The Deficit of the Isometric Tetanic Tension Redeveloped after a Release of Frog Muscle at a Constant Velocity

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ABSTRACT   Frog sartorius muscles tetanized isometrically were released at a constant velocity from lengths l_L to l_S ($\Delta l = l_L - l_S$; $l_S > l_0$). The tension P_S^* redeveloped after the release was lower than the isometric tension P_S at l_S, and higher than the isometric tension P_L at l_L. The tension deficit D is defined as the difference $P_S - P_S^*$. The timing of the release during the tetanus did not influence D. D/P_0 was proportional to $\Delta l/l_0$. The proportionality constant k was equal to 1.35 ± 0.19 ($n = 8$) when the velocity of release was 2.5 mm/s. When the muscles were released the same Δl, D was found to be an exponential decreasing function of the velocity. The tension deficit was also found in experiments performed in the region $l_S < l_0$. The proportionality constant k was smaller, but the influence of the velocity of the release on D was not modified. When the velocity of the release was changed during the release, D changed accordingly, showing that the effects of Δl and V are multiplicative. These facts suggest a working hypothesis based on the concept that the actin filaments which enter the overlap region during a release are strained by the tetanic stress and therefore unable to make normal cross-bridges.

INTRODUCTION

The present theories of muscular contraction rest on two basic postulates: (a) the isometric tetanic tension is a single-valued function of muscle length and therefore of the degree of overlap between the two sets of interdigitating filaments of the myofibrils; and (b) the isometric tetanic tension does not depend on the previous length changes that have occurred during the contraction (Huxley, 1974; White and Thorson, 1973).

Several experiments have been reported which seem to contradict these postulates. The tension-length diagram obtained by tetanizing the muscle isometrically at various lengths is not the same as that obtained by recording the active shortening of muscles which pull a free-hanging load (Buchthal, 1942; Maréchal, 1955; Delèze, 1961; Matsumoto, 1967). The velocity of isotonic shortening measured at some given length depends on the starting length (Carlson, 1957; Bahler et al., 1968). The tension during isovelocity release depends on the distance of the release, at least in frog slow muscle fibers (Floyd and Smith, 1971).

The tension which redevelops isometrically after a release at slow velocity is smaller than that which redevelops after a release at fast velocity (dogfish jaw

muscle, Abbott and Aubert, 1952; frog sartorius, Aubert, 1956; Aubert and Lebacq, 1971; frog semitendinosus, Delèze, 1961; isolated frog fibers, Buchthal, 1942). This paper reports a detailed analysis of this phenomenon.

The results of our experiments suggest an hypothesis which can account for the Abbott and Aubert phenomenon within the frame of the sliding filament theory, as well as for the other experiments described in this introduction.

METHODS

Muscles

The experiments were done on sartorius muscles of frogs (*Rana temporaria*) at 0°C. The Ringer solution contained 113.5 mM NaCl, 2.0 mM KCl, 1.8 mM $CaCl_2$, and 1.9 mM phosphate buffer, pH 7.5, and was gassed with O_2. The average tension of the muscles expressed as $P_0 l_0/W$ was 260 ± 5.0. N·mm/g (n = 68), where P_0 is the maximum isometric force, l_0 the reference length, and W the wet drained weight.

Mechanical Arrangements

FORCE MEASUREMENTS   Each muscle of a pair was tied with a cotton loop (compliance 0.1 mm/N) to a light silver chain connected to a strain gauge. The tension-time curves of each muscle were recorded on a fast UV recorder (S.E. Laboratories, Feltham, England, model 3006) equipped with galvanometers (type B160, S.E. Laboratories) having a natural frequency of 160 Hz. The records obtained with the UV-sensitive paper are unfortunately very poor for reproduction. We have therefore redrawn and superimposed them in order to save space.

The resting tension was never higher than 5% P_0 in the experiments performed at l_s = 1.14 l_0 and negligible in the other experiments; it was subtracted from the measured tension for the quantitative analyses. The active tension was measured at the time of the last stimulus. However, the tensions decreased somewhat during the tetanus, and we examined the results obtained with another method: as the tension decay was very nearly linear with time (see Fig. 1, curves A and B), it was possible to extrapolate the redeveloped tension back to the time at which the movement began, the isometric tensions also being measured at that time. As the results led to the same conclusions in both cases, we have reported only the data analyzed with the first method. All the tensions were expressed in P_0 units, P_0 being the maximal isometric tension produced at l_0 at the beginning of the experiments.

ERGOMETER   The principle of the electromagnetic ergometer was similar to that described by Machin and Pringle (1959). Two strain gauges were fixed to a small rod attached to the coil of an electromagnetic vibrator (Ling Altec Shaker, type 201, Altec Corp., Anaheim, Calif). This coil was activated by a ramp generator via a power amplifier. The magnitude and time-course of muscle length changes were determined by suitable choices of the voltage signal produced by the ramp generator. Accurate control of the coil movements by the input voltage was obtained by a feedback loop through a differential displacement transducer (type 7DCDT, Hewlett-Packard Co., Palo Alto, Calif.) which indicated the position of the vibrator. The velocity of the movement was adjustable in both directions from 0.2 up to 72.2 mm/s. Differentiation of the signal from the displacement transducer showed that the final speed was reached in <2 ms and that it did not vary by > 1% during the whole movement, within a range of 4.4 mm. There was negligible phase shift for sinusoidal movements up to 180 cycle/s. The compliance of the ergometer was 2 μm/N. The movement was triggered by a digital

electronic device either at the beginning of the tetanus or at a time when the tension had reached a steady level, i.e., 0.8 s later.

