

Some Properties of Linear Relaxation in Unfused Tetanus of Human Muscle

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Summary

The linear relaxation (LR) was studied in isometric unfused tetanus (UT) of the human flexor digitorum sublimis muscle. With a decrease of the force level, the shoulder on the relaxation curve, as measured from the last stimulus, shifted to the right. The length of the linear portion itself weakly depended on activation level. When steady force changed from 100 to 40-50 % of the maximum, the slope of LR decreased only by 15 ± 4 %. At smaller force levels the slope began to increase. LR can probably also be hidden in the twitch. With increased tetanus duration, LR becomes longer and slower at all force levels. LR was markedly diminished in contraction on the steep part of the exponential relaxation after a smooth tetanus. Its full recovery needed a train of 4-5 pulses (near 1 s) at the new stationary level. The form of the response to the additional pulse given during relaxation remained approximately constant during the most of LR portion and differed markedly before and after it. LR did not have direct relation to fatigue: in the first UT LR was always slower and longer than in subsequent ones.

Key words

Skeletal muscle – Unfused tetanus – Linear relaxation – Human

Introduction

The relaxation of skeletal muscle after isometric tetanic contraction often consists of two portions: the initial one with a low and approximately constant rate of force decay, and a later rapid exponential one (Huxley and Simmons 1970, Edwards *et al.* 1975, Allen *et al.* 1989, Edman and Lou 1990). The transition from one to the other is marked by a prominent shoulder or angle on the relaxation curve (Cannell 1986, Curtin and Edman 1989, Gurfinkel' and Levik 1981, Westerblad and Lannergren 1991). The initial slow phase of relaxation is referred to as delayed or linear relaxation (LR). We shall use the LR as an accepted term, although LR often differs from true linear decay. The LR modifies the contractile properties of the muscle as relaxation time and is thus important for motor control under natural conditions. The study of LR is also of theoretical interest, as commonly accepted models of muscular contraction meet more difficulties in explaining the linear force decay than the exponential one.

The LR has been mostly studied in smooth tetanus (Cannell 1986, Curtin and Edman 1989, Edman

and Lou 1990, Westerblad and Lannergren 1991). Yet, linear relaxation can also be observed in unfused tetanus. Gurfinkel' and Levik (1981) have shown that muscle at first develops tension with relatively small force oscillations (phase 1 of UT) in response to low frequency (8-12/s) stimulating train. Due to the staircase phenomenon, the amplitude of oscillations increases 2-3 fold after 60-100 pulses because of relaxation shortening and increased twitch amplitude. We have called this part of UT "phase 2". Subsequently, after 200 and more pulses, the relaxation begins to slow down, the force oscillations decrease significantly, the mean tension becomes greater than at the beginning of the tetanus (phase 3). The relaxation in this phase of UT is characterized by a more or less prominent "shoulder", and the slowing of relaxation is due mostly not to a change in the rate of exponential relaxation but to the appearance of linear relaxation that becomes longer with increased tetanus duration.

The study of relaxation in UT makes it possible to follow the process of LR development and to distinguish the long-term and short-term factors

modifying the relaxation rate. We have therefore investigated the dependence of linear relaxation parameters on tetanus duration and on the short-term changes of the activation pattern influencing the mechanical history of contraction.

Methods

The subject sat in a comfortable chair, the forearm, palm and fingers of the right hand immobilized by an individually adjusted fixation system lay on a horizontal platform in the supine position. The force of the portion of *m. flexor digitorum sublimis*, moving the forth digit, was recorded with an isometric strain gauge (Gurfinkel' and Levik 1985).

