J. Physiol. (1981), **311**, *pp.* 355–372
With 8 text-figures
Printed in Great Britain

ENHANCEMENT AND DIMINUTION OF MECHANICAL TENSION EVOKED BY STAIRCASE AND BY TETANUS IN RAT MUSCLE

BY CHRISTIAN KRARUP

From the Institute of Neurophysiology, University of Copenhagen and the Laboratory of Clinical Neurophysiology, Rigshospitalet, 9 Blegdamsvej, 2100 Copenhagen Ø, Denmark

(*Received 18 October 1979*)

SUMMARY

1. Potentiation of the isometric twitch tension was compared during and after the staircase and after tetanic stimuli in the fast-twitch extensor digitorum longus muscle of adult Lewis rats at 37–38 °C.

2. With up to 250 stimuli the potentiation rose with an increase in both the frequency and number of stimuli in the staircase (2–5/sec) and the tetanus (100–167/sec). After a tetanus of 375 stimuli (125/sec) the potentiation was smaller. The potentiation 2 sec after a tetanus of 250 stimuli (167/sec) was $+132 \pm 5\%$ ($n = 21$, S.E. of mean) which was greater ($P < 0{\cdot}001$) than at the 250th stimulus at 5/sec, $+92 \pm 3\%$ ($n = 21$, S.E. of mean).

3. After the staircase the decay of potentiation was initially slow and later more rapid. This was taken to indicate both the recovery of a process that diminished twitch tension and the decay of a process causing potentiation. After 250 stimuli (5/sec) the rate of decay of the processes causing diminution and potentiation had time constants of $34{\cdot}5 \pm 3{\cdot}8$ sec ($n = 18$, S.E. of mean) and $102{\cdot}2 \pm 6{\cdot}6$ sec ($n = 20$, S.E. of mean) respectively. Compared with the potentiation, the process causing diminution became relatively more pronounced the greater the frequency of stimuli.

4. The decay of post-tetanic potentiation showed an initial rapid and a later slower phase of decay. After a tetanus of 250 stimuli (167/sec) the rates of decay had time constants of $5{\cdot}7 \pm 0{\cdot}8$ sec ($n = 16$, S.E. of mean) and $113{\cdot}5 \pm 8{\cdot}7$ sec ($n = 19$, S.E. of mean) respectively.

5. Compared with the unpotentiated response the time course of the twitch was shortened initially in the staircase and when the post-tetanic potentiation was low. The contraction time was then increasingly prolonged the greater the potentiation and the greater the number of stimuli in the staircase and in the tetanus. The half-relaxation time was the more prolonged the greater the number of stimuli.

6. Potentiation can be described in terms of a two-compartment model of processes which show saturation. Both compartments were activated in a tetanus whereas only the compartment with a slow rate of decay was activated in the staircase. It is speculated that the two compartments are related to the excitation–contraction

Present address: Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG.

0022-3751/81/3770-0559 $07.50

coupling. The process that caused diminution of twitch tension during the staircase may be due to fatigue. It is suggested that the energy consumption in 250 twitches is about 10 times greater than in a tetanus of 250 stimuli which may explain the presence of fatigue after the staircase whereas it was absent after the tetanus.

INTRODUCTION

Repetitive stimuli to skeletal muscle cause potentiation of twitch tension (i) during trains of stimuli of low frequency and long duration (positive staircase phenomenon), and (ii) after a tetanus (post-tetanic potentiation). In mammalian muscle potentiation is prominent in fast-twitch fibres (rat: Close & Hoh, 1969; Hoh, 1974; Asmussen & Gaunitz, 1978; cat: Bagust, Lewis & Luck, 1974; human: Krarup, 1977), and occurs with only slight changes in the extracellular action potential (Slomić, Rosenfalck & Buchthal, 1968; Krarup, 1977; Krarup & Horowitz, 1979).

A number of workers assumed that potentiation is localized to excitation-contraction coupling (Desmedt & Hainaut, 1968; Close & Hoh, 1968*b*) and associated with a prolongation of the active state (frog, 0°C, Ritchie & Wilkie, 1955). On the other hand, the staircase in the papillary muscle of rabbit (29°C) was associated with an intensification of the active state (Edman, Grieve & Nilsson, 1966). The shortening in the contraction time during the staircase (Slomić *et al.* 1968) has been claimed to support the assumption that the active state is intensified during the staircase (Desmedt & Hainaut, 1968). Events that tend to diminish potentiation were attributed to fatigue (Kugelberg & Lindegren, 1979; Krarup & Horowitz, 1979) or to accumulation of Ca in the sarcoplasmic reticulum (Conolly, Gough & Winegrad, 1971).

The aim of this study was to examine the events of potentiation and of diminution of twitch tension by (i) comparing the potentiation evoked by a staircase and by a tetanus, and (ii) by studying the time course of the decay of potentiation. A short account of some of the results has been published (Krarup, 1978).

METHODS

Extensor digitorum longus muscles (EDL) from twenty-one 10–14-week-old female and male Lewis rats (170–300 g) were examined.

Anaesthesia. The rats were anaesthetized with halothane in 95 % O_2 and 5 % CO_2. The anaesthesia was induced with halothane *via* a mask, then the trachea was intubated. The anaesthesia was maintained such that the animals were hypotonic and the corneal reflexes absent. The animals were breathing spontaneously except when mechanical responses were evoked by direct stimulation of the muscle in the curarized animal. When the animal was curarized the halothane mixture, without further changes in the halothane concentration, was switched to a ventilator. Artificial ventilation was given at a frequency of 50–60/min (stroke volume 2·5–3 ml.). A slight hypercapnia (arterial $P_{CO_2} \sim 50$ mmHg, pH ~ 7·2, $P_{O_2} \sim 140$ mmHg) was produced to stimulate respiration and increase the blood pressure. A catheter was placed in the common carotid artery to record the blood-pressure by means of a Statham strain gauge. The blood-pressure was well maintained both before and after curarization (~ 250 mmHg).

Dissection of the muscle. The right EDL was exposed preserving the blood and nerve supply. That the blood supply was not arrested by the surgical procedure was assured by the block of neuromuscular transmission of the EDL when curare was administered systemically through the carotid artery. The nerves to other muscles of the leg were crushed or cut. The distal tendon of

the muscle was cut and attached to a tension transducer. The proximal attachment of the muscle was preserved.

Muscle chamber. The animal was placed in a prone position on a heated table. The hind limb was immersed in a fluid filled chamber (70 ml.) attached to the table. The chamber contained Liley's solution (NaCl, 136·8 mM; KCl, 5·00 mM; $CaCl_2$, 2·00 mM; $MgCl_2$, 1·00 mM; $NaHCO_3$, 24·0 mM; NaH_2PO_4, 1·00 mM; glucose, 11·0 mM) and 5 % CO_2 and 95 % O_2 were bubbled through the solution in a reservoir for at least 2 hr before the experiment (pH, 7·2–7·3, 37–38°C). $CaCl_2$ and $MgCl_2$ were obtained in stock solutions at a concentration of 1·000 M (Bie & Berntsen, Denmark). The temperature of the solution was kept at 37–38°C by exchanging the solution in the chamber at 1–2 ml./min *via* thin walled tubes in a heated surrounding jacket (Cyclotherm, Struers, Denmark). The temperature was monitored continuously by a thermocouple in the muscle chamber. The solution left the chamber through a 3 mm hole in the bottom.

Stimulation. To stimulate the muscle *via* the nerve, the sciatic nerve was exposed at the hip, crushed proximally, and placed on two silver hooks 5 mm apart with the cathode placed distally. The nerve was kept moist throughout the experiment. Rectangular current pulses (2–4 mA, 0·1 msec in duration), 1·5–2 times greater than those that evoked a maximal response, were delivered from a constant current stimulator. To stimulate the muscle directly, two platinum plates (5 × 15 mm) with the longitudinal axis transverse to the longitudinal axis of the muscle were placed 5 mm apart above and below the muscle. Supramaximal current pulses (1·5–2 times maximal, 500–700 mA, 0·1 msec in duration) were delivered from a condenser-coupled stimulator. The tension and the time course of the twitch were the same whether the stimuli were applied directly to the curarized or non-curarized muscle. Contractions in neighbouring muscle fibres (mainly in the gastrocnemius muscle) were often visible when stimuli were applied directly to the EDL. The distal tendons of these muscles had been cut and there was no detectable interference on the response from the EDL.