STIMULATION Each muscle of a pair was stimulated with supramaximal (120%) condenser discharges (time constant 5 ms) of alternating polarity applied through an assembly of eight platinum electrodes. The frequency of stimulation was 25/s, high enough to produce a well-fused tetanus at 0°C. The duration of the tetanus was usually 3.5 s, which was long enough to allow the tension redeveloped after the shortening to reach a plateau.

LENGTH MEASUREMENTS Before each experiment, the sarcomere length in the central zone of the muscle was measured by light diffraction and adjusted to 2.25 μm. The corresponding external length was taken as the reference length l_0; it was slightly longer (by about 0.5 mm on the average) than the reference length as defined by Hill (1952). All the lengths are expressed in l_0 units.

Experimental Design

Every muscle was tetanized seven or eight times, once every 15 min, the release during each tetanus being different either by its magnitude or by its velocity. The tetani were then repeated in a 'mirror' order (for an example of a 'series and reverse' records, see Fig. 2 A and B). All the data reported here are the means of measurements made on the two corresponding records.

A complex experimental design (balanced incomplete blocks) was used in the experiments described in section 5 of Results. The principle was to compare the two muscles of the same pair, subjected to exactly the same treatment but not at the same length, l_S. In order to keep the dimensions of this design to a manageable size, we limited to three the number of l_S's studied in one block, and we performed four such sets. The first set contained three muscle pairs, at lengths $(0.93 \, l_0, 1.07 \, l_0)$; $(0.93 \, l_0; l_0)$ and $(l_0, 1.07 \, l_0)$. The second block contained the pairs $(0.86 \, l_0, 1.14 \, l_0)$, $(0.86 \, l_0, l_0)$ and $(l_0, 1.14 \, l_0)$. Both sets were repeated once. In any particular group, the treatment pairs were assigned at random to the muscle pairs. The data were analysed according to Li (1964). For another example of this kind of experimental design, see Canfield et al. (1973).

RESULTS

1. Tension Deficit

Fig. 1 shows the basic experiment. A frog sartorius muscle was tetanized isometrically at 0°C for 3.5 s, either at a long length $l_L = 1.11 \, l_0$ or at a shorter length $l_S = 1.03 \, l_0$. Curve A shows the isometric tension P_S maintained at l_S, and curve B shows the isometric tension P_L maintained at l_L. P_S is larger than P_L because the overlap between the two sets of filaments increases when the muscle shortens from l_L to l_S.

Curve C shows a third tetanus with release. The tetanus was isometric at l_L for 0.8 s, the time-course of the tension being identical to curve B. The muscle was then shortened to L_S at a constant velocity of 2.5 mm/s, and redeveloped tension P_S^* after the end of release (the subscript S means that the tension is isometric at l_S, and the asterisk means that the tension is redeveloped after a release). We define the tension deficit D by the difference $P_S - P_S^*$.

$$D = P_S - P_S^*. \tag{1}$$

Abbott and Aubert (1952) reported three properties of D: (a) tension deficit depends on the velocity of the release, being 24% P_S at 0.5 mm/s and 6% P_S at 8 mm/s; (b) tension deficit lasts for the duration of the tetanus; and (c) if excitation is interrupted long enough for the tension to drop to zero, the full isometric value is reestablished when excitation is resumed. We fully confirm these observations. Moreover, we report in this paper detailed analyses on the effect a, and we bring new data on the effect of the extent of shortening and the effects of changes in the velocity of shortening during the release.

2. Influence of the Timing of the Release

This factor was tested in experiments similar to that shown in Fig. 1. The releases (from l_L = 1.13 l_0 to l_S = 1.05 l_0) at 2.5 mm/s began either with the stimulation or with a delay of 0.8 or 1.6 s. The tension deficit was exactly the same in three experiments of this type. This result indicates that the tension deficit is not linked to any of the many phenomena which vary during the tetanus: early pH changes (Distèche, 1960), labile maintenance heat (Aubert, 1956), phosphorylcreatine breakdown, Na influx, and K efflux.
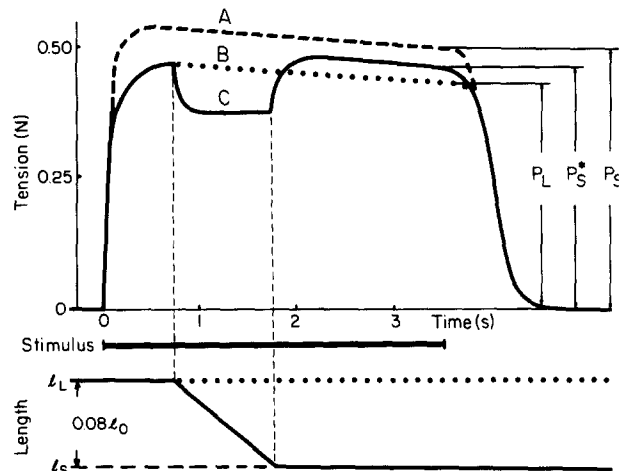

FIGURE 1. Three superimposed fused tetani of a frog sartorius muscle showing the Abbott and Aubert phenomenon. Upper curves: tension. Lower curves: length. (A) Bars: isometric at l_S = 1.03 l_0. (B) Dots: isometric l_L = 1.11 l_0. (C) Solid curves: release from l_L to l_S, after an isometric period at l_L of 0.8 s; Δl = 0.08 l_0; l_0 = 31 mm. Duration of tetani (horizontal black bar): 3.5 s. P_S and P_L are the isometric tensions at l_S and l_L; P_S^* is the tension redeveloped isometrically at l_S after a release from l_L to l_S. Weight = 64 mg. Experiment of 21 January 1971.

