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MECHANICAL DEACTIVATION INDUCED BY ACTIVE SHORTENING IN ISOLATED MUSCLE FIBRES OF THE FROG

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SUMMARY

1. The effect of active shortening on the time course and magnitude of isometric tension development during a single twitch and during an incompletely fused tetanus was studied at 0·2–1·2° C in isolated semitendinosus muscle fibres of the frog.

2. Active shortening caused a depression of the contractile force without markedly affecting the total duration of the twitch. The depressant effect increased with increasing amounts of sarcomere shortening. Sarcomere shortenings of 0·05 μm and 0·3 μm reduced the twitch force by approximately 5 and 20 per cent of the maximal tetanic tension, respectively.

3. A given sarcomere shortening induced the same absolute amount of depression of the contractile strength when the movement was carried out at different times during the initial 200–250 msec after the stimulus.

4. The influence of load and velocity of shortening during the movement phase was studied. Differences in load ranging between zero and 1/3 of the maximal tetanic tension (with concomitant changes in speed of shortening from V_{max} to approximately 1/5 of V_{max}) did not affect the degree of depression markedly. Under the conditions studied, the *extent* of movement appeared to be the only significant determinant of the depressant effect.

5. The reduction in force induced by active shortening persisted for 800–900 msec during an incompletely fused tetanus.

6. It is suggested that the depressant effect is based on a structural change in the myofilament system that is produced as the A and I filaments slide along each other during muscle activity.

INTRODUCTION

It has been demonstrated in several previous studies (Jewell & Wilkie, 1960; Joyce & Rack, 1969; Joyce, Rack & Westbury, 1969; Edman & Kiessling, 1971; Briden & Alpert, 1972; Sugi, 1972; Dickinson & Woledge,

1973; also see Hill, 1964) that a vertebrate skeletal muscle that is quickly stretched or allowed to shorten during a twitch loses some of its ability to produce tension during the remainder of the contraction period. A similar depressant effect in response to length perturbations has also been recorded in amphibian and mammalian cardiac muscle (Brady, 1966; Edman & Nilsson, 1971, 1972; Bozler, 1972; Kaufmann, Bayer & Harnasch, 1972). In a previous study on isolated skeletal muscle fibres (Edman & Kiessling, 1971) the effect of active shortening was shown to be distinct from the effect that is caused by altering the resting sarcomere length. A decrease of the initial sarcomere length (within the range 2·8–1·7 μm) was shown to reduce the duration of the mechanical activity. Shortening of the muscle fibre *during* activity, on the other hand, was found to reduce the tension output without any significant change of the total duration of the contractile response. The evidence obtained so far would seem to make clear that the movement effect is an entirely physiological phenomenon which appears both in the isolated muscle fibre preparation (Edman & Kiessling, 1971) and in the intact muscle in the body (Joyce & Rack, 1969; Joyce *et al.* 1969).

The present investigation was conducted with the aim of defining in more detail the conditions under which the contractility is reduced by active shortening in skeletal muscle. The extent of the depressant effect has been related both to the time during the contraction period and the degree of activity in the contractile system when the movement occurs. Experiments have also been designed to elucidate the time course of restoration of the contractility after the shortening phase. A brief account of some of these results has been published previously (Edman, 1971).

METHODS

Preparation. Single fibres were dissected from the ventral head of the semitendinosus muscle of *Rana temporaria*. The techniques used for mounting and recording of tension from the fibres were similar to those described previously (Edman & Kiessling, 1971). A schematic drawing of the experimental arrangement is illustrated in Fig. 1. The fibre (A) was mounted horizontally in a jacketed Perspex chamber (B) between a tension transducer (C) and a lever (D), which extended from the moving coil of an electromagnetic vibrator (E). The rest length of the fibre could be set to the desired value by adjusting the position of the vibrator-lever system or the tension transducer.

Stimulation. An assembly of six to eight platinum wire electrodes spaced at 2 mm intervals along the entire length of the fibre was used for stimulation. The distance between the wires and the fibre was approximately 1·5 mm. The electrodes were arranged as alternate anodes and cathodes and care was taken to ensure that each pair produced a supramaximal stimulus. Rectangular pulses of 1 msec duration were used. A single pulse or, in certain experiments, a series of four to five pulses of 3–5 Hz frequency were given at two minutes intervals to produce a single twitch or an incompletely fused tetanus. After 10–15 such contractions a 1 sec train of

pulses of a frequency of 18–30/sec was given to produce a completely fused tetanus. The fibres were usually mounted 2–4 hr before the experiment was started and were tetanized periodically during this time. Normally these fibres survived at least 2 days after dissection and the amplitude of the isometric tetanus declined by less than 5 per cent over a day of experimentation.

Bathing solution and temperature. A Ringer solution of the following composition was used (mM): NaCl 115·5, KCl 2·0, $CaCl_2$ 1·8, Na phosphate buffer 2·0, pH 7·0. The solution (8 ml.) was exchanged at approximately 1 hr intervals during the experiment. The temperature of the bath was adjusted by circulating a water-glycol mixture from a thermostatically controlled tank (Calora Ultrathermostat) through the jacket (Fig. 1J) surrounding the chamber. The bath temperature was checked periodically with a thermocouple and was maintained constant to $\pm 0{\cdot}2°$ C throughout an experiment. The temperature varied from 0·2 to 1·2° C between different experiments. Glass-distilled water was used for the washing of glass ware and for the preparation of solutions. All chemicals used were of analytical grade.

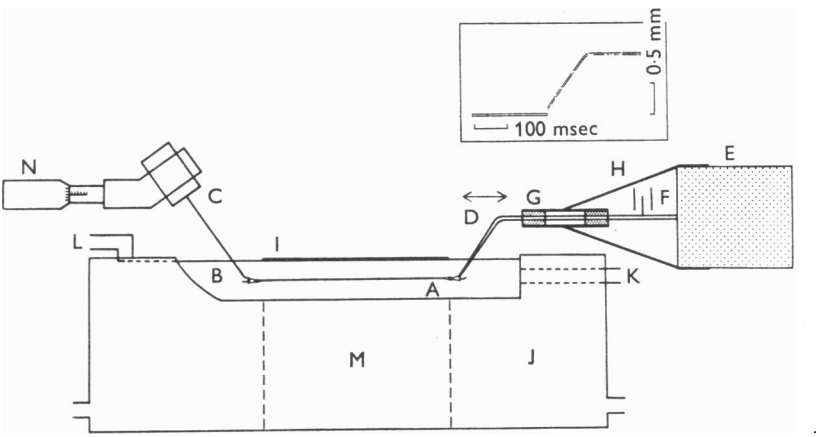

Fig. 1. A, muscle fibre. B, muscle fibre chamber. C, tension transducer. D, lever movable in the horizontal plane. E, electromagnetic vibrator. F, displacement transducer. G, Teflon blocks. H, brackets. I, glass slide placed on top of Ringer solution. J, jacket for circulation of thermostatically controlled water-glycol mixture. K, inlet for bath solution. L, suction drain. M, air space for passage of laser beam. N, micrometer screw. Inset: Simultaneous oscilloscope records of command signal (upper) and resulting lever movement (lower).