Programme of stimulation. The staircase phenomenon was evoked by 250 stimuli at 2, 3, and 5/sec. The post-tetanic potentiation was studied after trains of stimuli of 100, 125, and 167/sec, 1·5 sec in duration. In twelve rats the post-tetanic potentiation was in addition examined after tetani of 125/sec, 0·5, 1·0, 1·5, and 3·0 sec in duration. The effect of previous stimulation was examined by comparison of a repeated staircase or tetanus, e.g. in a run of staircases the potentiation of the 250th twitch at 2/sec was +69 %, at 3/sec it was +82 %, and at 5/sec +91 %. In a repeat 2/sec staircase, given after the 5/sec staircase, the potentiation was +72 %. In the study, the tetani were given in the sequence listed above. The post-tetanic changes in the twitch tension and in the time course of the twitch were not significantly different ($P < 0·2$) when the same tetanus (125/sec, 188 stimuli) was examined early and late in the experiment.

The potentiation after the staircase and the tetanus was examined by giving single or 3/sec trains of stimuli (1·5 sec in duration) once every min for 6 min and then at 10 min after the train of stimuli. Single stimuli were given once every 15 sec within the first min after the staircase and 2, 5, 10, and 30 sec within the first min after the tetanus. The stimuli up to 10 sec after the tetanus were delivered automatically by the programmed stimulator.

Recording of the electrical response. When the muscle was stimulated *via* the nerve the action potential was recorded through a platinum needle (0·3 mm in diameter) with a 2–3 mm bared tip and an a.c.-amplifier (20–10000 Hz, 3 db down, DISA 9014C0101). The tip of the needle was shaped as a hook and inserted into the end-plate zone. The remote electrode was a 10 cm^2 stainless-steel plate placed in the chamber. The action potential was displayed on one beam of a dual beam storage oscilloscope (Tektronix 549) and recorded on an ink-jet writer (upper limiting frequency 700 or 1200 Hz) *via* a digital memory store (Dahl & Buchthal, 1978). The memory store reproduced the action potential at a 10 times slower rate, i.e. the potential was recorded with an upper limiting frequency of 7000–10000 Hz.

Recording of the mechanical response. The proximal tendon was held by a clamp attached at a distance of about 1 mm from the insertion of the muscle fibres. The distal tendon was attached to a tension transducer by cotton thread, 0·5 mm in diameter, bound to the tendon less than 1 mm from the insertion of the muscle fibres. A load of 50 g stretched 1 mm of the thread by 0·02 mm and a load of 300 g by 0·05 mm. The length of the cotton thread was less than 1 mm. The tension transducer had a compliance of 0·75 mm/kg, an undamped oscillation frequency of 745 Hz, a rise time of 0·4 msec to 90 % of the amplitude to a step change in load, and was linear up to 600 g. In addition to the compliances of the tendons, the compliances of the tension transducer and of the

thread caused the muscle to shorten at most 0·2% (optimum length, see below) during a twitch and 0·9% during a tetanus.

The mechanical response was displayed *via* a d.c.-amplifier (0–1600 Hz, 3 db down) on the other beam of the storage oscilloscope and recorded directly on another channel of the ink-jet writer. In some instances the rate of force development was recorded directly on a third channel of the ink-jet writer. The first derivative was estimated by differentiation of the output of the d.c.-amplifier in an RC-coupled amplifier. The 'differentiator' amplified a sinusoidal signal of constant amplitude and varying frequency proportionally up to at least 1 kHz, and it was linear up to at least 500 V/sec. The output of the d.c.-amplifier during the twitch was 0·2–0·8 V, and the maximal rate of force development was 60–100 V/sec. The limitation of the use of the 'differentiator' was imposed by oscillations due to the natural frequency of the tension transducer. The twitch showed faint oscillations when evoked by stimuli to the nerve which were more marked during direct stimulation of the muscle. The amplified oscillations in the 'differentiated' twitch were damped by relaying the response through a low-pass filter with a limiting frequency of 2 kHz, 3 db down. The peak amplitude of the 'differentiated' response corresponded to the maximal rate of development measured manually from recordings.

Measurements. The time course and the amplitude of the electrical and mechanical responses were measured from polaroid photographs of oscilloscope tracings and from tracings of the ink-jet writer at a suitable sweep speed. The tracings were magnified two times and measured to an accuracy of 1–2%. Statistically the effects on the twitches of the frequencies and the number of repetitive stimuli were evaluated by the paired t test (ratio between the mean difference and the standard error of the mean of the difference) and were considered significant at $P < 0.05$.

Optimum length of the muscle. The muscle was stretched to a length at which it produced maximum twitch tension. The extension above load zero averaged 21% (14–32%), obtained by a load of 5·4 g (3·4–7·4 g). The cross-sectional area (cm^2) of fibres was calculated from (Close, 1964) the ratio of the wet weight of the muscle (g) and the length of fibres at optimum length (cm).

Reproducibility of the mechanical responses. Analysis of variance of the twitches before a staircase and a tetanus (six twitches of each of twenty-one muscles, ten twitches of each of twelve muscles) and of five tetani evoked by trains of 125/sec in each of twelve muscles, confirmed the null hypothesis that the mean and variance of the amplitude of the twitches and of the tetani were unchanged during the course of the experiment (mean, $P = 1.000$; variance $P = 0.900$). The ratio between the last and the first twitch tension in the experiment was 0·95 (0·82–1·09) and the corresponding ratio of tetani of 125/sec was 1·00 (0·84–1·16).

Evidence for the absence of block of neuromuscular transmission. The tension and the action potential during a tetanus evoked by trains of stimuli to the nerve (1·5 sec in duration, 125 and 167/sec) decreased gradually, whereas there was no decrement in tension when the frequency was 100/sec. The decrement was the more pronounced the longer the train. The decrement in tension was the same when the tetanus was evoked by stimuli directly to the muscle as when the tetanus was evoked by stimuli to the nerve. Therefore the decrement was not due to block of neuromuscular transmission, though a blocking effect of halothane has been described (frogs: Karis, Gissen & Nastuk, 1967; human: Miller, Way, Dolan, Stevens & Eger, 1972; guinea-pig: Waud, Cheng & Waud, 1973). The cause of the decrement in tetanic tension during direct stimulation was not further examined in this study.

Administration of curare. D-tubocurarine was injected into the catheter in the carotid artery in doses of 10 μg until the muscle did not respond to stimuli to the nerve (0·1–0·2 mg/kg body wt.). The neuromuscular block was maintained by injections of 10–20 μg at suitable intervals.

RESULTS

Some characteristics of the unpotentiated twitch and of the tetanic tension evoked by stimuli to the sciatic nerve are compiled in Table 1. The twitch tension, but not the tetanic tension, was 15–20% lower than reported by Close (1969) and Hoh (1974). The contraction time of twitches evoked by stimuli directly to the muscle was 0.8 ± 0.1 msec ($n = 17$, S.E. of mean, $P < 0.001$) shorter than in responses evoked by stimuli to the nerve.

TABLE 1. The amplitude and the time course of the unpotentiated twitch and the maximum tetanic tension (250 stimuli, 167/sec), evoked by stimuli to the sciatic nerve (37–38°C). Mean ± S.E. of mean, number of muscles = 21

Twitch tension (g/cm²)	382·6 ± 15·5
Contraction time (msec)	10·9 ± 0·1
Half-relaxation time (msec)	10·9 ± 0·1
Maximum rate of force development of the twitch (kg . sec^{-1} . cm^{-2})*	82·1 ± 3·1
Maximum tetanic tension (g/cm²)	2848·5 ± 105·4
Twitch/tetanus ratio	0·132 ± 0·003

*The rate of force development was measured manually from recordings. In those instances where the twitch tension was also differentiated the results of the two methods were similar.