3. Influence of the Extent of the Release

This influence is shown in Fig. 2 A and B. The muscle was first tetanized isometrically for 3.5 s at length l_S = l_0 (curve 1, Fig. 2 A). After relaxation, the resting muscle was lengthened by 0.02 l_0 (0.56 mm); 15 min later, the muscle was tetanized again (curve 2, Fig. 2 A) with a release at a constant velocity of 2.5

mm/s from $l_L = 1.02\,l_0$ to $l_S = l_0$, starting 0.8 s after the first stimulus. The following tetani were similar, except that l_L was gradually increased by steps of $0.015\,l_0$ (curves 3–7, Fig. 2 A) and then decreased by the same steps (curves 8–13, Fig. 2 B). The last tetanus (No. 14) was isometric at the short length l_S. The figure shows that the tension redeveloped decreases as the magnitude of shortening increases. The effect is obviously more prominent in Fig. 2 A than in Fig. 2 B. It seems therefore that fatigue enhanced the effect in Fig. 2 A but reduced it in Fig. 2 B. To correct partially for this effect we averaged the measurements made on two corresponding tetani (i.e., No. 2 and No. 13, No. 3 and No. 12, etc.).
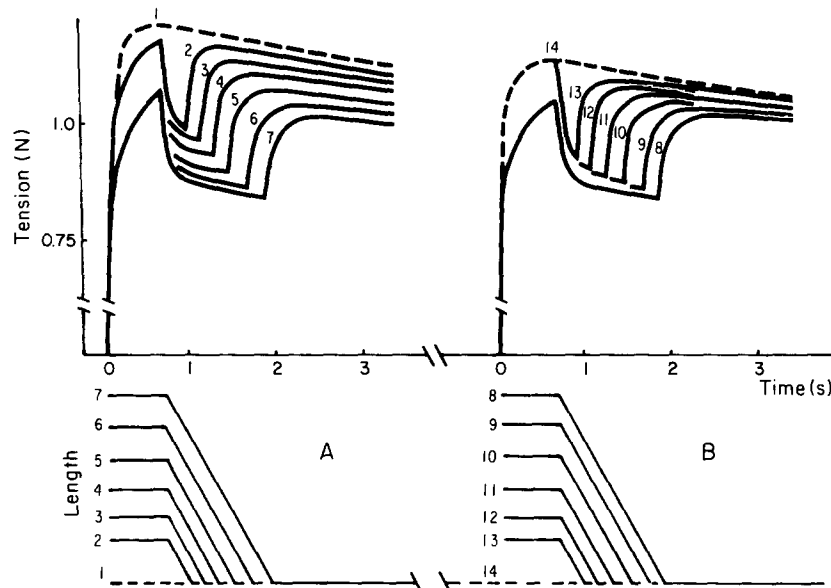

FIGURE 2. The effect of the extent of shortening on the tension deficit. The tetani are arranged in a "mirror" sequence. The numbers refer to the number of the tetanus in the series. Upper curves: tension; lower curves: length. Curves 1 and 14: isometric at $l_S = l_0$. Curves 2–7 in A, and in reverse sequence, curves 8–13 in B: releases to l_S at a velocity of 2.5 mm/s from 1.02, 1.03, 1.05, 1.06, 1.08, and 1.10 l_0. The fast initial tension transients during the shortening have not been redrawn in eight curves in order not to distort the figure. $l_0 = 28$ mm. Weight = 121 mg. Experiment of 10 November 1971.

Fig. 3 shows that the tension deficit D (as defined by Eq. 1 and expressed in P_0 units) is a linear function of the extent of shortening Δl (expressed in l_0 units). A linear regression analysis of these data according to the equation:

$$D = k\,\Delta l, \tag{2}$$

yielded k: 1.32 (statistical probability < 0.001). Seven other experiments gave similar results. The mean k of the eight experiments is 1.35 ± 0.19 (SEM).

4. Influence of the Velocity of the Release

A typical experiment is shown in Fig. 4. The muscle was tetanized isometrically at l_L = 1.18 l_0 (dots) and at l_S = 1.11 l_0 (bars) or with releases from l_L to l_S at various velocities, starting at a constant time. The tension redeveloped after the release is always larger than P_L but smaller than or equal to P_S. The tension deficit is maximal after the release at the slowest velocity (1.4 mm/s). It decreases as the velocity of shortening increases and, eventually, becomes imperceptible when the velocity of shortening is 25.6 mm/s.