Tension recording. An RCA 5734 mechano-electric transducer fitted with a glass extension (compliance 1·5 μm/mN) was used for recording of isometric tension. The frequency response of the tension transducer was approximately 750 Hz when the end of the glass lever was submerged in the bathing fluid.

Determination of sarcomere length. The sarcomere length at rest and during activity was determined by means of laser diffraction technique (Cleworth & Edman, 1972). A helium-neon continuous-wave laser (Spectra-Physics models 124 and 133) was used, and the diffraction pattern was displayed on a partially transmitting horizontal screen placed at a distance of 118 mm above the fibre. A glass cover slip of 0·1 mm thickness (Fig. 1 I) was placed on the surface of the bathing solution during

these measurements. Diffraction patterns were taken up from several different places along the middle segment of the fibre (approximately the middle 90 per cent of the fibre length). The sarcomere length was calculated from the zero- to first-order line spacing using the formula for light diffraction. For convenience a calibrated scale was placed on the screen so as to enable a direct reading of the sarcomere length from the zero- to first-order line spacing. With this method it was possible to determine the sarcomere length to 0·02 μm within the range of sarcomere lengths studied in this investigation. Control experiments were performed in which the resting sarcomere length was determined both by diffraction technique and by light microscopy (Edman & Kiessling, 1971) for various settings of the fibre length. Sarcomere lengths derived by the two methods agreed to the accuracy stated above for the diffraction technique. The changes in sarcomere length that occurred in response to various amounts of release of the fibre during activity were calculated from the change in overall fibre length and the sarcomere length at the onset of the lever movement.

Determination of fibre length and cross-sectional area. The overall fibre length (the distance between the insertions of the fibre to the tendons) was measured to the nearest 0·05 mm using a Zeiss Stereo II microscope at 6× magnification. For determination of the cross-sectional area the fibre was moved to a separate trough. The fibre length was adjusted to give a sarcomere length of approximately 2·2 μm. With the arrangement used the preparation could be turned 90° around its longitudinal axis so that two perpendicular diameters could be measured at any selected place along the fibre. The measurements were carried out by means of an ocular micrometer at 500× magnification using a Zeiss Standard Universal microscope. Measurements were made at 1–2 mm intervals along the fibre. The cross-sectional area was calculated from the two diameters obtained at each measuring place assuming that the cross-section had an elliptical shape. A mean value of the cross-sectional area was obtained from six to eight such measurements. Typically the individual values deviated from the mean by 5–7 per cent.

Electromagnetic puller. The purpose of this device was to produce controlled, stepwise changes in length of the muscle fibre during contractile activity. The principal unit was an electromagnetic vibrator (Ling Shaker model 101), the moving coil of which was provided with an aluminium tube serving as a lever for the muscle fibre. The part of the lever that was submerged in the bath was a glass tube. The essentials of the mechanical arrangement are illustrated in Fig. 1. In order to stabilize the lever movements the horizontal part of the lever (90 mm long) was fitted into two Teflon blocks (G) placed 20 mm apart in a brass holder. The latter was firmly attached by means of two brackets (H) to the body of the electromagnetic vibrator. The channel in the Teflon blocks was 0·1 mm wider than the diameter of the lever and was filled with thin silicon oil. A steel wire (0·2 mm diameter) passed through a hole in the lever, perpendicular to the long axis of the lever, and was fixed to two leaf springs that were mounted on either side of the vibrator body. The object of having the lever spring-loaded was to decrease the series compliance of the puller and, hence, to increase its resonant frequency. With the spring tension existing at resting position of the lever the resonant frequency was 170 Hz; it increased to 310 Hz at the most advanced position of the lever.

Displacement transducer. The position of the lever (D) was monitored by a capacitance-type transducer (F) similar to that described by Arlock (1970). A grounded brass plate (15 mm × 15 mm) was mounted on the lever as is illustrated in Fig. 1. Two other brass plates of similar size were mounted on a separate holder on either side of the lever plate so as to obtain two differentially coupled variable capacitators. The difference in capacitance between these two units was measured with a transformer bridge that was provided with a phase sensitive detector. The frequency

response of the transducer was approximately 1 kHz. The transducer output was calibrated by measuring the movement (to the nearest 5 μm) of the lever tip at 40 × magnification using a Zeiss Stereo II microscope fitted with an ocular micrometer. A rectilinear response was obtained for lever movements within a range of 2 mm. The calibration did not change over a day's use of the apparatus.

Servo system. A pulse generator produced either of three different signals: (1) a single fast step, (2) a ramp signal or (3) a combination of (1) and (2), yielding an initial rapid step followed by a ramp. The amplitudes of (1) and (2), as also the slope of (2), could be varied independently. The pulse, after amplification, controlled the current output of a power amplifier which operated the electromagnet of the vibrator unit. The signal from the displacement transducer was used for feed-back control of the lever movements, both in its original form as position feed-back and, after differentiation, as velocity feed-back. In order to avoid oscillation in the system a separate feed-back loop was introduced from the output of the power amplifier.

The triggering of the pulse generator was synchronized with the electric stimulus of the muscle fibre. By means of a delay unit the onset of the lever movement could be set (with an accuracy of 2 msec) to any selected time after stimulation of the fibre.

Display and recording of transducer signals. The signals from the tension and displacement transducers were displayed on a Tektronix 5103N storage oscilloscope and photographed on 35 mm orthochromatic film. In some of the early experiments a Tektronix 502 A oscilloscope was used. The signals were also monitored on a polygraph jet-ink writer (Elema-Schönander Mingograph) which had a frequency response of approximately 680 Hz under the conditions used in the present experiments. In order to achieve a composite recording like that illustrated in Figs. 2 and 6 the timing of the individual releases was first tested out by trial and error. During this procedure the records stored on the oscilloscope screen, or the ink-writer records, were used as a guide. After the appropriate settings of the servo system had been found for each separate run, a complete set of releases was repeated in a sequence using a 2 min interval between the individual recordings as described.