The active electrode (1 cm in diameter) was placed over the corresponding motor point, the reference electrode (6 x 8 cm) was on the dorsal side of the wrist. Rectangular current pulses (10 mA, 1 ms) were used. A further increase of the stimulating current produced pain during tetanic stimulation. The current used evoked maximal tension at 50 Hz. This tension was equal to 30-40 % of the maximal voluntary contraction (MVC). It was difficult to assess this value more precisely, as the subject usually recruited other flexors of fingers and the wrist during MVC. Because of the complex geometry of the multijoint muscle under study and inaccurate information about the force arm, we expressed the force in percentage of the force of a smooth tetanus or in absolute values of the strain gauge readings.

Unless otherwise stated, the stimulating trains consisted of 400 pulses giving UT of 40-60 s duration depending on the stimulation frequency. The stimulator with a stabilized current output was triggered by short rectangular pulses from the digital output of an IBM PC AT computer. Force data were fed into a computer *via* an A/D converter, the sampling rate being equal to 500/s.

We used two main stimulation modes: stimulation at a constant frequency and a "force clamp" mode. In the latter case, the force feedback controlled the stimulation pattern applying the next pulse when the tension fell to a specified level.

Six subjects aged from 20 to 69 years participated in this study. Altogether 96 unfused tetani were recorded.

In contrast to a smooth tetanus, UT under physiological conditions is more sensitive to regulation and varies significantly in repeated trials (Jami *et al.* 1983, Gurfinkel' *et al.* 1990b, Powers and Binder 1991). We observed especially large differences between the first UT and subsequent ones, even with 1-4 hours of rest inbetween. Complete recovery giving the UT identical with initial one could not be obtained in the same subject on the same experimental day. So we indicate the sequential number of each tetanus when

describing the experimental results. In our study the period of rest between subsequent recordings was not less than one hour.

Results

Gurfinkel' and Levik (1985) have shown that the linear relaxation was absent at the beginning of UT and appeared during transition into its third phase. One could observe the process of LR development by inserting short 500 ms gaps in 8/s stimulating trains (Fig. 1). During such stimulation, the mean force of UT grew slowly and reached a maximum to the 300th-400th pulse.

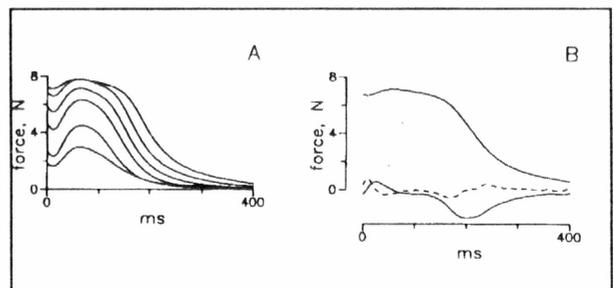


Fig. 1

The development of linear relaxation during sustained UT (8 Hz, 400 pulses). Relaxation curves are obtained by introducing short 500 ms gaps in the stimulus train. First tetanus. A – relaxation after 20, 50, 100, 150, 200 and 300 pulses (from bottom to top). B – relaxation after the 400th pulse. Upper curve – force, at the bottom first (solid line) and second (broken line) time derivatives of force. Abscissa – time after last pulse.

At first a portion with a smaller curvature appeared on the relaxation curve and only afterwards did it become longer and began to resemble a straight line. This process did not appreciably influence the time to peak. Random fluctuations of the slope of LR from one "twitch" of UT to another did not exceed 7 % of the mean value.

Fig. 1 shows that the initial part of relaxation changes much more than the exponential part. It can thus be concluded that the linear and exponential portions of tension decay are due to different mechanisms. Perhaps, LR should not be considered as true relaxation but as a process impeding real exponential relaxation.