D is plotted as a function of the velocity of shortening V in Fig. 4 B (filled circles). The solid curve drawn through the points is described by Eq. 3:

$$D = A \ e^{-V/\alpha}, \tag{3}$$
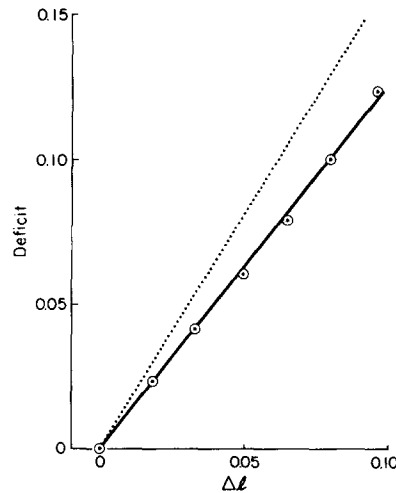
with A = 0.10 and α = 5.7 mm/s.

FIGURE 3.   The tension deficit $D = P_S - P_S^*$ (measured on the curves shown in Fig. 2 and expressed in P_0 units) is plotted as a function of the magnitude of the release Δl expressed in l_0 units (solid line). Each point is the mean of the "series" (Fig. 2 A) and the "reverse" (Fig. 2 B). The stippled line, slope = 1.61, is a theoretical line explained in the Discussion.

Table I shows the results obtained for five other experiments. The means A and α (with their SEM) observed are 0.101 ± 0.006 and 6.1 ± 0.24 mm/s, respectively.

The next question to consider is whether D depends on the tension maintained during the release, P_r. The answer is difficult because P_r varies during the release. The initial fast change arises from the discharge of the series elastic elements; in fast releases, this change is quantitatively the more important but it seems that it is not a major factor as D is then very small. The tension after the fast tension drop is maintained during the longer part of the slower shortenings at a level which does not change much. We have chosen the tension measured just at the end of the release, P_r', as an estimate of P_r (expressed in P_0 units) because this estimate allows us to evaluate the force-velocity of the muscle at

length l_S. Using the force-velocity relationship and knowing the function which relates D to V, D can be expressed as a function of P'_r. It is convenient to use here the exponential form of the force-velocity curve of Aubert (1956), neglecting the small force f, rather than using the classical hyperbolic form of Hill. Substituting V solved in the force-velocity equation into Eq. 3 we obtain

$$D = A' \ (P'_r)^\gamma. \tag{4}$$

Each experiment was analyzed according to this equation. If the reasoning is true, than A' should equal A and γ should equal B/α (B being the velocity constant of the exponential form of the force-velocity relation).
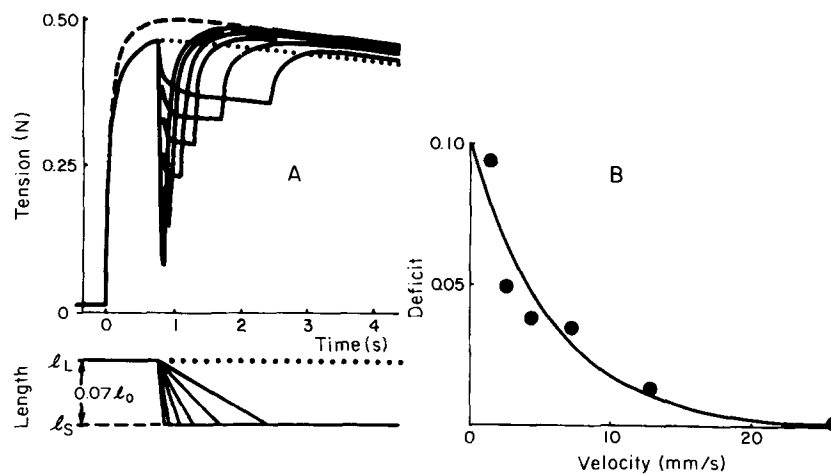

FIGURE 4. The effect of release velocity on the tension deficit. (A) Eight superimposed tetani (series part of the experiment). Upper curves: tension. Lower curves: length. Bars: isometric at $l_S = 1.11 \ l_0$. Dots: isometric at $l_L = 1.18$. Solid curves: releases from l_S to l_L ($\Delta l = 0.07 \ l_0$, at velocities of 1.4, 2.5, 4.4, 7.2, 12.9, and 25.6 mm/s). The fast initial tension transients have not been redrawn in four curves, in order not to distort the figure. (B) The tension deficit $D = P_S - P_S^*$ expressed in P_0 units, as a function of the velocity of the release. Means of the series (shown in A) and the reverse of the experiment $l_0 = 30$ mm. Weight = 45 mg. Experiment of May 1977.

Table I shows that A' is somewhat higher than A; the mean ratio B/α is equal to 2.57 ± 0.10, and is thus equal to γ (2.50 ± 0.11). We conclude that D is as well described by Eq. 4 as by Eq. 3. A and A' are two different estimates of the maximal D obtained for an infinitely slow velocity of release. As $A < P_S - P_L < A'$, it is reasonable to conclude that the maximal D is approximately equal to $P_S - P_L$. This conclusion is important insofar as it suggests that the tension deficit is brought about in that part of the filaments which enter the overlap region during the shortening.

5. Influence of the Length on the Effect of Shortening

All the experiments reported above were performed at a final length l_S longer than l_0. In this case, $P_L < P_S^* < P_S$. In these experiments, the mean sarcomere

spacing was larger than 2.25 μm, before as well as after the release, and therefore there was a direct proportionality between muscle length and the fraction of bridges at either end of thick filament which overlap the corresponding thin filaments (stage 1-2 of Gordon et al., 1966).