Performance of servo system. Fig. 1 (inset) illustrates simultaneous recordings of the command signal and the resulting lever movement. During the initial phase the puller was allowed to move at approximately 200 mm/sec. This velocity far exceeded the maximal speed of shortening, about 18 mm/sec, produced by a 10 mm long muscle fibre at 1–2° C (Mulieri, Mulieri & Edman, 1974). The puller could be made to follow the command signal faithfully during the second phase when velocities < 200 mm/sec were produced, yielding an almost perfectly straight ramp. A slight curvature of the ramp was obtained in some experiments, due to overdamping of the feed-back loop, but this had no obvious effect upon the results obtained. Small (0·2 mm) step changes could be performed with a rise time of 1 msec.

RESULTS

1. *Depressant effect of different amounts of active shortening*

Fig. 2 illustrates the depressant effect on the isometric twitch response caused by various amounts of unloaded shortening during the activity period. The contraction was initiated at 2·45 μm sarcomere length, and the fibre was released at selected times (records *a–e*) to shorten and redevelop isometric force at a shorter sarcomere length. The redevelopment of tension shown in records *a–e* occurred between 2·25 and 1·95 μm, i.e. at sarcomere lengths on the plateau of the length–tension curve where

tetanic tensions are quite constant (cf. Gordon, Huxley & Julian, 1966; Edman, 1966). The speed of the lever movement was sufficiently high to prevent any force production during the shortening phase. The time of release was adjusted appropriately to allow the fibre to redevelop isometric tension at very nearly the same time after the stimulus in each case.

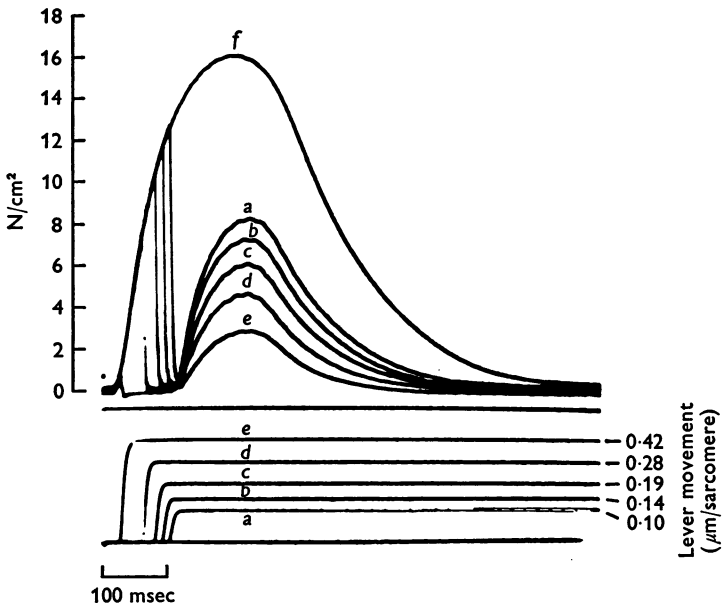

Fig. 2. Superimposed records illustrating redevelopment of tension (upper traces) after different amounts of active shortening during a single twitch of isolated muscle fibre. Speed of lever movements (lower traces) high enough to prevent tension development during shortening of fibre. Lever movements (calibrated in μm sarcomere shortening) timed so as to allow fibre to redevelop tension at nearly the same time in all cases. Release *a* (control) was just sufficient to cause a complete drop in tension and an immediate redevelopment of tension. Note the uniform depression of isometric myograms *b*–*e* compared to control myogram *a*. Top trace (*f*) is the unreleased isometric twitch. Maximal tetanic tension: 27 N/cm^2.

It can be seen from Fig. 2 that active shortening reduced the fibre's ability to produce tension, and that the depressant effect increased with the amount of shortening. In confirmation of previous findings (Edman & Kiessling, 1971) movement did not markedly affect the duration of the mechanical activity: The tension output was reduced to nearly the same degree at all times after the shortening phase.

Fig. 3 illustrates the magnitude of the depressant effect at various degrees of sarcomere shortening as determined in four experiments of the

type described in Fig. 2. In this plotting the peak tension recorded after the smallest amount of shortening (causing a complete drop in tension, see myogram *a*, Fig. 2) has been used as a control. The decrease in tension below this control level (*a–b*, *a–c*, *a–d*, *a–e*, upper traces, Fig. 2) has been plotted in relation to the sarcomere shortening that occurred in excess of the control (*b–a*, *c–a*, *d–a*, *e–a*, lower traces, Fig. 2). The reduction in force has been expressed in per cent of the maximal tetanic tension for each respective fibre. It can be seen that the depression of active tension was not a rectilinear function of the length change; each new increment of sarcomere shortening resulted in a progressively smaller increase of the

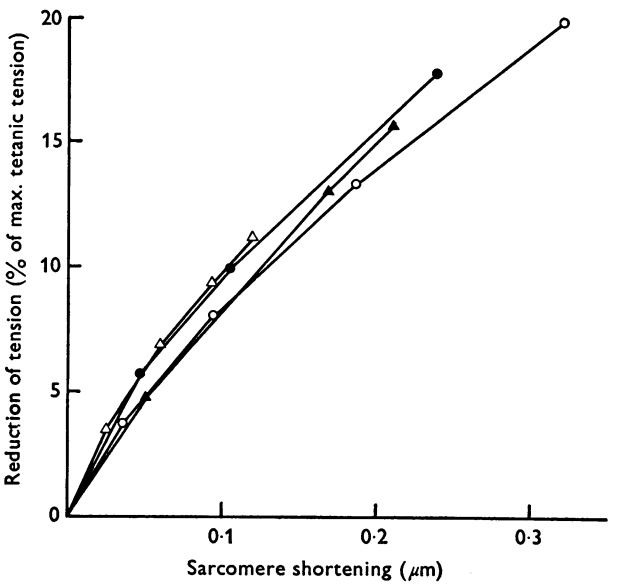

Fig. 3. Depression of contractile force in response to different amounts of sarcomere shortening during a single twitch. Maximal tetanic tension: △, 18 N/cm². ●, 24 N/cm². ▲, 28 N/cm². ○, 27 N/cm² (the same experiment as illustrated in Fig. 2). For further information, see text.

depressant effect. The reduction in twitch tension after 0·3 μm sarcomere shortening corresponded to approximately 20 per cent of the maximal tetanic tension. A 0·05 μm sarcomere shortening caused a depression of the twitch tension that corresponded to 4–6 per cent of the maximal tetanic tension. As is demonstrated in the following section the amount of depression (expressed in per cent of maximal tetanic tension) is not critically dependent on the time at which the movement occurs during the twitch period.