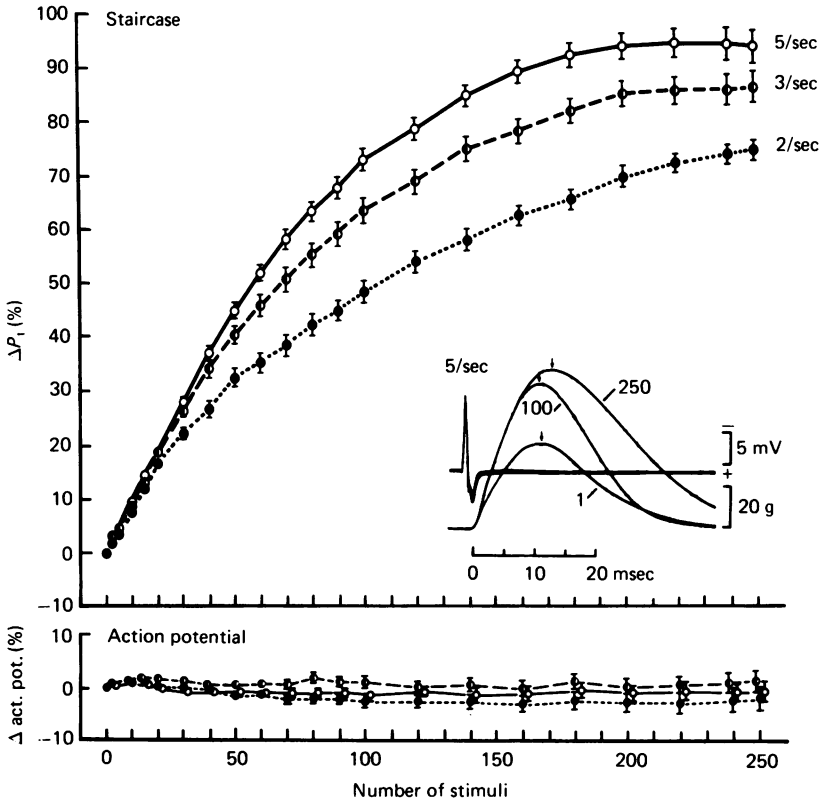

Fig. 1. *Above:* the potentiation of twitch tension ($\triangle P_t$ %) as a function of the number of stimuli during the staircase at 2/sec (●----●), 3/sec (◐--◐), and 5/sec (○——○). *Inset:* the superimposed action potentials (upper traces) and twitches (lower traces) to the 1st, 100th, and 250th stimulus in the staircase. Arrows show the contraction times of the twitches. 37·7°C. *Below:* changes in the amplitude of the muscle action potentials. The points are means of 20–21 muscles and the vertical bars denote the S.E. of mean. 37–38°C.

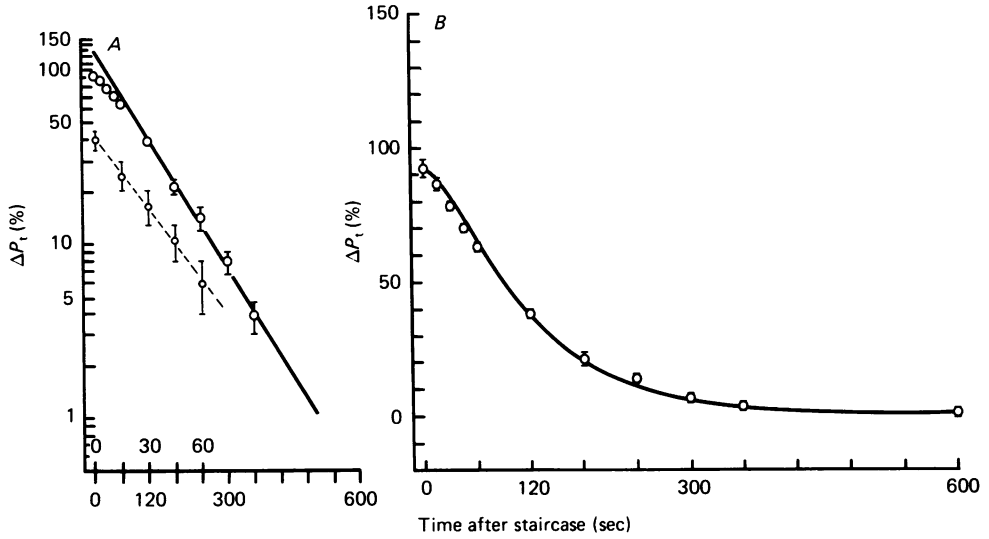

Fig. 2. The decay of potentiation after the staircase evoked by 250 stimuli (5/sec). *A*: the potentiation ($\triangle P_t$%) is shown on a logarithmic scale as a function of time after the staircase (lower abscissa). The continuous line is the regression line (least squares fit) of points 120–360 sec after the staircase ($r = -0{\cdot}9974$, $P < 0{\cdot}001$). The dashed line represents the regression of the numerical difference of the potentiation and the extrapolated continuous line within the first min after the staircase as a function of time (expanded time scale, upper abscissa, $r = -0{\cdot}9952$, $P < 0{\cdot}001$). *B*: shows the decay of potentiation on an arithmetic scale. The continuous line represents the average of the decay in each of twenty experiments:

$$P_{TS}(t) = 132\exp(-0{\cdot}0104t) - 40\exp(-0{\cdot}0318t),$$

where $P_{TS}(t)$ is the calculated potentiation (in %) as a function of time (t in sec) after the staircase. In a goodness of fit test the curve did not deviate significantly from the observed values ($\chi^2 = 13{\cdot}159$, $P < 0{\cdot}2$). The points are average values in twenty muscles and the vertical bars denote the S.E. of mean. 37–38°C.

Potentiation of twitch tension

The staircase phenomenon. Action potentials and twitches evoked by a train of stimuli delivered at 5/sec are shown in Fig. 1, *above*, *inset*. The twitch tension increased gradually, the more the higher the frequency ($P < 0{\cdot}005$, Fig. 1, *above*). At 3 and 5/sec the potentiation reached a maximum after 200 stimuli; at 2/sec it continued to increase throughout the duration of the train. The amplitude of the action potential was only slightly changed (Fig. 1, *below*).

After the staircase, the potentiated twitch decreased to the pre-staircase level within 5–10 min. The decay, as shown in Fig. 2*A* after 250 stimuli at 5/sec, had two phases. Within the first minute after the train the decay occurred more slowly than later, when the logarithm of potentiation decayed linearly with time (Fig. 2*A*, continuous line). The logarithm of the numerical difference between the experimentally determined potentiation and the extrapolated decay of potentiation within the first minute after the staircase decayed linearly with time (Fig. 2*A*, dashed line). Hence, the decay of potentiation could be fitted by combining two exponential