We performed some experiments to explore the region in which all the myosin molecules make bridges, i.e., when the sarcomere spacing is < 2.20 μm. If the release is made from $l_L = l_0$ to some $l_S < l_0$, then $P_L > P_S$, which is the inverse of the situation represented in Fig. 1. The tension redeveloped after the release is still smaller than P_S. There is thus still a tension deficit which can be defined by Eq. 1.

This tension deficit depends on the extent of shortening Δl. The phenomenon was studied in experiments similar to those described above in section 3 (with the same Δl and the same velocity of release). Eq. 2 applies in this case as

TABLE I

INFLUENCE OF THE VELOCITY OF A RELEASE ON THE TENSION REDEVELOPED AFTERWARDS ($l_S > l_0$)

l_S	$P_S - P_L$	Velocity of release $D = \times 1,000$						A	α	A'	γ
		25.6	12.9	7.2	4.4	2.5	1.4				
	$\times\,1,000$			mm/s				$\times\,1,000$	mm/s	$\times\,1,000$	
1.17	170	1	13	35	38	48	94	100	5.7	134	2.54
1.10	140	1	26	32	52	68	99	127	5.7	253	2.58
1.10	120	2	17	33	49	71	98	108	6.5	206	2.29
1.06	120	1	18	24	31	51	78	86	6.1	124	2.66
1.08	120	0	7	28	42	68	94	103	5.4	135	2.85
1.11	140	2	17	29	37	50	83	83	7.0	143	2.07
1.10	140	1	16	30	42	59	91	101	6.1	166	2.50
±0.02	±80	±0.3	±2.6	±1.6	±3.2	±4.4	±3.5	±6.5	±0.24	±21	±0.11

In the six experiments shown, the muscles were released from l_S + 0.08 l_0 to l_S at various velocities, starting 0.8 s after the beginning of the stimulation. The tension was measured at 3.5 s, and the difference between P_S (isometric tension at l_S) and P_S^* (tension redeveloped after the release) is given for various velocities of release, being expressed in 1,000P_0. These results are analyzed according to Eqs. 3 (A and α) and 4 (A' and γ). The last two lines of the table show the mean and standard error of each parameter for the six experiments.

well but with a lower constant k. For $l_S = 0.87$ l_0, $k = 0.92$ (mean of four experiments). When l_S was equal to 0.93 l_0, k increased to 1.20 (mean of four experiments). These results suggest that k increases with l_S. Therefore, we made further experiments to see whether the increase of k with l_S would still occur when l_S is higher than l_0. In three series of experiments in which l_S was equal to 1.00 l_0, 1.07 l_0, and 1.14 l_0, we found that k did not change any more with l_S: it was equal to 1.35, 1.35, 1.38, respectively (each value is the mean of four experiments). As these experiments were made according to a special design (incomplete randomized blocks; see Methods), the standard errors of all the means quoted above are the same and equal to 0.18.

The tension deficit when $l_S < l_0$ depends on the velocity of release in a manner similar to that found when $l_S > l_0$ (section 3 above). Table II shows the results obtained in six experiments which were in every respect similar to those

reported in Table I, except for one thing: l_s was lower than l_0. It is clear that the dependence of the tension deficit on the velocity of the release is only slightly influenced by l_s: α is slightly higher (7.6 ± 0.8 against 6.1 ± 0.2), as well as A (0.135 ± 0.015 against 0.101 ± 0.006), but A' (0.144 ± 0.020 against 0.166 ± 0.021) and γ (2.46 ± 0.33 against 2.50 ± 0.11) are not different.

6. Influence of a Change in the Velocity of Shortening during the Release

The experiments described above have shown that the velocity and the extent of the release control the tension deficit. They give no information on the influence of the tension maintained at each instant during the release, P_r. This is so because P_r does not change much during isovelocity releases, except for a fast initial transient. The question now is whether D depends only on the state (V and P_r) at the end of the release, or whether it depends on the state (V and P_r) at each moment of the release. In order to answer it, we made experiments in which V was changed suddenly *during* the release.

TABLE II

INFLUENCE OF THE VELOCITY OF A RELEASE ON THE TENSION REDEVELOPED AFTERWARDS ($l_s < l_0$)

		Velocity of release									
		$D = \times 1,000$									
l_s	P_s-P_L	25.6	12.9	7.2	4.4	2.5	1.4	A	α	A'	γ
	$\times 1,000$			*mm/s*				$\times 1,000$	*mm/s*	$\times 1,000$	
0.92	160	12	50	72	100	118	136	155	9.8	163	2.03
0.92	110	6	19	37	57	67	88	89	9.2	96	2.39
0.93	70	1	16	34	55	75	93	125	5.4	107	2.17
0.93	130	3	29	70	89	120	167	199	6.3	220	2.80
0.92	70	1	8	17	53	64	112	124	5.4	171	3.89
0.92	20	7	30	51	68	82	118	117	9.2	109	1.46
0.92	90	5	25	47	70	88	119	135	7.6	144	2.46
	±20	±1.7	±5.9	±8.8	±8.3	±10.2	±12	±15	±0.84	±20	±0.33

Experiments reported are similar to those reported in Table I, except that the length reached at the end of the release is smaller than l_0 rather than larger.