2. *Effect of shortening at different times during the activity period*

The aim of the following experiments was to find out as to what extent the depressant effect depends on the time during the activity period at which the shortening is carried out. The fibre was stimulated to produce four incompletely fused isometric twitches at 2·20 μm sarcomere length. During the fourth cycle the fibre was released at different times after the stimulus to redevelop tension at 2·13 μm sarcomere length. This amount of shortening was sufficient to cause an almost complete drop in tension and an immediate redevelopment of isometric force (Fig. 4, inset). In a second series the fibre was released to redevelop tension at 1·98 μm sarcomere length. Thus, in both series of releases the redevelopment of tension occurred at sarcomere lengths where the fibre's tetanic output was maximum (for references, see section 1).

Fig. 4 illustrates the peak redevelopment of tension at various times after the stimulus in the two series of releases. The difference in tension recorded between the two series (dashed vertical lines) represents the depressant effect of the extra amount of shortening (0·15 μm/sarcomere) that occurred during the second series. It is seen that the percental decrease in redeveloped force was progressively larger as the movement was carried out later during the activity period. For example, the isometric force was reduced to approximately 65 per cent of the control value (symbol 1) when the movement occurred 17–47 msec after the stimulus (horizontal bar 1). By comparison the same amount of motion carried out 140–178 msec after the stimulus (horizontal bar 9) reduced the isometric force (symbol 9) to 18 per cent of the control. The results show, however, that the *absolute* amount of depression was nearly the same for movements performed over a wide interval of time. This is further illustrated in Fig. 5 (filled circles) which is a replotting of the data given in Fig. 4. Here the amount of depression (dashed vertical bars in Fig. 4) has been plotted in relation to the time at which the large movement ended in each case. Similar measurements from two other experiments are also included in Fig. 5. As can be seen the magnitude of depression was nearly the same (to $\pm$ 7 per cent) irrespective of the time at which the movement was carried out within the initial 200–250 msec after the latent period.

It should be pointed out that the results presented in Figs. 4 and 5 do only apply if the test and control releases are carried out from the same *initial* sarcomere length. An alternative way of performing the experiments would be to initiate the contraction at two different resting sarcomere lengths and to record the redevelopment of tension at a given shortened length. This, however, would lead to a difference in duration of the twitches recorded in the two release series. The twitches initiated at the more stretched length would have a longer duration of mechanical activity

and a longer time to peak tension (Edman & Kiessling, 1971). This would to some extent mask the depressant effect produced by the larger movement (cf. Fig. 8 in Edman & Kiessling, 1971).

3. *The depressant effect in relation to load and velocity of shortening*

It was of interest to find out whether the depressant effect of active shortening might be related to the speed of the shortening, the force and

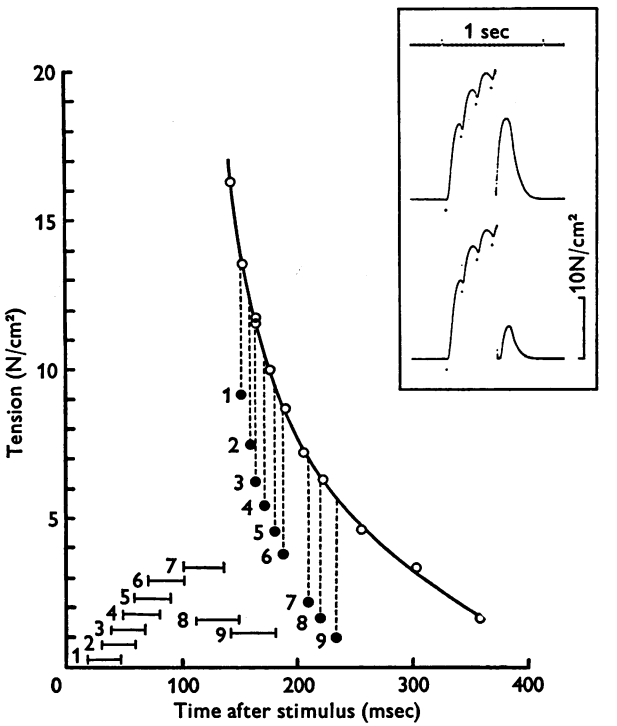

Fig. 4. Depressant effect of active shortening performed at different times during activity period. Measurements carried out during fourth cycle of a series of four incompletely fused twitches. Open circles: peak redevelopment of tension after a small release (control), 0·07 μm/sarcomere. Filled circles (numbered 1–9): peak redevelopment of tension in response to a large release (test), 0·22 μm/sarcomere. Contraction was initiated at the same sarcomere length, 2·20 μm, in both test and control. Numbered horizontal bars: time intervals during which the respective test releases occurred. Dashed vertical distances: tension reduction caused by the extra amount of sarcomere movement that occurred during test release. Inset: complete isometric myograms from ink writer illustrating tension redevelopment after control release (upper) and test release (lower). Maximal tetanic tension: 28 N/cm^2.

the work output of the fibre during the movement. Fig. 6 demonstrates an experiment designed to test these points. In designing these experiments advantage has been taken of the fact (see previous section) that a given amount of shortening at a constant speed is equally effective whenever it occurs during the initial 250 msec after the latent period. In Fig. 6*A* is illustrated the effect of a given amount of movement at maximum speed of shortening. The general approach was the same as described