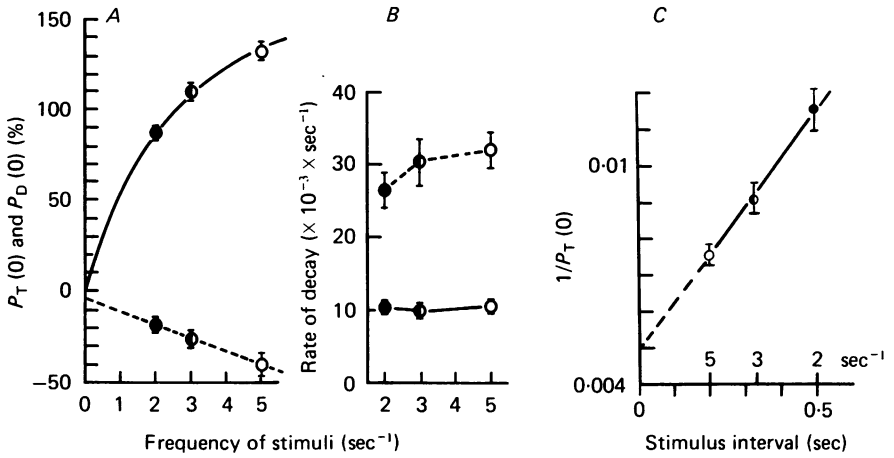

Fig. 3. Calculated events of potentiation ($P_T(0)$, A and C) and of diminution ($P_D(0)$, A), at time zero after the staircase of 250 stimuli, and their rates of decay (B). The points (symbols as in Fig. 1) are average values of twenty or twenty-one muscles and the vertical bars denote the S.E. of mean. A: the continuous line represents $P_T(0)$ and the dashed line the linear regression of $P_D(0)$ (least squares fit, $r = 0{\cdot}9994$, $P < 0{\cdot}05$) as a function of the frequency of the staircase. C: the hyperbolic function of $P_T(0)$ after linear transformation (least squares fit, $r = 0{\cdot}9977$, $P < 0{\cdot}05$) with maximum potentiation shown by extrapolation (dashed line). B: the rates of decay of the event of potentiation (α, continuous line) and of the event of diminution (γ, dashed line). 37–38 °C.

functions, one of positive and the other of negative amplitude, suggesting that the decay of the increased twitch tension was determined both by the recovery of a process that had diminished the twitch tension and by the decay of a process that had potentiated the twitch:

$$P_{TS}(t) = P_T(0)\exp(-\alpha t) + P_D(0)\exp(-\gamma t) \quad (1)$$

where $P_{TS}(t)$ is the calculated potentiation (in %) as a function of time (t in sec) after the staircase; $P_T(0)$ is the process (in %) at time zero after the staircase that caused potentiation and $P_D(0)$ is the process (in negative %) that caused diminution. The rates of decay of the potentiation, α, and of the process causing diminution, γ, were calculated in sec^{-1}. The continuous line in Fig. 2B represents the average decay calculated from eqn. (1) in each of twenty muscles; the decay showed good fit to the measured potentiation.

After 250 stimuli the potentiation at time zero after the staircase, $P_T(0)$, could be fitted by a rectangular hyperbolic function of the frequency of stimuli in the staircase (Fig. 3A). By contrast, the size of the process causing diminution at time zero, $P_D(0)$, became linearly more pronounced with increasing frequency, intercepting the ordinate near zero (Fig. 3A). Thus, as the frequency of the staircase increased, the relative size of $P_D(0)$ increased more than did $P_T(0)$. After 250 stimuli at 5/sec the potentiation was $+92 \pm 3$ %; at time zero after the staircase (i.e. at the 250th response) the $P_T(0)$ was $+132 \pm 5$% and $P_D(0)$ was -40 ± 6% (S.E. of mean).

The rate of decay of the process causing diminution, γ, was three times faster than that of potentiation, α($P < 0{\cdot}001$, Fig. 3B). After the 5/sec staircase γ corre-

sponded to a time constant of $34{\cdot}5 \pm 3{\cdot}8$ sec, and α to a time constant of $102{\cdot}2 \pm 6{\cdot}6$ sec (S.E. of mean). Neither γ nor α changed significantly with a change in the frequency of the staircase (Fig. 3*B*).

The assumption that the increase in twitch tension during the staircase was the result of processes with opposite effects was examined by linearisation of the staircase phenomenon. As illustrated for the 5/sec staircase (Fig. 4), the increase in twitch tension within the first 120–140 contractions of the train could be described by a rectangular hyperbolic function of the number of stimuli. The staircase phenomenon, however, reached a maximum earlier and was lower than predicted from the hyperbola (Fig. 4, *inset*). This suggested that the process causing diminution was absent until after 120–140 contractions in the train, which was supported by the finding (one experiment) that after 50 and 100 contractions the decay of potentiation could be described by a single exponential function. For comparison the potentiation at time zero after the staircase, $P_T(0)$, is shown in Fig. 4, *inset*, and was slightly ($13 \pm 3\%$, S.E. of mean) larger than the potentiation predicted from the hyperbola. The maximal potentiation, as estimated from extrapolation of the hyperbola (Fig. 4) was $+213 \pm 9\%$. The corresponding potentiation of the 2/sec staircase was $+207 \pm 14\%$, and of the 3/sec staircase $+203 \pm 14\%$ (S.E. of mean). The number of stimuli necessary to evoke half-maximum potentiation was higher the lower the frequency of the train. The extrapolated maximum of $P_T(0)$ as a function of the frequency of the train was similar to that estimated from the staircase, $+205 \pm 19\%$ (S.E. of mean, Fig. 3*C*).

Post-tetanic potentiation. Action potentials and twitches evoked before and 2 sec after tetani of 63–375 stimuli (125/sec) are shown in Fig. 5. With up to 250 stimuli (100–167/sec) the post-tetanic potentiation 2 sec after the tetani increased with increasing number of stimuli. When the tetanus was evoked by 375 stimuli (125/sec), the post-tetanic potentiation (Table 2, Fig. 5) was lower ($P < 0{\cdot}005$) than after 188 stimuli (125/sec), both when evoked early and late in the experiment, and after 250 stimuli (167/sec).

After the tetanus the potentiated twitch decrease to the pretetanic level within 5–10 min. An example of the decay of post-tetanic potentiation after 250 stimuli at 167/sec is shown in Fig. 6*A*, and as seen from Fig. 6*B* the decay had two phases. Within the first 10–30 sec after the tetanus the decay occurred rapidly (Fig. 6*B*, dashed line) and then more slowly (Fig. 6*B*, continuous line). The logarithm of both phases of post-tetanic potentiation decayed linearly with time suggesting that the decay of post-tetanic potentiation as a function of time after the tetanus could be fitted by two potentiating events with different rates of decay:

$$P_{TP}(t) = P_{T1}(0) \exp(-\beta t) + P_{T2}(0) \exp(-\alpha t) \qquad (2)$$

where $P_{TP}(0)$ is the calculated potentiation (in %) as a function of time (t in sec) after the tetanus; $P_{T1}(0)$ and $P_{T2}(0)$ are the events of potentiation (in %) at time zero after the tetanus. The rates of decay of the events of potentiation, β and α, were calculated in sec^{-1}. The continuous line in Fig. 6*C* represents the average decay of potentiation calculated from eqn. (2) in each of nineteen muscles; the decay showed good fit to the measured potentiation.

The degree of potentiation of $P_{T1}(0)$ and $P_{T2}(0)$ could be described by rectangular