In the first experiment, shown in Fig. 5 A, V was changed from 2.5 to 25.6 mm/s when the release was half-way. The tension dropped sharply during the release at high velocity, but it did not redevelop to the isometric control P_s (bars) as it would if D depended only on the tension maintained at the end of the release. The tension deficit was half that observed when the release was made at the slow velocity all the way down (dots). It was not changed when the order of the velocities was reversed. In another experiment, the velocity of the release was increased from 2.5 to 25.6 mm/s when the release was 27, 50, 76, or 100% complete. The tension deficit D was measured as a fraction of that observed when the release was slow all the way, D_S. Fig. 5 B shows the linear relationship obtained between D/D_S and the percentage of the movement at low velocity.

The last series of experiments shows that the effect of V on D is not modified by a previous slow shortening (Fig. 6). The velocity of shortening is slow (2.5 mm/s) for half the release; it is then decreased to 1.4 mm/s (open circles) or increased to 7.2 mm/s (fine bars) or 25.6 mm/s (dots). The tension deficit which is built up during the second half of the release can be estimated by comparison with a release which is stopped half-way (thick line), and analyzed according to Eq. 3. The mean velocity constant α ($n = 4$) was 7.9 ± 1.0, a value which is not different from that reported above (Table I: 6.10 ± 0.24). Thus, α is not sensitive to a previous shortening.
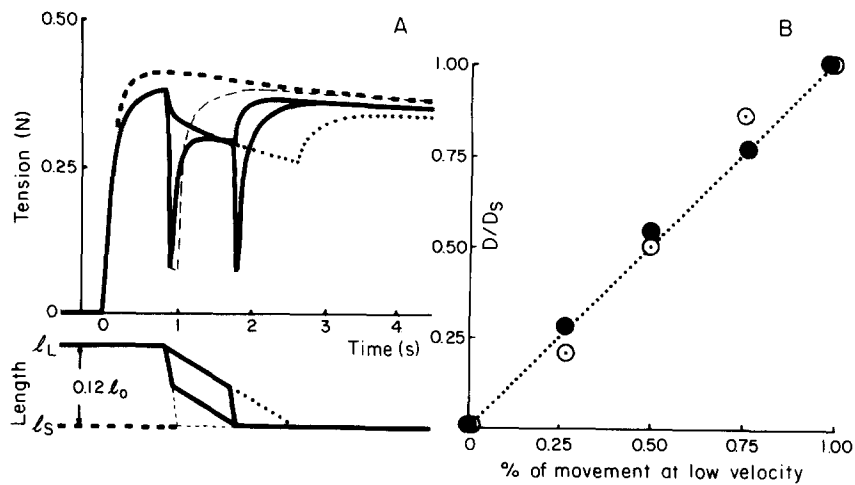

FIGURE 5. (A) Five superimposed tetani showing the interaction between velocity and extent of release. Upper curves: tension. Lower curves: length. Bars: isometric at $l_S = 1.05 \, l_0$. The other curves show tetani with releases from 1.16 l_0 to 1.05 l_0 ($\Delta l = 0.12 \, l_0$). Dots: velocity of the release, 2.5 mm/s (this curve is partly covered by the continuous lines). Fine bars: release velocity, 25.6 mm/s. Solid curves: the velocity of the release is changed from 2.5 to 25.6 mm/s, or from 25.6 to 2.5 mm/s when the muscle has shortened half-way. $l_0 = 32$ mm. Weight = 48 mg. Experiment of 28 April 1977. (B) The effect of a change in velocity during the release on the tension deficit. Six tetani: isometric at 1.03 l_0; with releases ($\Delta l = 0.12 \, l_0$) at a constant velocity of 25.6 mm/s or of 2.5 mm/s; with releases at two velocities—the release begins at 2.5 mm/s and its velocity is increased to 25.6 mm/s when the magnitude of the shortening is 0.27, 0.50, and 0.76 Δl, respectively. The tension deficit $D = P_S - P_S^*$ is expressed in percent of D_S, the tension deficit measured when the whole release is at 2.5 mm/s. It is plotted (●) as a function of the percentage of the release at the slow velocity. (O) The same measurements made on the heterolateral muscle. The dotted line has a slope = 1. $l_0 = 32$ mm. Weight = 52 mg. Experiment of 28 April 1977.

DISCUSSION

Our experiments show that the tension P_S^* which is redeveloped after a release to a length l_S remains usually lower than the tension P_S maintained at l_S in a tetanus isometric from the start. The tension deficit defined as $D = P_S - P_S^*$ has

the following properties: (a) Its magnitude is set by a process which occurs during the shortening, because D depends on the mechanical characteristics of the release (extent and velocity). (b) It is not influenced by processes which occur before or after the release, because the timing of the release is without effect, and D persists after the release as long as the muscle is tetanized. (c) The process responsible for D is active at each moment during the shortening, because D is proportional to Δl (Eq. 2) and it is enhanced by increasing the load (Eq. 4). (d) The state of the muscle (V and P_r) during the second half of a release does not influence the tension deficit which is created during the first half and reciprocally. More specifically, it is not possible to inhibit a tension deficit by suddenly unloading the muscle at the end of the release.
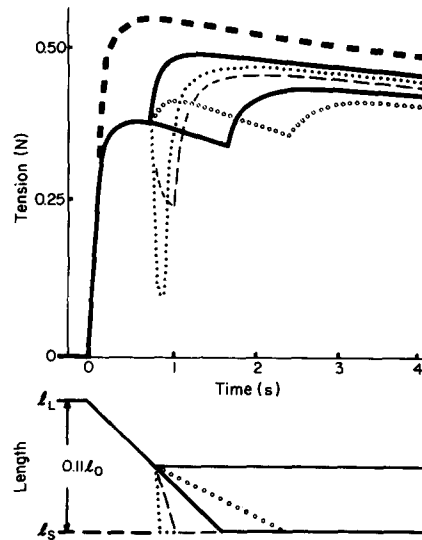