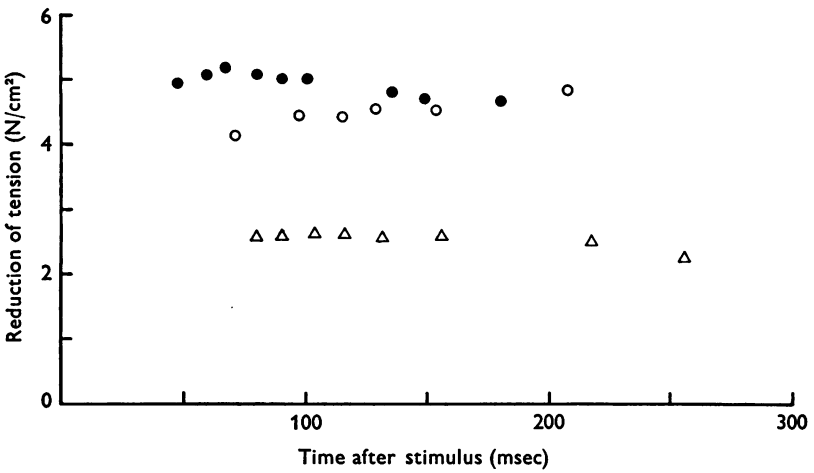

Fig. 5. Reduction of active force plotted as a function of time (after the stimulus) at which the movement ended. Sarcomere shortening in excess of control release: ●, 0·15 μm. ○, 0·16 μm. △, 0·12 μm. Maximal tetanic tension: ●, 28 N/cm². (the same experiment as illustrated in Fig. 4). ○, 32 N/cm². △, 27 N/cm².

in section 1, except for the fact that the analysis was carried out during the last cycle of a series of four incompletely fused twitches. In myogram *a* (control) the amount of release was just enough to cause a complete drop in tension and an immediate redevelopment of force. In myogram *b* the fibre was allowed to shorten over an additional distance (0·15 μm/sarcomere) before being stopped to redevelop isometric tension. The lever movement was fast enough to prevent any tension production during the shortening phase in myogram *b*, which means that the fibre was allowed to shorten at maximum speed. The redevelopment of tension in myograms *a* and *b* occurred at 2·10 and 1·95 μm sarcomere lengths, respectively. As can be seen the extra amount of shortening that occurred in myogram *b* caused a substantial (approximately 4·8 N/cm²) decrease in peak force.

Fig. 6*B* shows the redevelopment of isometric tension after the same

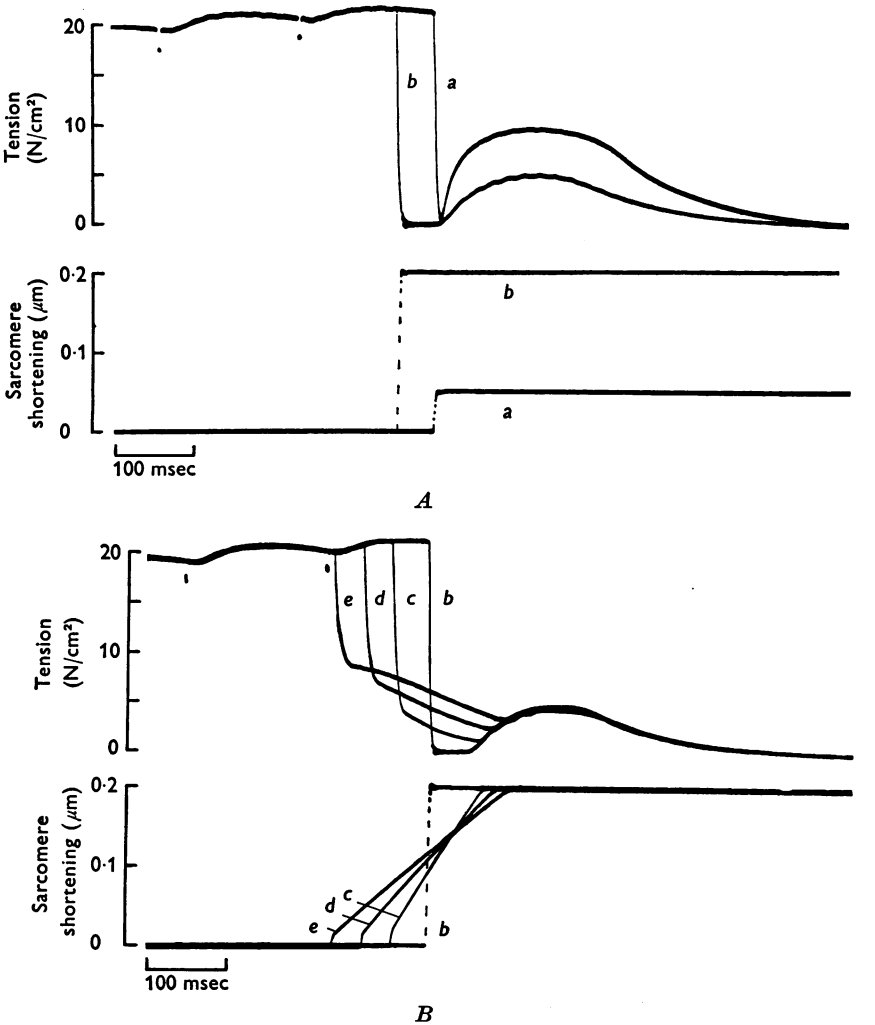

Fig. 6. Depressant effect of active shortening at varied force and velocity during the shortening phase. Release carried out during the fourth cycle of an incompletely fused tetanus. Upper traces: fibre tension. Lower traces: lever movements calibrated in μm sarcomere shortening. *A*, redevelopment of tension in response to a small (0·05 μm/sarcomere, myogram *a*) and a large (0·2 μm/sarcomere, myogram *b*) movement. Lever movement performed in one rapid step so as to prevent force development during shortening phase. *B*, superimposed records (*b*–*e*) illustrating redevelopment of tension after the same amount of sarcomere shortening as in myogram *b* in *A*. In myograms *c*–*e* shortening occurs in two steps, an initial rapid phase and a second slower phase to allow the fibre to produce a certain amount of tension during the movement. Note that the same isometric force is developed after the shortening period in all cases. Maximal tetanic tension: 24 N/cm^2.

amount of active shortening as used in myogram *b* of Fig. 6*A* but at varied velocities of shortening and, hence, varied active force during the shortening phase. The recording illustrated by myogram *b* in Fig. 6*A* has been repeated in Fig. 6*B* and serves here as a control. In myograms *c–e* the lever was programmed to produce an initial rapid length change followed by a less rapid phase. In this way the fibre was constrained to produce a certain force during the shortening period. The time of release was adjusted appropriately so as to get the redevelopment of tension to start at some point on the rising phase of myogram *b* in each case.