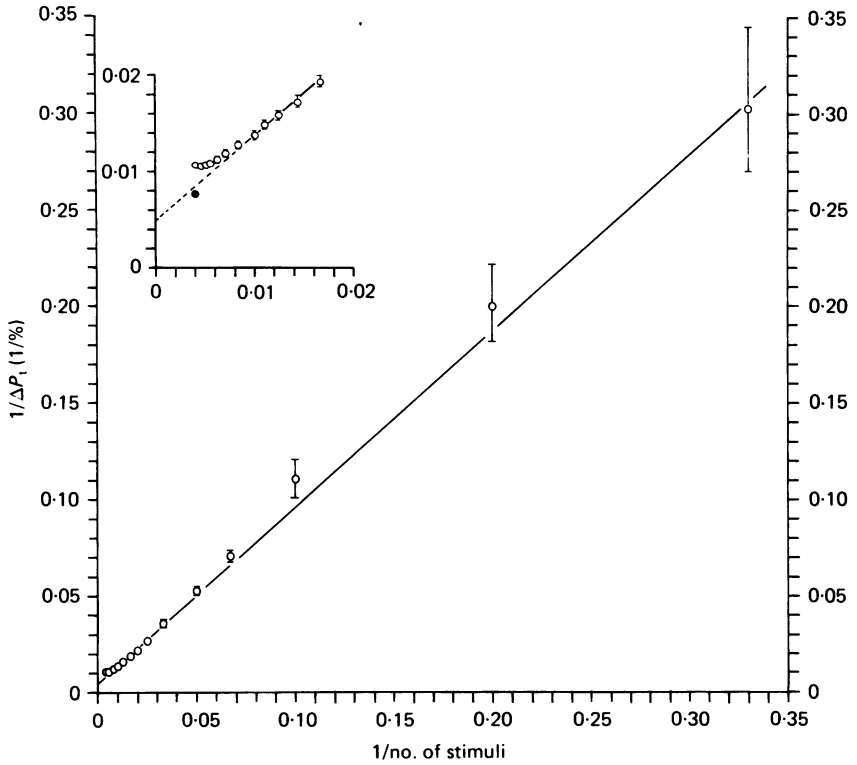

Fig. 4. Linearized plot of the staircase as a function of the stimuli in the 5/sec train. The number of points above 100 stimuli has been diminished for clarity. *Inset:* the linearized plot of potentiation at 60–250 stimuli on a five times larger scale. The filled circle represents the event of potentiation at time zero ($1/P_T(0)$) after the 5/sec staircase. The regression line is the average of each of twenty-one muscles. The dashed line in *inset* is the extrapolated hyperbola. The points are averages of 21 muscles and the vertical bars denote the S.E. of mean. 37–38 °C.

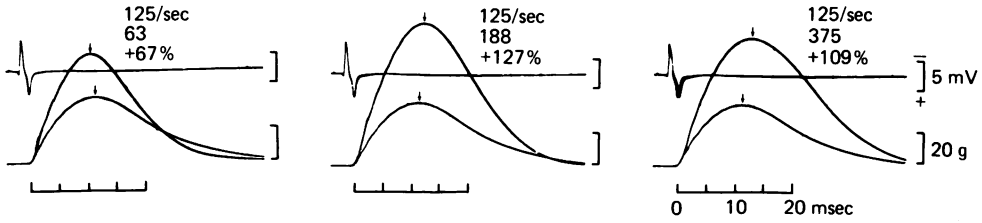

Fig. 5. Examples of post-tetanic potentiation after tetani (125/sec) evoked by different number of stimuli. The upper traces in each recording are the superimposed action potentials, the lower traces the pre- and the 2 sec post-tetanic twitches. The numbers at the post-tetanic twitches represent the frequency and the number of stimuli that evoked the tetani, and the percentage degree of post-tetanic potentiation. The arrows show the contraction times. 37·3–37·7 °C.

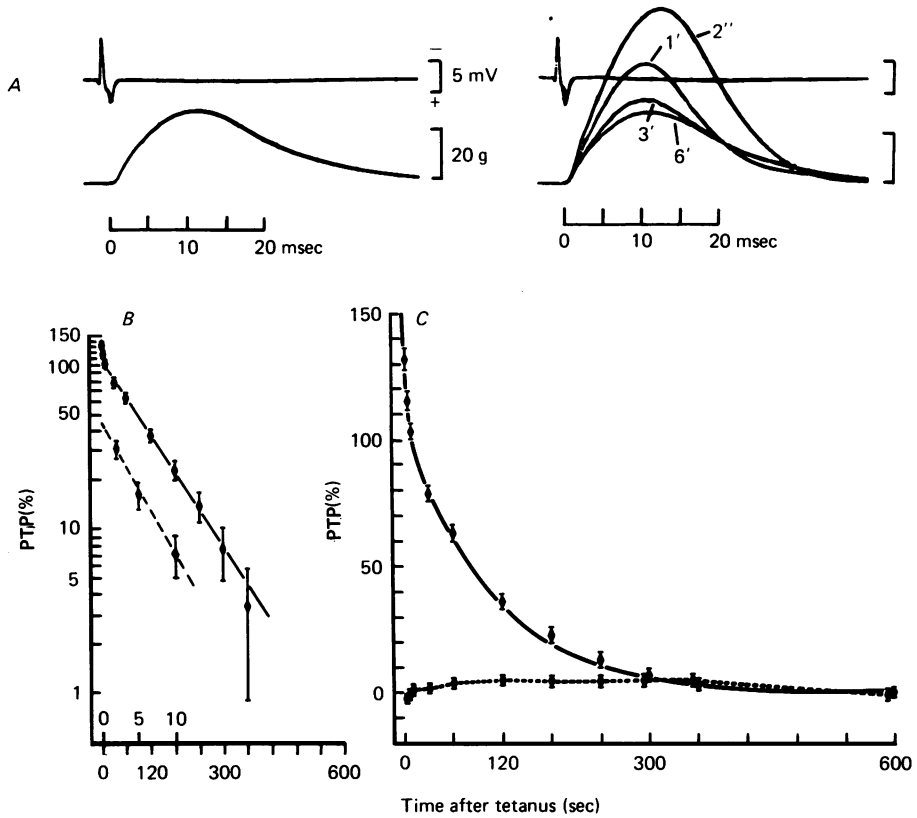

Fig. 6. The decay of potentiation after the tetanus evoked by 250 stimuli (167/sec). *A*: action potentials (upper traces) and twitches (lower traces) evoked before (left) and 2 sec, 1, 3, and 6 min after the tetanus (right). *B*: the potentiation (PTP(%)) is shown on a logarithmic scale as a function of time after the tetanus (lower abscissa). The continuous line is the regression line (least squares fit) of points 30–300 sec after the tetanus ($r = -0{\cdot}9990$, $P < 0{\cdot}001$). The dashed line represents the regression of the difference between the measured potentiation and the extrapolated continuous line within 10 sec after the tetanus as a function of time (expanded time scale, upper abscissa, $r = -0{\cdot}9985$, $P < 0{\cdot}05$). *C*: shows the decay of potentiation on an arithmetric scale. The continuous line represents the average of the decay in each of nineteen muscles:

$$P_{TP}(t) = 44 \exp(-0{\cdot}2362t) + 107 \exp(-0{\cdot}0094t)$$

where $P_{TP}(t)$ is the calculated potentiation (in %) as a function of time (t in sec) after the tetanus. In a goodness of fit test the curve did not deviate significantly from the observed values ($\chi^2 = 7{\cdot}583$, $P < 0{\cdot}5$). The dashed line shows the change in the amplitude of the action potential. The points are average values in nineteen muscles and the vertical bars denote the S.E. of mean. 37–38°C.

hyperbolic functions of the number of stimuli in the tetanus (Fig. 7*A*). The functions had extrapolated maxima at +75% and +192% for $P_{T_1}(0)$ and $P_{T_2}(0)$ respectively. After a tetanus of 250 stimuli (167/sec) $P_{T_1}(0)$ was $+44 \pm 4$% and $P_{T_2}(0)$ was $+107 \pm 3$% (S.E. of mean). The decay of post-tetanic potentiation after the tetanus of 375 stimuli suggested that the low post-tetanic potentiation 2 sec after the tetanus was due to the process causing diminution. The event of potentiation $P_{T_2}(0)$ at time