FIGURE 6. The additive effects of different release velocities on the tension deficit. Six superimposed tetani. Upper curves: tension. Lower curves: length. Bars: isometric at 1.03 l_0. The other curves show releases from 1.13 l_0 to 1.03 l_0 at a velocity of 2.5 mm/s, which starts with stimulation. When the shortening is half-way (i.e., 0.7 s after the start), it is either stopped (middle solid curves), slowed to 1.4 mm/s (open circles) or quickened to 7.2 or 25.6 mm/s (fine bars and dots), or it remains at 2.5 mm/s (lower solid curve). l_0 = 30 mm. Weight = 66 mg. Experiment of 25 April 1977.

Our results demonstrate that the tension deficit is precisely controlled by the mechanical parameters of the release. This fact is sufficient in our opinion to rule out many possible explanations for the tension deficit: geometric distortions of the fibres which make up a muscle; redistribution of sarcomere lengths during the release (Hill, 1953); accumulation or exhaustion of a substance in the sarcoplasm (phosphorylcreatine splitting, pH changes, or ionic fluxes); failure of the activation system (the depressant effect of Edman [1975] is not sensitive to wide variations of V). We can think of two possible explanations to account for

D in terms of mechanochemical processes: (a) inhibition of the cross-bridges being the result of the work done during the release or (b) stress inhibition of the cross-bridges which are formed by that part of the filaments which enter the overlap region during the shortening.

Hypothesis a explains the proportionality between D and Δl (Eq. 2) and the positive correlation between D and P_r (Eqs. 4 and 5 b), for which it would, however, predict a linear relationship rather than the power function experimentally found. Also, this hypothesis does not provide any reason why there is a limit to the tension deficit which is never larger than $P_S - P_L$ (when $l_S > l_0$). These difficulties are, however, not decisive.

Hypothesis b seems more attractive, as it explains quantitatively Eq. 2, qualitatively Eq. 4, and accounts also for the fact that $D < P_S - P_L$ (when $l_S > l_0$). It is worthwhile to state it more explicitly. During a shortening such that the sarcomere spacing is never shorter than 2.25 μm, actin filaments enter from the I band into the overlap region and make cross-bridges which are "new" in the sense that their actin parts have not reacted with myosin until allowed to by the shortening. In the H region, the tips of the actin filaments move toward the center of the A band; they react with myosin heads, which are also new, in a sense similar to that used for new actin. By hypothesis, these new cross-bridges have a lower capacity for tension development than the "old" cross-bridges which were already working before the shortening. There are two subclasses of hypotheses according to whether the new myosin cross-bridges or the new actin cross-bridges are inhibited. We think that the first subclass can be excluded, because the Abbott and Aubert phenomenon exists also for length $l_S < l_0$ at which there can be no new myosin cross-bridges as a result of shortening. But new actin cross-bridges will still form, as long as shortening allows I filaments to enter the overlap region.

It is easy to see that this hypothesis accounts for the proportionality between the tension deficit and the extent of shortening (Eq. 2), and the fact that the proportionality constant (k) does not depend on length (Results, section 5) at least if $l_S > l_0$. It allows to calculate a theoretical estimate of k, in the case where none of the new cross-bridges produce tension. Taking 3.65 and 2.25 μm as sarcomere length corresponding to zero tension and maximum tension, the extent of shortening (expressed in l_0 units) for a tension deficit equal to 1 (i.e., equal to P_0) is theoretically $1/(3.65/2.25 - 1.00) = 1.61$ (in l_0 units). Fig. 3 shows a line of this slope. The maximal value K of k in Eq. 2 (for $V \to 0$) probably ($P > 0.95$) lies between 1.26 and 2.08, mean 1.67. Thus, we reach the remarkable conclusion that the maximum tension deficit observed for an infinitely slow movement equals that which should be observed if all the actin sites which enter the overlap region during shortening did not make any cross-bridges (or made cross-bridges unable to produce any force). This hypothesis explains also why the tension deficit cannot be larger than the difference $P_S - P_L$ (when $l_S > l_0$). It remains to understand why the activity of the new actin sites is modulated by the velocity of the release. A possibly fruitful speculation is that the tetanic stress strains the actin filaments in the I band, but very little in the A band. This seems acceptable as the actin filaments are anchored to the myosin rods in the A band, whereas they are free in the I band. If the velocity of the release is high, the stress is small, and the actin filaments are little strained when they enter the

overlap region during the shortening. The tension which is developed by these new actin cross-bridges would thus be almost normal, and $P_S^* = P_S$. On the other hand, if the velocity of the release is low, the tetanic stress on the actin of the I bands remains high. The actin filaments of the I band enter into the overlap region in a strained condition and make "new actin" cross-bridges which are somehow unable to produce full tension and P_S^* would therefore be less than P_S.

