contraction had to be greater than the distance of release. Rate of tension redevelopment was initially equal in the two types of contraction. But as the redevelopment peaks were ap-

TABLE IV
DIRECT COMPARISON OF QUICK-RELEASE AND ISOTONIC-ISOMETRIC
CONTRACTIONS

		Distance	Redevelop-	Redevelopment	Time of peak
		release or shortening	ment begun	peak force	
		<i>mm</i>	<i>msec</i>	<i>N</i>	<i>msec</i>
(1) Quick release	0.00 N	0.6	36	0.230	129
shortening		0.7	36	0.245	132
(2) Quick release	0.00 N	0.7	40	0.231	138
shortening		1.1	40	0.235	136
(3) Quick release	0.00 N	0.5	28	0.211	130
shortening		0.63	28	0.214	135
(4) Quick release	0.02 N	0.7	53	0.199	128
shortening		0.8	53	0.203	125
(5) Quick release	0.03 N	0.4	45	0.239	128
shortening		0.6	45	0.248	124
(6) Quick release	0.10 N	0.7	53	0.206	132
shortening		1.1	53	0.210	132
(7) Quick release	0.10 N	0.7	55	0.198	135
shortening		1.2	55	0.199	137
(8) Quick release	0.007 N	1.0	56	0.192	142
shortening		1.2	56	0.191	142
(9) Quick release	0.06 N	0.5	88	0.174	144
shortening		1.2	88	0.180	142
(10) Quick release	0.00 N	0.4	32	0.228	130
shortening		1.0	32	0.239	128

proached, the rate of tension development fell in the muscle which had been released and tension peaked at a lower value (Fig. 7). In most of the comparisons, peak tensions were greater following the isotonic-isometric intervention. The average difference between the contractions was 0.006 N (Table IV).

DISCUSSION

The frog sartorius muscle was chosen for these studies in order to relate the data to the great body of literature on active state. The muscles used performed qualitatively (length-tension responses) and quantitatively (force output) like others reported in the literature (1, 7, 10, 11). Active state measurements were reproducible within 1% under any set of conditions. It is consequently felt that these data can be applied to the literature on active state measurements in whole muscles and can be used to explain conflicts within that literature.

In the past, discrepancies in active state measurement may have resulted from inadequate control of muscle length. Although experiments were carried out at muscle lengths near the plateau of the active length-tension curve, no checks were run to determine specifically if the active state was altered by a

change in length over the length range employed. Subsequent work (3-5, 11, 13, 14) revealed that active state did change as a function of muscle length. In the present experiments, rigid controls of muscle length were employed. A muscle was not allowed to shorten or lengthen outside a length range where isometric twitches were superimposable. This restriction limited the scope of the active state decay curve which could be measured, but within the length restriction changes in active state were not due to differences in muscle length.

In the present experiments, an attempt was made to keep within the confines of previously established rationales for active state measurements. Many investigators have used quick release to measure the active state. The theoretical basis for its use has been discussed (Rationale). In these experiments, the quick-release technique was employed with an additional restriction. The largest release employed was 1.4 mm, equal to the length range over which twitches were congruent. This restriction was employed to insure that the release technique did not produce differences in active state decay curves that were dependent on the change in sarcomere length.

The isotonic-isometric and quick-release techniques employed essentially the same rationale. The perturbation, a change from isotonic to isometric contraction, was reasonable since skeletal muscles undergo a combination of isotonic and isometric phases in contraction when working against the tendons which anchor them to bones.

The isotonic-isometric technique of lifting varying loads through constant distances revealed that the height of the active state decay curve at any moment was a function of the distance of isotonic contraction that the muscle had shortened during the perturbation phase of the experiment. This confirms and extends the phenomenon discovered by Edman and Kiessling (5) on single frog semitendinosus fibers. The greater the distance shortened (within the range of congruent twitches), the less the active state value at a later moment in the contraction cycle over the spectrum of active state values examined. When a linear regression analysis of active state force at 100 and 110% time to peak tension on distance of external shortening was run, the regression line was significantly different from zero ($P < 0.001$). The slopes of the regression lines (Fig. 5) indicate that around the time of peak tension, the force developing potential of the muscle decreased 20% for every millimeter of external shortening (or 3.33% decrease in muscle length) incurred during the contraction.

The subsequent rate of active state decay as measured by the isotonic-isometric technique also increased as the distance of external shortening increased. The work of Edman and Kiessling (5) has previously shown that the later movement occurs in the contraction cycle, the more the active state is diminished by the movement. In the generation of an isotonic-isometric

decay curve, a larger amount of external shortening imposed during the perturbation would result in more of the shortening motion during the decay period of the active state on the basis of Edman and Kiessling's work. One would expect the active state to be depressed more by these larger movements. Under these circumstances the increase in slope found is not surprising.

The idea that the active state varies with the previous history (amount of perturbation) of the contraction is supported by the quick-release results as well as the isotonic-isometric results. When measured by quick release, active state decay rate is a function of distance of release. Release causes more inactivation than the isotonic-isometric perturbation when the postperturbation force redevelopment phases are compared at equivalent sarcomere lengths at equivalent points in time.

The fact that ongoing shortening "turns off" the active state can be offered as a possible explanation for the discrepancy between the isometric myogram and the myogram reconstructed from force-velocity and stress-strain data (10). If, during the early phase of tension rise in an isometric myogram, the internal shortening taking place is causing the active state to be turned off somewhat, rise of tension would be slowed. In contrast, in the calculation from stress-strain force velocity data, active state was assumed to be maximal throughout the time period examined. The actual tension increase curve should, indeed, be less than the reconstructed curve if active state levels were diminished in the real measurement, even temporarily. An alternative explanation is that the series compliance is underestimated by the quick-release technique. This results from extrapolation of the shortening record back to the initial time of quick release to ascertain the instantaneous undamped length change associated with the release to a given load (Fig. 3B of reference 10). If the final velocity used in the extrapolation is somewhat faster than the shortening velocity immediately after the release the compliance would be underestimated by using the final velocity for extrapolation. Similar analysis can be applied to the differences found between isometric myograms following a release to zero tension and those obtained immediately following activation. Consideration must be given to the quantitative effects of any given manipulation on the intensity and time-course of the active state.

One of the foremost conflicts in recent years is based on results from the heat data, which demonstrate that there is a heat of shortening, and the biochemical data which indicate that there is no heat of shortening. Hill (9) asserted that the biochemists found less breakdown of creatine phosphate in an isotonic contraction, despite a "heat of shortening" because the active state was "turned off" by the shortening process. The present results indicated that he may be correct, although future experiments must confirm whether heat production is reduced in the same way by shortening as the mechanically deter-

mined active state. Chemical data (2) must be appropriately corrected for active state inactivation of shortening. When this is done a significant shortening heat appears.

The data, then, indicate that the shortening or release movement allowed in many of the attempts to measure the active state actually causes the active state to change. These experiments do not directly suggest a mechanism whereby the active state is changed. Movement may affect calcium fluxes, binding, or cross-bridge functioning during the contraction. Whatever the mechanism of action, the effect of motion on the active state must be acknowledged when employing the active state to evaluate muscle performance. It must be recognized that the active state, in terms of its measurability, is only a relative quantity, for even the process of isotonic shortening causes it to change.

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