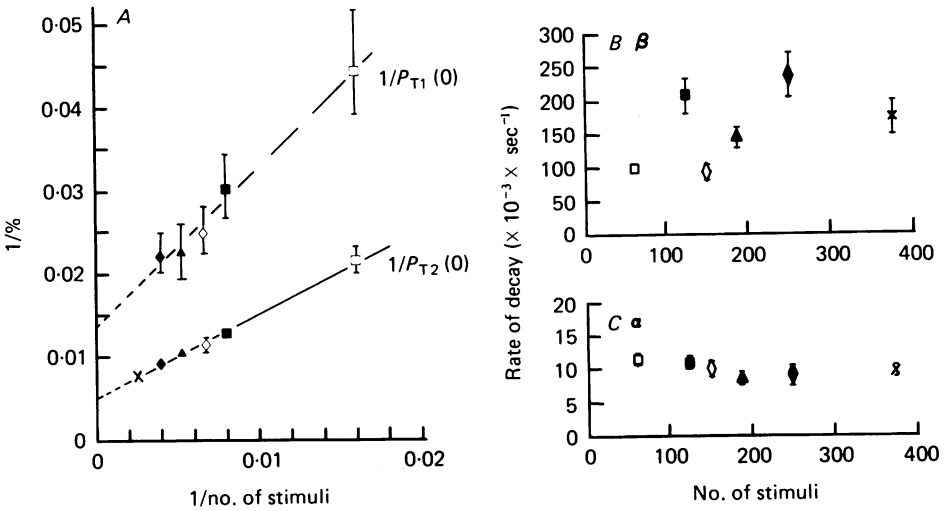

Fig. 7. Calculated events of potentiation at time zero after the tetanus $P_{T1}(0)$ and $P_{T2}(0)$, (*A*) and the rates of decay of the events (*B* and *C*) as functions of the number of stimuli in the tetani. *A:* linearized plots of the events of potentiation. The regression lines are fitted by the least squares method (continuous line, $r = 0{\cdot}9964$; dashed line, $r = 0{\cdot}9918$, $P < 0{\cdot}001$). The maximum potentiation of the events is shown by extrapolation of the hyperbola (stippled lines). *B:* rates of decay of the event of potentiation $P_{T1}(\beta)$; *C:* rates of decay of $P_{T2}(\alpha)$. The points represent average values of ten to twenty-one muscles and the vertical bars denote the S.E. of mean. The value from tetani evoked by 188 stimuli (125/sec) is taken from the first series in the experiments. Symbols: 125/sec, 63 stimuli (□), 125 stimuli (■), 188 stimuli (▲), 375 stimuli (×); 100/sec, 150 stimuli (◇); 167/sec, 250 stimuli (◆). 37–38 °C.

zero after the tetanus had the size, $+130 \pm 7\%$ (S.E. of mean) expected from the hyperbolic relationship (Fig. 7*A*). The size of the event of potentiation $P_{T1}(0)$ could only be determined with accuracy in few muscles and was not included in Fig. 7*A*.

The rate of decay of the event of potentiation P_{T1}, β, was 10–20 times faster than that of P_{T2}, α ($P < 0{\cdot}001$, Fig. 7*B* and *C*). The rate of decay, β, did not change significantly with an increase in the number of stimuli of the tetanus (Fig. 7*B*). α after a tetanus of 63 stimuli was about 35% greater ($P < 0{\cdot}01$) than after a tetanus of 188 or 250 stimuli, but did not change significantly above 188 stimuli (Fig. 7*C*). After the tetanus of 250 stimuli β had a time constant of $5{\cdot}7 \pm 0{\cdot}8$ sec and α had a time constant of $113{\cdot}5 \pm 8{\cdot}7$ sec (S.E. of mean). After 250 stimuli in a staircase and in a tetanus there was no significant difference in α ($P < 0{\cdot}4$), and the time constant was similar to those described by Close & Hoh (1968) and Hoh (1974).

The time course of the potentiated twitch

As compared to the pre-staircase twitch, the time course of the twitch, whether evoked by stimuli to the nerve or directly to the muscle, was shortened initially in the staircase. The shortening was not due to increased synchronisation of excitation as the action potential was not shortened in duration or increased in size. The maximum shortening occurred about the 20th response and the shortened time course was then gradually prolonged. The shortened contraction time early in the staircase

TABLE 2. Percentage potentiation of twitch tension and change in the time course of the potentiated twitch during the staircase and 2 sec after the tetanus evoked by stimuli to the sciatic nerve (37–38°C)

	Staircase				Post-tetanic				
Frequency of stimuli (sec^{-1})	2	3	5	5	125	125	125†	125	167
Number of stimuli	250	250	100	250	63	125	188	375	250
Number of muscles	21	20	21	21	11	11	21	10	21
Potentiation of the twitch (%)	+74	+85	+74	+92	+64	+100	+127	+112	+132
S.E. of mean (%)	2	3	2	3	4	5	4	6	5
Contraction time (%)	+4*	+6*	−8*	+8*	−6*	+1	+9*	+20*	+12*
S.E. of mean (%)	1	1	1	1	1	2	1	2	1
Half-relaxation time (%)	−9*	−1	−22*	+3	−23*	−17*	−20*	+16*	−17*
S.E. of mean (%)	3	3	2	4	2	2	3	3	3

*When measured in msec the changes in the contraction time and the half-relaxation time were statistically significant ($P < 0{\cdot}01$–$0{\cdot}001$).

† The first series in the experiments. The post-tetanic potentiation from the second series was $+119 \pm 8\%$ ($n = 10$, S.E. of mean), not significantly different from that in the first series ($P < 0{\cdot}2$).

was associated with an increase in the rate of force development of the twitch which was about one-third greater than the potentiation (20th–40th response, $P < 0.05$). At the 100th response the increase in the rate of force development and in the twitch tension was similar. From the 100th to the 250th response the further potentiation was associated with only little change in the rate of force development, and the contraction time was markedly prolonged as exemplified in twitches in Fig. 1. Also the time course of the potentiated twitch, as compared to the pretetanic twitch, was shortened after a tetanus of 63 stimuli (125/sec, Fig. 5). The time course of the twitch was then prolonged the more the greater the number of stimuli in the tetanus (Fig. 5).

In Table 2 the percentage changes in the amplitude and the time course of the potentiated twitch are compared. (i) After 250 stimuli in the staircase and the tetanus the potentiation and the prolongation of the contraction times were the more marked the higher the frequency of stimuli. (ii) The potentiation after 100 stimuli in the 5/sec staircase was similar to that after 250 stimuli at 2/sec; however, the contraction time was shortened after 100 whereas it was prolonged after 250 stimuli. (iii) The contraction time after 375 stimuli (125/sec) was more prolonged ($P < 0.01$) than after 188 stimuli (125/sec) and after 250 stimuli (167/sec) though the post-tetanic potentiation was lower, This greater prolongation was associated with a prolongation of the duration of the action potential (Fig. 5). These relationships suggested that the change in the contraction time was related both to the potentiation of the twitch and to the number of repetitive stimuli as shown in Fig. 8*A* (straight line fit by least squares method, $n = 13$, $r = 0.9783$, $P < 0.001$). In the 225 individual responses examined the correlation coefficient was 0·8350 ($P < 0.001$). The change in the contraction time as a function of the potentiation ($n = 13$, $r = 0.7581$, $P < 0.01$) and of the number of stimuli ($n = 13$, $r = 0.9164$, $P < 0.001$) separately had a greater scatter. The potentiation increased with increasing frequency of stimuli but the contraction time did not seem directly related to this variable.

As seen from Table 2 and Fig. 8*C* there was no relationship between the change in the half-relaxation time and the amount of potentiation of twitch tension. However, there was a rough relationship between the changes in the half-relaxation time and the number of stimuli in the staircase and in the tetanus (Fig. 8*B*, straight line fit by the least squares method, $n = 13$, $r = 0.8317$, $P < 0.001$). The gradual prolongation of the initially shortened half-relaxation time during the staircase (e.g. Table 2, 5/sec, 100 and 250 stimuli) could be related to the size of the process causing diminution. Thus, at the 250th twitch in the 2, 3, and 5/sec staircases the prolongation of the half-relaxation time was a linear function of the size of the process causing diminution, $P_D(0)$ (least squares method, $n = 58$, $P < 0.001$). After 250 stimuli in the tetanus the half-relaxation time was markedly shortened, whereas after 375 stimuli, when the process causing diminution was present, the half-relaxation time was prolonged.