For description of LR we selected two parameters: duration and the mean rate of force decay. We assumed that LR begins from the tension peak, and ends with a "shoulder" on the relaxation curve. The shoulder was defined as the point of maximal curvature on the relaxation curve, i.e. as the local minimum of second time derivative of force (Fig. 1B). Firstly, we

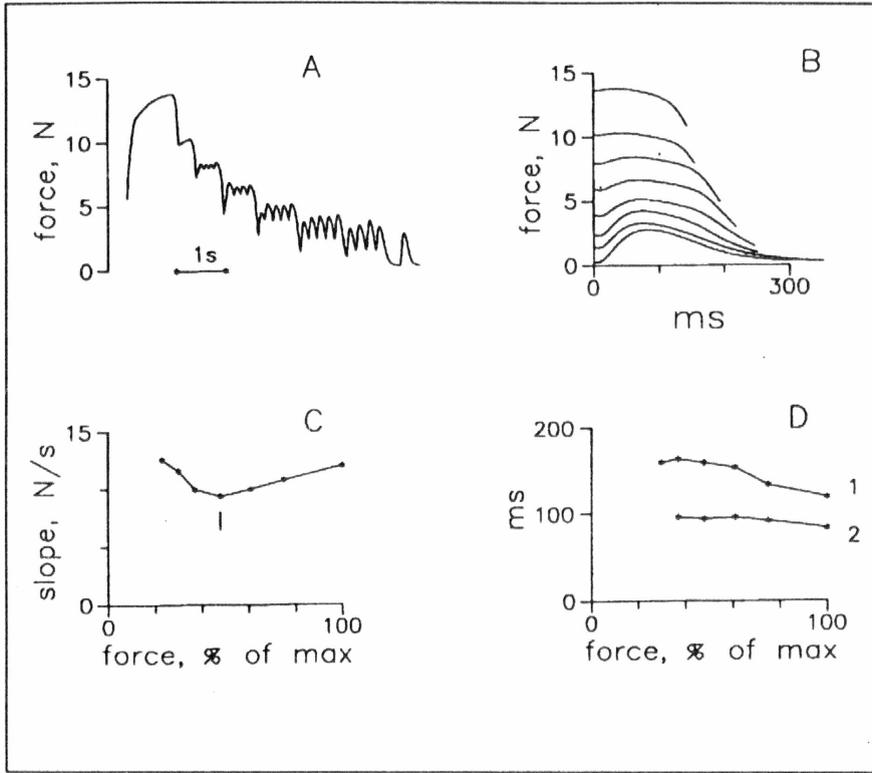


Fig. 2

The dependence of LR on the force level. First tetanus. A – the force response to cascade-type stimulation was the basing for constructing Figures 2B, C and D. B – the superposition of curves of relaxation from different force levels. C – the mean slope of force decay in LR as a function of peak force (in % of smooth tetanus force) from which the relaxation begins. The arrow marks the force level below which the force oscillations begin to exceed the range of LR. D – the dependence of temporal parameters of LR on force. Curve 1 – the time from last pulse to end of LR; curve 2 – the duration of LR measured from peak of force.

smoothed the signal with a low pass filter to suppress small oscillations due to the non-zero sampling intervals. The duration of linear relaxation was measured with a precision of about 4-8 ms and was a reasonable characteristic of linear relaxation for all contractions except the ones with a very low force level. In the latter case the shoulder was hardly observable as linear relaxation changed smoothly into an exponential one. Thus, it is necessary to keep in mind some non-linearity of the LR and the inaccuracy in the evaluation of its duration when evaluating the data. The mean rate of force decay was defined as the slope of the central part of the linear portion.

To study the dependence of LR on the force level, we used stimulation of the "cascade" type, consisting of force clamp steps. For this we inserted a short 1 s smooth tetanus into the 8 Hz stimulating train with a stepwise tension decrease after it (Fig. 2a). Each step in the cascade consisted of 5 stimulating pulses in the force clamp mode. Naturally, the mean stimulation frequency decreased after the transition to the lower force step.