It is evident from Fig. 6*B* that the depressant effect of active shortening was not markedly influenced by the velocity of the shortening within the limits investigated. The isometric portions of the myograms can be seen to be almost identical, no matter whether the movement occurred at maximal speed of shortening (myogram *b*) or at a speed (0·4 sarcomere lengths/sec, myogram *e*) that was only 1/5 of the maximum speed of shortening at the temperature considered (cf. Mulieri, Mulieri & Edman, 1974). The same results as illustrated in Fig. 6*B* have been obtained in altogether six experiments. In two of these a conventional spring-loaded isotonic lever (Edman & Kiessling, 1971) was used.

The results illustrated in Fig. 6*B* also demonstrate that the depressant effect was not critically dependent on the tension, or the work output of the fibre during the shortening phase. As can be seen, the tension in the fibre during the movement varied between zero at the highest velocity of shortening (myogram *b*) and 3·4–8·8 N/cm^2 (approximately 1/7–1/3 of the maximal tetanic tension) at the lowest velocity studied (myogram *e*). The external work of each sarcomere segment varied between zero and about 10^{-6} J/cm^2 under these conditions.

4. *The long-lasting nature of the movement effect*

The depressant effect of active shortening is retained over a considerable time and does not disappear merely by restimulation of the fibre. This is demonstrated by the experiment presented in Fig. 7. The fibre was stimulated to produce four incompletely fused twitches. Myogram *a* illustrates the isometric force throughout cycles 1–4 at 2·11 μm sarcomere length. Myogram *b* shows for comparison the force development during cycles 2–4 at 2·11 μm sarcomere length after the fibre has been allowed to preshorten from 2·34 μm sarcomere length during the first cycle. The shortening was ended 5 msec before the second stimulus. The speed of the lever movement during the first cycle was adjusted appropriately to prevent the fibre from producing any appreciable tension during the shortening phase.

It is evident by comparing myograms *a* and *b* in Fig. 7 that movement during the first contraction cycle affects the force production during the subsequent cycles. The peak tension recorded during the second cycle in myogram *b* is thus seen to be considerably smaller than that exhibited during the first cycle in myogram *a*. This is of relevance in view of the fact that the force development starts from approximately the same tension level in both cases and the two records refer to the same sarcomere length. The reduction in peak amplitude of the second cycle in myogram *b* thus reflects a true depression of the fibre's ability to produce force caused by active shortening during the preceding cycle. Clearly, the fibre had not even regained its contractile strength completely during the third contraction cycle as indicated by the fact that the peak amplitude during the third cycle in myogram *b* is lower than that of the first cycle in myogram *a*.

Attempts were made to quantify the depressant effect during the different contraction cycles after an initial shortening phase. The same general approach was used as illustrated in Fig. 7, i.e. the fibre was stimulated to produce four or five incompletely fused twitches in the presence and absence of active shortening during the first cycle. The isometric tension (corresponding to myogram *a* and cycles 2–4 of myogram *b* in Fig. 7) was recorded at 2·28–2·09 μm sarcomere length in the different experiments, i.e. at lengths where the tetanic force was maximal. Preshortening during the first cycle was initiated at 2·95–2·34 μm sarcomere length, the amount of shortening being 0·23–0·75 μm/sarcomere. In the runs where no shortening occurred during the first cycle, a small release (0·05–0·11 μm/sarcomere) was carried out at different times during cycles 2–5 in order to find out the fibre's ability to redevelop isometric force after a complete drop in tension.

The results of a typical experiment are illustrated in Fig. 8. Here the open circles (II–V) represent the peak force recorded during the 2nd–5th contraction cycles when shortening had occurred during the first cycle. The filled circles indicate the peak redevelopment of tension in response to the test releases during cycles 2–5 when there had been *no* shortening during the first cycle. Using these latter data as controls it was possible to estimate the amount of reduction of the contractile force for each of the values II–V.

The results in Fig. 8 show that shortening during the first cycle reduced the peak twitch force during the 2nd cycle to approximately 75% of the control value. The depressant effect diminished during the subsequent contraction cycles and had virtually disappeared by the attainment of the peak of the 4th cycle. Fig. 9 illustrates the results from another experiment in which the interval between the stimuli was smaller. In this case a clear depression of the contractile force can be seen to exist during the

4th contraction cycle. Note, however, that value IV in Fig. 9 occurs about 200 msec earlier after the shortening phase (which ended just before the second stimulus) than value IV in Fig. 8.

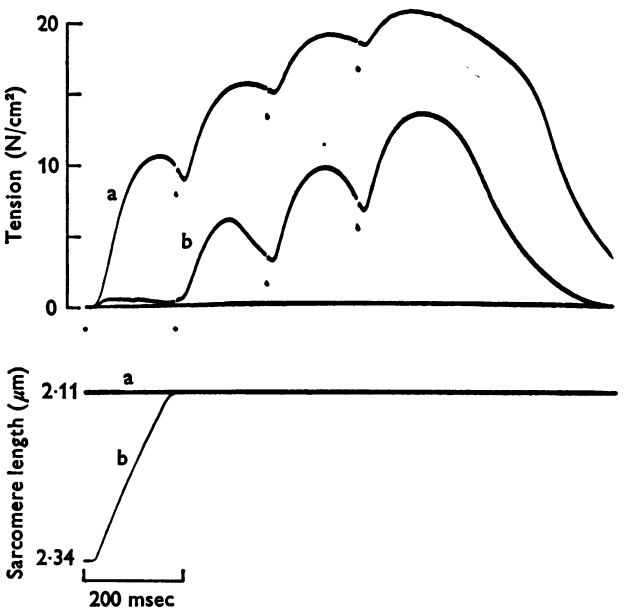

Fig. 7. Isometric tension development at 2·11 μm sarcomere length during incompletely fused tetanus in the absence (myogram *a*) and in the presence (myogram *b*) of shortening during first cycle. Contraction initiated at 2·34 μm sarcomere length during first cycle in myogram *b*. Lower traces: position of electromagnetic lever indicating sarcomere length during myograms *a* and *b*. Maximal tetanic tension: 24 N/cm².

Fig. 10 illustrates the decay of the depressant effect in four experiments performed as described in Figs. 8 and 9. The amount of depression has been plotted as a function of time after the shortening phase in each preparation. With the technique used the first value could be obtained 100–150 msec after the shortening was finished, i.e. at the attainment of peak twitch tension during the second cycle. The depressant effect can be seen to decline steadily, as indicated by the slope of the individual lines in Fig. 10. Note that in spite of the difference in magnitude of the depressant effect and the different frequencies of stimulation between the various fibres, 800–850 msec were required in each experiment for the complete disappearance of the effect.