DISCUSSION

In this study of muscle mechanics in the fast-twitch extensor digitorum longus muscle of rat, the change in twitch tension (37–38°C) during and after a staircase and after a tetanus was described in terms of events with opposite effects: (i) that

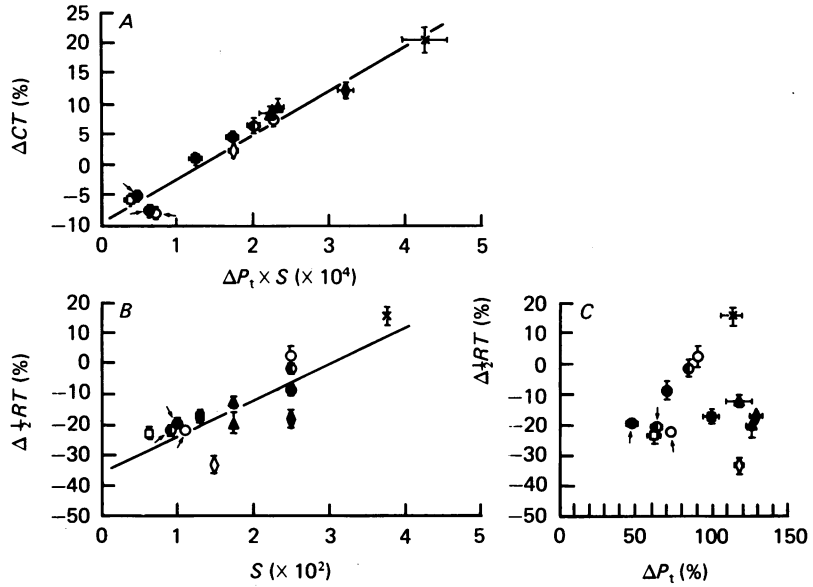

Fig. 8. The change in the time course of the twitch during the staircase and after the tetanus, determined in the 100th and 250th twitch of the staircase and 2 sec after the tetanus. *A*: The change in the contraction time ($\Delta CT\%$) as a function of the product of the potentiation of twitch tension ($\Delta P_t\%$) and the number of stimuli (S) that evoked the staircase or the tetanus. The continuous line was fitted by the method of least squares, see text. *B*: The change in the half-relaxation time ($\Delta\frac{1}{2}RT\%$) as a function of the number of stimuli. The continuous line was fitted by the method of least squares, see text. *C*: the change in the half-relaxation time as a function of the potentiation of twitch tension. Symbols: staircases evoked by 2/sec (●), 3/sec (◐), and 5/sec (○); arrows at twitches after 100 stimuli. Tetani evoked by 125/sec, 63 stimuli (□), 125 stimuli (■), 188 stimuli (▲, two sets of data), 375 stimuli (×); 100/sec, 150 stimuli (◇); 167/sec, 250 stimuli (◆). The points are averages of ten to twenty-one muscles and the vertical bars denote the S.E. of mean. 37–38 °C.

of potentiation, and (ii) that of diminution. The decay of potentiation after the staircase and the tetanus was similar over a large time interval with the exception that the rate of decay was faster immediately after the tetanus. The rapid rate of decay after the tetanus was evaluated by giving single stimuli with short time intervals. At frequencies of 0·5–0·2/sec slight potentiation would be caused by the stimuli which would counteract the decay of potentiation and may cause it to be delayed. However, this effect is presumably small. The time constants of the rapid and slow phases of decay of potentiation were close to those described in papillary muscle in rabbit at 36–37 °C (2·6 and 92·0 sec respectively, Edman & Jóhannsson, 1976). Hoh (1974) also noticed two rates of decay in the EDL and ascribed the initial fast decay to a simultaneous depression of twitch tension. However, the findings here are more compatible with two events of potentiation. Edman & Jóhannsson (1976) suggested the potentiation in heart muscle to be explained by a two-compartment model of calcium metabolism. A two-compartment model could also be assumed in the EDL, the potentiation in compartment I, associated with a fast rate of decay, being one half to one third the size of the potentiation in compartment II, associated

with a slow rate of decay. The potentiation in compartment I is not activated during the staircase and at low temperature (Krarup, 1981*a*). The potentiation in compartment II is present both after the staircase and after the tetanus and has similar rates of decay.

The potentiation in the compartments could be fitted by hyperbolic functions of the number of stimuli in the staircase (Fig. 4) and in the tetanus (Fig. 7) and of the frequency of stimuli in the staircase after 250 stimuli (Fig. 3). This may suggest that the processes involved in potentiation show saturation. Moreover, though large extrapolations were involved, the maximum potentiation in compartment II was similar (about +200%) when evoked by a staircase and a tetanus. The maximum potentiation in compartment I was about one third that in compartment II.

Activation of mammalian muscle fibres is considered to be incomplete at 35–38°C (Close & Hoh, 1968*a*, *b*; Desmedt & Hainaut, 1968; Ranatunga, 1977). The active state of the unpotentiated twitch in the EDL was calculated to increase from 60 to 90% that attained in a fused tetanus when the muscle was potentiated (Rosenfalck, 1974). Changes in the excitation-contraction coupling with an increased release of activator Ca have been proposed to account for the increased activation during potentiation (Close & Hoh, 1968*b*; Desmedt & Hainaut, 1968). However, in frog muscle the level of free Ca was found to decline during the staircase and in the post-tetanically potentiated twitch (Blinks, Rüdel & Taylor, 1978). The experiments reported here do not give evidence as to the nature or localization of the processes involved in the two-compartment model for potentiation. Assuming that the excitation-contraction coupling is involved in the increased activation (Ranatunga, 1977), it could be speculated that a finite number of sites in the transverse tubular system-terminal cicternae, associated with the excitation-contraction coupling, have differential sensitivity to the fraction of time in a train of impulses during which the membrane is depolarized. In mammalian heart muscle the potentiation was directly proportional to the duration of the depolarization (Edman & Jóhannsson, 1976).

The contraction time of the potentiated twitch seemed to be influenced in opposite directions by the repetitive activity and by the potentiation of tension: as compared with the unpotentiated twitch, the contraction time was shortened initially in the staircase and at low post-tetanic potentiation. The shortening was even more marked when the potentiation was decreased by low temperature (Krarup, 1981*a*). Potentiation tended to decrease this shortening and eventually to prolong the contraction time. A prolonged contraction time of the potentiated twitch has been interpreted to be due to (i) a prolongation of the time during which the muscle was activated (Ritchie & Wilkie, 1955; Close & Hoh, 1968*b*) thus increasing both the tension and the contraction time, and (ii) an increased activation causing a prolonged contraction time (Ranatunga, 1977). The finding in this report of an initial greater and a later smaller increase in the rate of force development than in twitch tension during the staircase seems, however, more compatible with the hypothesis that the potentiation is due initially to an increase in activation and later to a prolongation of the time during which the muscle is activated (Close & Hoh, 1968*b*).

The contraction time of the twitch was also prolonged by an increase in the number of stimuli in the staircase or in the tetanus which was independent of potentiation, unrelated to neuromuscular transmission and unrelated to the presence or absence

of the process causing diminution. Although not quantitatively investigated in the study, the prolongation of the contraction time may be related to a prolongation of the action potential, evident after tetani of 375 stimuli (Fig. 5). Independently of the potentiation, the intracellular action potential was prolonged during the staircase in frog muscle (Colomo & Rocchi, 1965). Hanson (1974) showed the intracellular action potential in fast-twitch rat muscle after repetitive activity to be 25% prolonged; the peak amplitude was only slightly diminished, and the negative afterpotential was increased.

After 250 stimuli in the staircase the rate of decay was initially slower than later. The initial decay was evaluated by stimuli once every 15 sec as compared with stimuli once every 60 sec after the first minute. The potentiating effect of repetitive stimuli at a frequency of 0·067/sec was considered to be small and would only cause very little delay on the decay of potentiation. The main cause of the delay in decay was suggested to be due to the recovery of a process causing diminution of twitch tension. This process was absent until after about 140 contractions in the staircase and increased with increasing frequency of stimuli. This is compatible with fatigue Edwards, Hill & Jones (1975) linked the slowing of relaxation in fatigued mouse muscle to a decline in adenosine triphosphate (ATP) concentration; in frog muscle the prolonged relaxation was linearly related to the decline in the free energy change of ATP hydrolysis during fatigue (Dawson, Gadian & Wilkie, 1980). In this study the half-relaxation time seemed the more prolonged the greater the diminution. Diminution occurred both in twitches evoked by stimuli to the nerve and directly to the muscle, confirming that 'contractile fatigue' occurs after fewer stimuli at low frequency of stimulation than 'fatigue of neuromuscular transmission' (Kugelberg & Lindegren, 1979). The diminution seemed related to the tension-time area of the twitch since it was increased during the staircase at low temperature (Krarup, 1981*a*) whereas the diminution disappared during partial block of the excitation-contraction coupling by Dantrolene (Krarup, 1981*b*).