Figures 2b and 2d show that the decrease of force led to the time shift of the shoulder to the right with respect to the last stimulating pulse. Yet, if measured as the duration of the linear portion, i.e. not from the last stimulus but from the last peak of force, the length of LR turned out to be much less dependent on the activation level (curve 2 in Fig. 2d). It is thus possible that processes underlying the slow phase of relaxation, begin at the point of maximal force and only depend slightly on the activation level. The slope of LR also did not vary much (Fig. 2c). A 2-fold force decrease from 100 to 40-50 % of the maximum produced a decrease of the slope only by 15 ± 4 % (mean \pm S.D. on the average in 6 subjects. Only for forces smaller than 40-50 % of the maximal value the slope began to increase slightly. At such force levels, the oscillations of tension in unfused tetanus became greater than the force decrement in LR, and began to include a part of "exponential" relaxation.

In the "cascade" experiments, we also tried to find out whether the linear relaxation is present in the twitch. The curves in Fig. 2b show that, at smaller

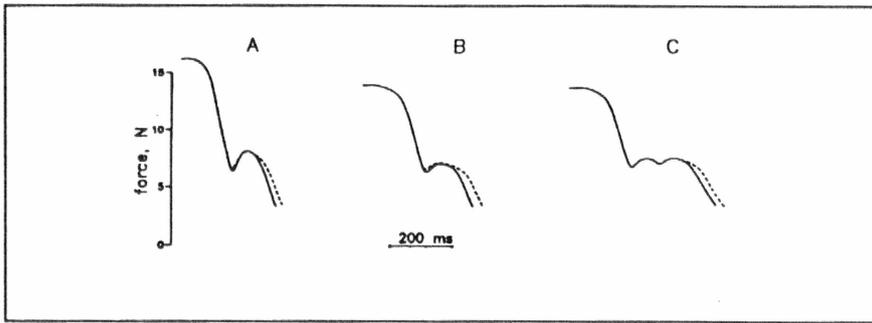


Fig. 3

The "destruction" of LR in a contraction given during relaxation after smooth tetanus (1 s, 50 Hz). Second UT. A – phase 2, B,C – phase 3 of UT. The dashed line shows the relaxation after sustained unfused tetanus with force level equal to peak force in additional contraction (see text).

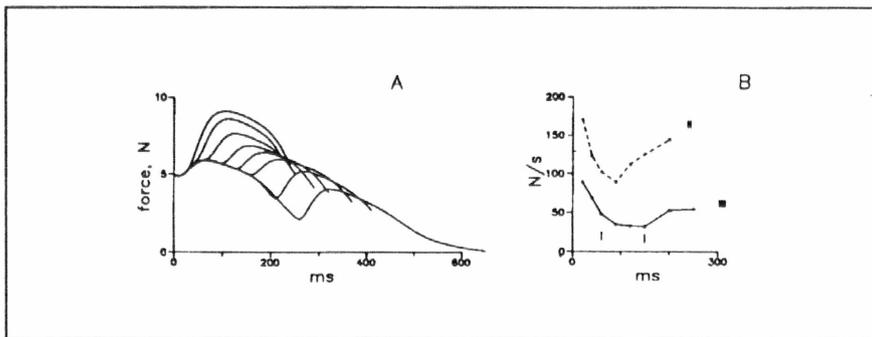


Fig. 4

The responses to additional pulses given 20, 40, 60, 90, 120, 150, 200 and 250 ms after the preceding one. First tetanus. A – superposition of the contractions (phase 3 of UT) B – the maximal rate of force development in additional contraction as a function of the interpulse interval in phase 2 (broken line) or phase 3 (solid line) of UT. The small vertical bars mark the beginning and the end of LR.

tensions, the transition from LR to exponential relaxation becomes more smooth, as the rate of exponential relaxation is approximately proportional to the force level. By extrapolating, one can assume that the twitch hides the LR, which is hardly discernible from the exponential part.