The duration of the depressant effect is likely to be somewhat underestimated by the approach described in Figs. 8–10 due to the fact that the

test releases, although small, will cause some deactivation of the fibre. According to the results presented in section I, a sarcomere shortening of 0·05–0·11 μm (equivalent to the amount of test release used in the above

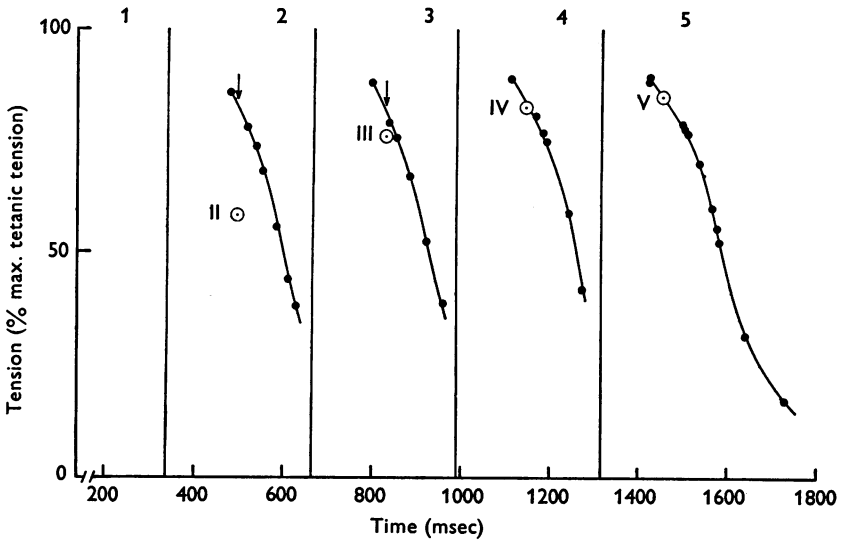

Fig. 8. Reduction of contractile force during cycles 2–5 of an incompletely fused tetanus after shortening during the first contraction cycle. Filled circles: peak redevelopment of tension at 2·09 μm sarcomere length after a small (0·05 μm/sarcomere) release carried out at different times during cycles 2–5, in the absence of shortening during first cycle. Open circles (II–V): peak isometric tension recorded at 2·09 μm sarcomere length during cycles 2–5 after the fibre had shortened from 2·40 μm sarcomere length during the first cycle. Intervals between stimuli (vertical lines): 327 msec. Arrows indicate tension values used as controls for values II and III. Differences between values II–V and their respective control tensions are plotted in Fig. 10 (open triangles). Maximal tetanic tension: 23 N/cm^2.

analysis) can be expected to reduce the contractile strength by 5–10 per cent of the maximal tetanic tension. If the data points in Fig. 10 are corrected to account for this release effect, the curves will intersect with the abscissa at approximately 900 msec instead, i.e. at a slightly higher value than shown in Fig. 10.

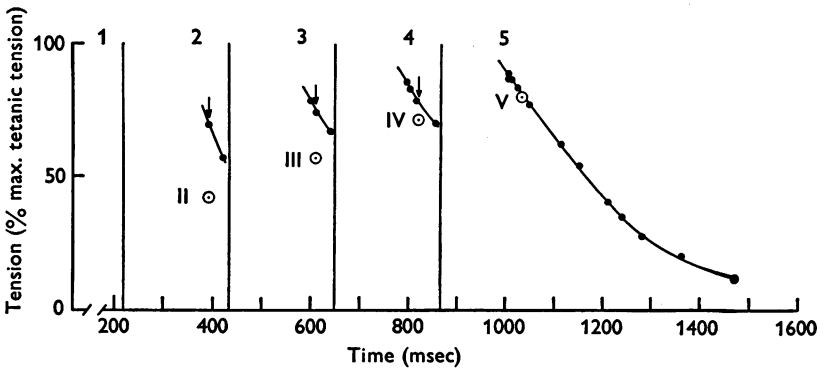

Fig. 9. The same kind of experiment as illustrated in Fig. 8. Stimulation intervals 216 msec. Filled circles: peak redevelopment of tension at 2·20 μm sarcomere length after a small (0·11 μm/sarcomere) release carried out at different times during cycles 2–5, in the absence of shortening during first cycle. Open circles (II–V): peak isometric tension at 2·20 μm sarcomere length during contraction cycles 2–5 after shortening from 2·95 μm sarcomere length during the first cycle. Differences between values II–V and their respective control tensions are plotted in Fig. 10 (filled circles). Maximal tetanic tension: 24 N/cm^2.

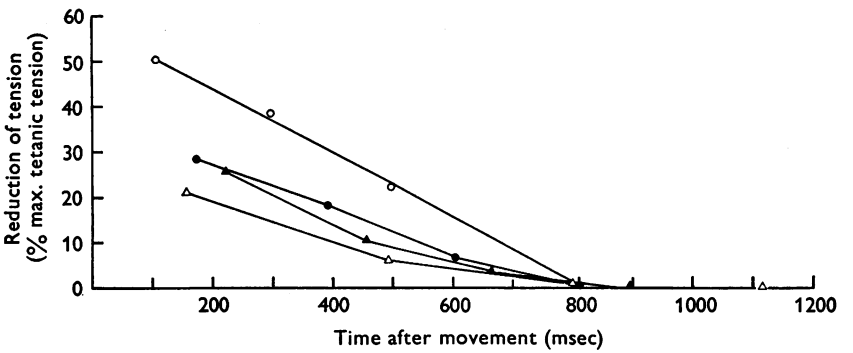

Fig. 10. Time course of disappearance of the depressant effect after active shortening during incompletely fused tetanus. Results of four experiments performed as described in Figs. 8 and 9. Stimulation intervals: ○, 182 msec. ●, 216 msec. ▲, 242 msec. △, 327 msec.

DISCUSSION

Quantitative aspects of the movement effect

Active shortening during a twitch causes a depression of the mechanical activity without markedly affecting the duration of the twitch response. Before discussing the nature of the deactivation it is of interest to consider

some quantitative aspects of the movement effect during muscle contraction. The results have shown that the depressant effect is a function of the amount of sarcomere movement, and it is possible to conclude that the amount of sarcomere shortening that normally takes place during isometric recording is large enough to cause a substantial depression of the contractile output during a twitch. For example, a 5 per cent series compliance, which is a reasonable value in an isolated muscle preparation, would allow the sarcomeres to shorten by 0·1 μm during a twitch at optimal length (2·2 μm sarcomere length). This degree of active shortening can be expected (Fig. 3) to reduce the peak isometric twitch tension by an amount that corresponds to 8–10 per cent of the maximal tetanic tension. The decrease in peak twitch amplitude that is seen after the introduction of extra series compliance in the recording system has previously been explained by Hill (1951) as being due to the longer time it takes to stretch the series elastic element. The present results would seem to make clear, however, that the reduction in force can be attributed, in part, to deactivation of the contractile machinery caused by the extra amount of sarcomere shortening that takes place under these conditions.