Fig. 5 A demonstrates that unloading the muscle at the end of a shortening by increasing V does not restore the contractile output. This is true even if the tension drops to zero (unreported experiments). We interpret this to mean that the actin filaments which are in the A band are not free to unload their strain as long as the nearby cross-bridges maintain the quasi-crystalline structure of the A band. Reciprocally, their strain is not increased by the isometric tetanic stress built up during the redevelopment of tension after the release. Thus, the actin filaments in the A band are mechanically isolated from the stress which acts on them in the I band. This speculation predicts that a short interruption of the stimulation, just long enough to relax the muscle should restore the tension output. This fact has been reported by Abbott and Aubert (1952), and observed by us as well.

Unfortunately, this hypothesis might be difficult to prove by direct X-ray measurements as the expected strain of the I filament is probably well below the limit of resolution of present X-ray diffraction analyses (cf. White and Thorson, 1973, p. 227). On the other hand, it provides new interpretations of some experiments reported in the literature.

Buchthal (1942), Maréchal (1955), Delèze (1961), and Matsumoto (1967) have shown that the 'isotonic' length-tension diagram is not the same as the 'isometric' length-tension diagram. The latter is obtained by tetanizing the muscle isometrically at various lengths, the former by recording the active shortening of muscles pulling a free-hanging load. For light loads, the muscle shortens to the lengths at which it can develop isometrically a force equal to that load. But for heavy loads the shortening stops at a length at which the muscle can develop an isometric force much higher than the load. Hypothesis *b* can at least qualitatively account for these observations. In an isometric tetanus, no cross-bridges are inhibited; in an isotonic contraction, some cross-bridges are made with new actin sites which are partially or completely inhibited; their proportion increases with the extent of the shortening, and their inhibition increases with the load. When the load is light, they are but slightly inhibited, and the isotonic contraction has the same end point as the isometric one. When the load is heavy, they are partially or completely inhibited and, consequently, the isotonic contraction will fall short of the isometric. In principle, the hypothesis could explain quantitatively these results; further experiments will show whether this expectation will turn out to be true. This interpretation seems to contradict the classical studies of Gordon et al. (1966) and Edman (1966) who reported that the ability to produce tension in single muscle fibers is determined by the actual sarcomere spacing during the activity of the fiber, without reference to the sarcomere length existing at the moment when the contraction is initiated. However, both Gordon et al. (1966) and Edman (1966) derived their results from lightly afterloaded contractions: since the tension maintained during the

release was low, the deficit of isometric tension after the shortening should have been low as well. Eqs. 2 and 4 can be used to calculate the size of the tension deficit expected in the experiments of these authors. In Fig. 6 of Gordon et al. (1966) the tension during the shortening does not exceed $0.2 \, P_0$; using Eqs. 2 and 4 with $K = 1.61$, $\Delta l = 1.00/2.20 = 0.44$, $P_r = 0.2 \, P_0$, D should equal $1.61 \times 0.44 \times (0.2)^{2.50} = 0.013$. Gordon et al. (1966) could not observe such a small effect, even if it existed. The case of Edman (1966) is similar; in the experiments shown on his Fig. 4, the maximum load lifted isotonically by the fiber is $0.3 \, P_0$; as $\Delta l = 1.9 \, \mu m$, according to our Eqs. 2 and 4, the tension deficit should be equal to $1.61 \times 1.9 \times (0.3)^{2.50}/2.20 = 0.07$; his Fig. 4 shows a tension deficit of 7%; so here again, the observations of Edman (1966) agree with ours. Edman (1966, p. 412) adds that with loads larger than those used in the experiments reported in his Fig. 4, "the tension deficit was larger, the more extensive the preceding shortening." This is exactly what we expect from our results.

Carlson (1957) and Bahler et al. (1968) have described another interesting effect. A muscle shortening isotonically, lifting a load F from various initial lengths $l_1, l_2, l_3 \ldots$; the velocity of shortening $V_1, V_2, V_3 \ldots$ is measured when the muscle is at some l_S ($l_S < l_1 < l_2 < l_3 \ldots$). It is found that $V_1 > V_2 > V_3 \ldots$ and that the differences $(V_1 - V_2)$, $(V_1 - V_3) \ldots$ are larger, the larger F. If indeed new actin cross-bridges are inhibited, the velocity of the shortening should accordingly decrease or, in release contractions of the type used in the experiments reported here, the tension maintained during a release should be lower than that which would have been observed if these cross-bridges were not inhibited. If this is true, then the hypothesis accounts for the results of Carlson (1957) and Bahler et al. (1968). It explains also the results of Floyd and Smith (1971), who reported that the tension during isovelocity release depends on the distance of the release. It may explain the findings of Edman et al. (1976) who have reported that the force-velocity relationship is not hyperbolic in the high-force region, the curvature reversing at tensions higher than 78% P_0. These authors favor the idea that some cross-bridges undergo different kinds of behavior in the high-force region and in the low-force region. Whether this population can be identified with the new actin cross-bridges population is a problem for future experiments.

Edman (1964) has reported that glycerinated fibers produce a tension at a given length which is critically dependent on the sarcomere spacing at which the contraction is initiated. It seems to us that this observation indicates that new cross-bridges might perhaps occur in glycerinated fibers. If further work confirm this inference, then it would be feasible to initiate direct biochemical studies on the mechanism of the tension deficit.

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