The next series of trials had to establish if the form of linear relaxation is unambiguously related to the force level. During phases 2 and 3 of UT we evoked a short smooth tetanus and then switched to the force clamp mode at the 7 N level for one pulse. We thus obtained a twitch superimposed on the steep part of relaxation after a smooth tetanus (Fig. 3). As in the steep phase of relaxation after a smooth tetanus, significant internal displacements and redistribution of sarcomere lengths do appear (Curtin and Edman 1989), this contraction was probably not strictly isometric. When force decayed to 3 N, force clamp was again switched on for 10 pulses. In response to this sequence, the force increased to 7 N and was sustained at this level for some time. The relaxation from this

"stationary", steady level is shown in Fig. 3 by a broken line. Such experiments demonstrate that the contraction under non-stationary conditions is shorter than under "stationary" ones, and the increment of force is greater. However, the time to peak did not decrease and even increased by 2-4 ms. It was also demonstrated that two pulses at a given level were not sufficient for the full recovery of LR (Fig. 3c). The recovery needed 4-5 pulses, i.e. about 1 s. It is necessary to stress that the "shortening" of the initial part of relaxation also occurred in phase 2 of UT, where the linear portion was absent (Fig. 3a).

Thus, both in phase 2 and 3 the exponential relaxation began earlier due to a shortening of the initial slow part of relaxation. In this connection, the old observation of Rack *et al.* (1969), who reported the marked increase of force oscillations in UT during linear stretch deserves to be mentioned. Furthermore, the switch to rapid relaxation is accelerated by stretch-induced cross-bridge detachment (Lombardi *et al.*

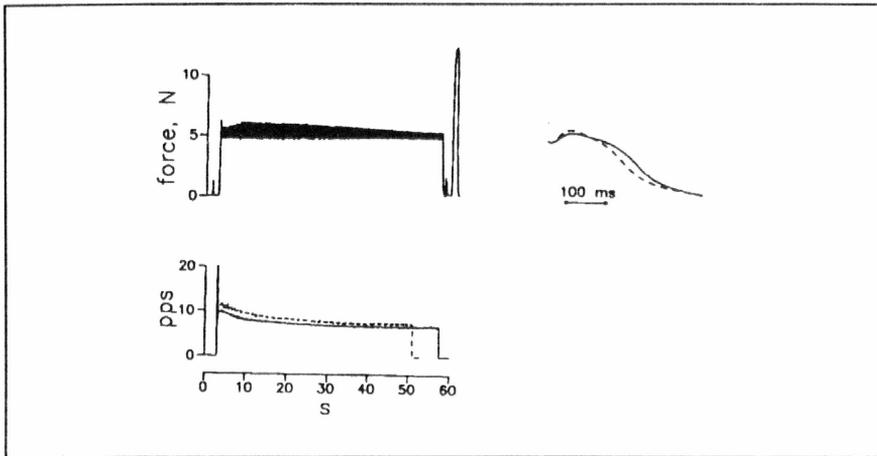


Fig. 5

Force clamp at the 5 N level in first and second UT. The twitches and smooth tetanus (1 s, 50 Hz) after UT are also shown. On the right – the last contraction of UT on an extended time scale. At bottom – the stimulation frequency necessary for the maintenance of constant force. Broken lines show the curves for the second UT (evoked one hour after the preceding tetanus). The subject with the most prominent difference between first and second tetanus was selected for illustration.

1990). All this proves that linear relaxation is better expressed in the steady isometric mode.

If a muscle does not relax from the maximal force but from some lower level (for example, 10 N), the differences between stationary and non-stationary contraction become almost imperceptible. We also used other ways for obtaining "non-stationary" relaxation, for example, after rapid tension transient from a low to a more elevated level induced by a short high frequency (50 Hz) sequence of 2-3 pulses. By its duration and slope such "non-stationary" relaxation differed from the stationary one of the same level but not markedly so. Of course, the time to peak was significantly prolonged, but the whole LR portion was also correspondingly shifted to the right.