The above conclusion that active shortening reduces the isometric force also applies to the redevelopment of tension after a quick release similar to that employed in the classical active state analysis (Hill, 1951; Ritchie, 1954; Ritchie & Wilkie, 1958). By releasing the muscle during an isometric twitch the sarcomeres have to shorten over an additional distance in order to restretch the external series elastic elements, and this leads to further deactivation of the muscle. This validates to some extent the objection against the active state analysis that was raised in the past (e.g. Pringle, 1960) when it was pointed out that the release process itself might affect the outcome of the analysis. As can be deduced from the data in Fig. 3, however, the source of error in a quick-release recording that is caused by active shortening can be made small by limiting the release to 2–3 per cent of the resting muscle length. This corresponds to approximately 0·05 μm sarcomere shortening which would reduce the isometric force by no more than 5 per cent of maximum tetanic tension.

Evidence obtained in studies on whole frog's sartorius muscle has been interpreted to show (Briden & Alpert, 1972) that the abrupt fall in tension that occurs during a quick release may exert a depressant effect on the contractile process which adds to that induced by muscle shortening. Account has been taken of this possibility in the present study by allowing the same tension drop in both test and control in the experiments used to quantify the movement effect. The results give no reason to believe, however, that a drop in tension *per se* affects the mechanical activity to any significant degree. As shown in Fig. 6 the initial tension drop may be varied

over a considerable range without noticeable effect on the redevelopment of tension after the length change.

Nature of movement effect

Excitation-contraction coupling

The depressant effect of active shortening is probably not caused by an altered release of activator calcium. This is inferred from the fact that a given sarcomere shortening is equally effective in reducing the contractile force when carried out at various times during the rising phase of the twitch, i.e. at instants when the rate of release of calcium can be assumed to differ greatly (Jöbsis & O'Connor, 1966; Ashley & Ridgway, 1970). A movement effect can indeed be demonstrated late during the relaxation phase when the release of activator calcium is likely to have ceased completely and the concentration of free calcium in the myoplasm has been restored to a very low level (Jöbsis & O'Connor, 1966; Ashley & Ridgway, 1970).

The experimental findings also provide evidence against the idea that the movement effect is due to an enhanced elimination of calcium from the myofibrillar space. An increased rate of removal of the activator agent would cause an abbreviation of the twitch response. The results would seem to make clear, however, that movement causes a diminution of the tension output but does not change the total duration of the twitch significantly. The experimental findings are thus consistent with the view that the movement effect represents a decreased response of the myofilament system to the activator calcium rather than altered kinetics of the release and elimination of the activator.

It appears unlikely that the reduction in contractility is due to non-uniform shortening of the sarcomeres along the length of the fibre. A decrease in active force would be anticipated if some sarcomeres were shortening to lengths below 2·0 μm where they would form a weaker segment of the fibre. Evidence against this possibility is provided by the finding (K. A. P. Edman, unpublished) that a given sarcomere shortening causes approximately the same amount of depression of the contractile force when carried out between 2·7 and 2·5 μm sarcomere length as between 2·1 and 1·9 μm sarcomere spacing. Furthermore, examination of the laser diffraction pattern in different regions of the fibre, using the technique previously described (Cleworth & Edman, 1972), did not reveal any obvious difference in the distribution of sarcomere lengths after a small (0·05 μm/sarcomere) and large (0·25 μm/sarcomere) fibre movement.

There is no reason to believe that the decrease in active force after shortening is due to depletion of chemical energy for the contractile process. All the evidence

would seem to suggest that the supply of chemical energy does not under normal conditions limit the force production during a twitch or a brief tetanus of amphibian skeletal muscle (e.g. Mommaerts, 1969). This is supported by the present results which show that the depressant effect is not significantly affected by variations in the work output during the shortening phase.

Structural change

The interesting possibility emerges from the present analysis that the movement effect is ultimately based on a structural change in the myofilament system caused by active sliding of the A and I filaments. Such a change may account for the longlasting nature of the depression and the fact that the contractile strength cannot be restored merely by restimulation of the fibre. There is evidence from *in-vitro* studies (Morawetz, 1972) that a protein structure may undergo a rapid change in response to a physical or chemical intervention and that such a change may stay over a relatively long time. Conformational changes with a life time in the range of seconds have been reported (Antonini & Brunori, 1970; Morawetz, 1972). The 800–900 msec lifetime recorded for the movement effect in the present study is thus fully compatible with the idea of a structural change in the myofilament system.

While a structural change in the myofilament system would seem to be a plausible cause of the movement effect, the precise nature and location of the change is still unknown. It is clear that the muscle fibre has to shorten actively for the depressant effect to appear; passive shortening carried out immediately before the stimulus is thus without influence on the twitch amplitude (K. A. P. Edman, unpublished). It is therefore logical to assume that the *active* propulsion of the A and I filaments causes the change that is responsible for the decrease in contractile force. The results would seem to make clear, however, that the movement effect is not related in a quantitative way to the degree of interaction between the A and I filaments. This is inferred from the finding that the velocity of shortening, and hence the force generated by the contractile system during the movement, can be varied over a wide range without affecting the depressant effect. It would seem that the displacement *per se* of the thin filaments into the array of thick filaments during contraction brings about a change in the contractile system that causes the transitory decline in contractile force.

The lack of dependence of the depressant effect on the active force during the shortening phase may be interpreted to mean that the structural change is not contained within the crossbridge unit itself. Another conceivable locus of the change is the I filament. Results obtained in X-ray diffraction studies (Hanson, Lednev, O'Brian & Bennet, 1972; Haselgrove, 1972; Huxley, 1972) support the view that the ability of the A and I filaments to interact is governed by the sterical arrangement of the

troponin-tropomyosin complex along the length of the thin filament. A possibility worthwhile considering in the light of the present results would be that the physical state of this regulatory protein complex is affected as the thin filament slides during contraction, leading to a transitory impairment of the interaction between the actin and myosin units.

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