Despite the small force fall during LR, the state of contractile machinery could change significantly in the mean time. For example, the marked decrease of free calcium concentration in the cytoplasm or the change in the state of attached cross-bridges would be conceivable. However, the mechanical response to the additional pulse remained almost constant over the whole period of LR, except in its initial part (Fig. 4a,b). The rate of tension development after an additional pulse and the value of force increment was minimal and approximately constant. In phase 2 of UT both the horizontal portion of the rate plot and linear relaxation were absent. The reference contraction was obtained after the force clamp at 5 N level for 5 pulses. Thus, for obtaining all curves in Fig. 4 we needed 50 pulses (from the 350th to 400th), which did not significantly modify the "stationary" reference LR. Probably, the responsiveness

of the contractile machinery to the additional pulse during the linear portion of relaxation remained constant, and in this respect the state of the contractile apparatus did not change.

The force of UT (8 Hz) after 300-400 pulses was 1.5 to 2.0 times greater (depending upon the sequential number of the tetanus) than the initial tension. Yet, a short smooth tetanus (1 s, 50 Hz) given at the beginning and at the end of UT of 400 pulses showed that the maximal muscle force after UT was $19 \pm 2\%$ lower than the initial one. The marked shifts in metabolite levels can also appear in phase 3 of UT (Edwards *et al.* 1975). With a further increase in tetanus duration from 400 to 800 pulses, the exponential and linear relaxation became monotonously slower and longer, and the curve in Fig. 2c was lower approximately in parallel to its original course. One could thus suppose that linear relaxation develops only because of fatigue. Still, there are some arguments against such a supposition. First, the fatigue of UT at 8 Hz was absent as the tension increased up to the 400th pulse and if we maintained a constant force the frequency of stimulation fell (Fig. 5). It should be noted that the exponential part of relaxation changed at this time much less prominently than the initial one (Fig. 1).

On the other hand, in the second UT (1-2 hours after the first) the force is smaller and the maintenance of the same tension requires a higher frequency of stimulation. Simultaneously, the LR is shorter and more rapid (Fig. 5). Still, the second trial did not reveal any significant changes in the force of the smooth tetanus. Accordingly, responses to the

cascade given in the second tetanus were markedly shortened and curves in Fig. 2c and 2d were shifted correspondingly upwards and downwards. Evidently, a fresh muscle can hardly be more fatigued during the first tetanus than in the following ones. Besides that, we have already seen a prominent LR in a few cases in the first tetanus after 100-150 pulses. Such early LR could not be observed in subsequent tetani. All this proves that LR is not directly connected with fatigue and is subjected to regulation under physiological conditions.

Physiological experiments on human muscles raise an important question whether electrical stimulation is equivalent to voluntary activation and whether, for example, the difference between the first and second UT can be due to the synchronous contraction of muscle fibres and possible structural damage. To test this assumption, we asked the subject to sustain a voluntary contraction of about 10 N (approximately 25 % of MVC) for 45 s using a visual feedback provided by an oscilloscope. Immediately after that, short UT (15 pulses in the force clamp mode at 5 N) followed as a test for LR. After one hour the experiment was repeated. It turned out that after the second voluntary contraction LR was also shorter and more rapid.

Discussion

In this paper we have described some properties of LR in UT of human muscle. Some of them have already been discussed above. Since measurements of tension were performed *in situ*, they cannot provide direct information about subcellular processes. Some effects at tissue level could not be excluded. For example, the tendon compliance is not zero and depends on the tension (Proske and Morgan 1987). Delayed "low-frequency fatigue" differs in fast fatigable and fatigue-resistant motor units (Jami *et al.* 1983, Powers and Binder 1991). But we think that a qualitative description and behaviour of LR is the same for the whole muscle and for single fibres. Besides that, the investigation on intact human muscle is important for the understanding of how the muscles behave under physiological conditions.

Jami *et al.* (1983) and Powers and Binder (1991) described the difference in the force of first and second UT in different motor units of the cat muscle. They did not find large changes in maximal tetanic force and in the muscle action potential. They explained low-frequency fatigue by a temporary failure of excitation-contraction coupling. Our results suggest that a change also occurs at later stages of the contraction-relaxation cycle. We conclude that contractile properties are modified after previous activity under natural conditions.

In principle, the development of the slow linear part of relaxation could be due to the following reasons: a) the slowing of kinetics of cross-bridge cycling or the appearance of long-lived (rigor ?) bonds, and b) the slowing of Ca^{2+} sequestration or Ca^{2+} release by troponin. The linear relaxation was studied mainly in the smooth tetanus. These studies revealed the following facts. The LR markedly lengthens in a state of fatigue and when pH is lowered (Curtin and Edman 1989, Westerblad and Lannergren 1991). Quick stretches (greater than 5-10 nm per half of a sarcomere) applied during the slow phase of relaxation accelerate and terminate the LR. On the other hand, quick releases of less than 2-3 nm per half sarcomere prolong the slow phase (Lombardi *et al.* 1990). The end of linear portion coincides with the beginning of significant internal displacements and non-uniform chaotic redistribution of sarcomere lengths during exponential relaxation (Huxley and Simmons 1970). Correspondingly, during LR itself stretching or shortening of separate segments does not occur. This peculiarity is a principal difference between exponential and linear relaxation. Curtin and Edman (1989) believe that the movement of segments occurs when the difference in the number of active cross-bridges between them becomes sufficiently large and cannot be compensated by small stretches of the segments with fewer cross-bridges. The fact that during LR high-frequency short-range stiffness decreases even more slowly than the tension (Edman and Lou 1990) supports the supposition about small changes in the number of attached cross-bridges during LR.

The concentration of free calcium in the cytoplasm falls during LR (Cannell 1986, Allen *et al.* 1989), yet it is not known whether it is released from troponin. Cannell (1986), for example, explains the abrupt slowing of the free calcium decay after the end of LR by massive calcium release from troponin just at the moment of shoulder. The definite role in the dynamics of calcium concentration during relaxation is assigned to intracellular buffers, for example, parvalbumin (Pechere *et al.* 1977, Cannell 1986, Westerblad and Lannergren 1991). Westerblad and Lannergren (1991) when discussing the processes which determine the speed of LR during fatigue list factors connected both with the speed of calcium sequestration and with the rate of the detachment of cross-bridges.

The question of changes of short-range stiffness, of sarcomere length and of the behaviour of cross-bridges during LR in unfused tetanus still remains to be clarified definitely. In UT the internal displacements are greater, there are the gaps in the active state, and the contractile apparatus is not fully activated. Because of lesser density of attached cross-bridges and of the non-stationary active state the UT may represent an essentially different mode of muscular work and have different characteristics of LR.

For example, in contrast to the smooth tetanus, the change of muscle length during UT markedly suppressed the constant component of tension and increased its oscillations (Joyce *et al.* 1969).

However, it seems interesting that over a great force range the length and slope of the linear portion only weakly depend on the force (Fig. 2). Relaxation from any level cannot be obtained by simple scaling of relaxation from the level of the fused tetanus. Westerblad and Lannergren (1991) also mention that the LR slope is independent of the force level. This can mean that the processes, underlying linear relaxation, actually begin near the tension peak, and that the rate of detachment of cross-bridges during LR is, for some reason, independent of their number. This peculiarity is

also an essential difference between exponential and linear relaxation.

It is interesting that the appearance of LR in phase 3 of UT is accompanied by a rise of low-frequency stiffness and muscle viscosity (Gurfinkel' *et al.* 1990a) and by a decrease of ATP consumption (Edwards *et al.* 1975, Gurfinkel' and Levik 1985) in relation to the produced force. Now we are not disposed to make any definite conclusions about the nature of LR, but we believe that the properties of LR in unfused tetanus revealed in the present study are important. They should be taken into account in any discussion of the LR mechanism.

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Reprint requests

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