That diminution was present after 250 stimuli in a staircase but not after 250 stimuli in a tetanus may be due to a greater energy consumption when there is relaxation between contractions. Thus in human muscle the rate of heat production was six times greater per unit force at a stimulus rate of 5/sec than in a fused tetanus (Edwards, Newham & Wiles, personal communication). The reasons for this difference may be (i) a greater external and internal work performed during 250 twitches than in a tetanus, and (ii) a greater activation heat in a series of twitches than in a tetanus. Disregarding the compliance of the tendons, the work performed during the staircase was 10–11 times greater during the staircase than during the tetanus:

$$\frac{P_t \times X_1 \times 250}{P_0 \times X_2} = \frac{575 \times 0{\cdot}2 \times 250}{2849 \times 0{\cdot}9} = 11$$

where P_t is the twitch tension (g/cm^2) which on average was 50% greater than the pre-staircase twitch (Table 1); P_0 is the tetanic tension (g/cm^2); X_1 is the 0·2% shortening of the muscle during the twitch, and X_2 is the 0·9% shortening during the tetanus (see Methods p. 358). The work resulting from this compliance was 3×10^{-4} J/g wet wt. of muscle per twitch and 80×10^{-4} J/g per tetanus. The activation heat of frog muscle in the second of two contractions decreased with a

decrease in the stimulus interval (Homsher & Keen, 1978), and in the EDL of rat (27 °C) the activation heat was 2–3 times greater per stimulus at a rate of 5/sec than during a fused tetanus (80/sec) (Wendt & Gibbs, 1973). Although metabolites are presumably replaced during the staircase, there seems to be a deficit in energy since a fast-twitch muscle is fatigued at a frequency of as low as 3–4/sec (Kugelberg & Lindegren, 1979). However, diminution was present after a tetanus of 375 stimuli and the decrease in post-tetanic potentiation was associated with a prolongation of the half-relaxation time of the twitch. It is of interest in this connexion that Close & Hoh (1968*b*) found the potentiation in the EDL to be more diminished after 500 stimuli at 20/sec than after 900 stimuli in a tetanus of 300/sec. In the human elbow flexors both diminution and potentiation occurred simultaneously in a staircase whereas only potentiation was present after a tetanus (Krarup & Horowitz, 1979).

I am grateful to Professor F. Buchthal for his help, criticism, and encouragement. The study was supported by grants from the Muscular Dystrophy Group of Denmark, the Foundation for Experimental Neurology (Copenhagen, Denmark), the Danish Medical Research Council, and by a grant to Professor Buchthal from the Muscular Dystrophy Association of America, New York.

REFERENCES

ASMUSSEN, G. & GAUNITZ, U. (1978). Kontraktile Eigenschaften der quergestreiften Muskelfasern des Ösophagus im Vergleich mit ausgewählten Skelettmuskeln der Ratte. *Acta biol. med. germ.* **37**, 335–346.

BAGUST, J., LEWIS, D. M. & LUCK, J. C. (1974). Post-tetanic effects in motor units of fast and slow twitch muscle of the cat. *J. Physiol.* **237**, 115–121.

BLINKS, J. R., RÜDEL, R. & TAYLOR, S. R. (1978). Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *J. Physiol.* **277**, 291–323.

CLOSE, R. (1964). Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol.* **173**, 74–95.

CLOSE, R. (1969). Dynamic properties of fast and slow skeletal muscles of the rat after nerve cross-union. *J. Physiol.* **204**, 331–346.

CLOSE, R. & HOH, J. F. Y. (1968*a*). Influence of temperature on isometric contractions of rat muscle. *Nature, Lond.* **217**, 1179–1180.

CLOSE, R. & HOH, J. F. Y. (1968*b*). The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle. *J. Physiol.* **197**, 461–477.

CLOSE, R. & HOH, J. F. Y. (1969). Post-tetanic potentiation of twitch contractions of cross-innervated rat fast and slow muscles. *Nature, Lond.* **221**, 179–181.

COLOMO, F. & ROCCHI, P. (1965). Staircase effect and post-tetanic potentiation in frog nerve-single muscle fibre preparations. *Archo Fisiol.* **64**, 189–266.

CONNOLLY, R., GOUGH, W. & WINEGRAD, S. (1971). Characteristics of the isometric twitch of skeletal muscle immediately after a tetanus. A study of the influence of the distribution of calcium within the sarcoplasmic reticulum on the twitch. *J. gen. Physiol.* **57**, 697–709.

DAHL, K. & BUCHTHAL, F. (1978). Digital memory recorder in electromyography and nerve conduction studies. *Electroenceph. clin. Neurophysiol.* **45**, 538–544.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1980). Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorous nuclear magnetic resonance. *J. Physiol.* **299**, 465–484.

DESMEDT, J. E. & HAINAUT, K. (1968). Kinetics of myofilament activation in potentiated contraction: Staircase phenomenon in human skeletal muscle. *Nature, Lond.* **217**, 529–532.

EDMAN, K. A. P., GRIEVE, D. W. & NILSSON, E. (1966). Studies of the excitation-contraction mechanism in the skeletal muscle and the myocardium. *Pflügers Arch. ges. Physiol.* **290**, 320–334.

EDMAN, K. A. P. & JÓHANNSSON, M. (1976). The contractile state of rabbit papillary muscle in relation to stimulation frequency. *J. Physiol.* **254**, 565–581.

EDWARDS, R. H. T., HILL, D. K. & JONES, D. A. (1975). Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. *J. Physiol.* **251**, 287–301.

HANSON, J. (1974). The effects of repetitive stimulation on the action potential and the twitch of rat muscle. *Acta. physiol. scand.* **90**, 387–400.

HOH, J. F. Y. (1974). Neural regulation of muscle activation. *Expl Neurol.* **45**, 241–256.

HOMSHER, E. & KEAN, C. J. (1978). Skeletal muscle energetics and metabolism. *A. Rev. Physiol.* **40**, 93–131.

KARIS, J. H., GISSEN, A. J. & NASTUK, W. L. (1967). The effect of volatile anesthetic agents on neuromuscular transmission. *Anesthesiology* **28**, 128–133.

KRARUP, C. (1977). Electrical and mechanical responses in the platysma and in the adductor pollicis muscle: In normal subjects. *J. Neurol. Neurosurg. Psychiat.* **40**, 234–240.

KRARUP, C. (1978). The effect of dantrolene sodium (Dantrium) and temperature on excitation contraction coupling. *IVth International Congress on Neuromuscular Diseases.* Montreal, Canada. (Abstract 298).

KRARUP, C. (1981*a*). Temperature dependence of enhancement and diminution of tension evoked by staircase and by tetanus in rat muscle. *J. Physiol.* **311**, 373–387.

KRARUP, C. (1981*b*). The effect of Dantrolene on enhancement and diminution of tension evoked by staircase and by tetanus in rat muscle. *J. Physiol.* **311**, 389–400.

KRARUP, C. & HOROWITZ, S. H. (1979). Evoked responses of the elbow flexors in control subjects and in myopathy patients. *Muscle & Nerve* **2**, 465–477.

KUGELBERG, E. & LINDEGREN, B. (1979). Transmission and contraction fatigue of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. *J. Physiol.* **288**, 285–300.

MILLER, R. D., WAY, W. L., DOLAN, W. M., STEVENS, W. C. & EGER, E. I. (1972). The dependence of pancuronium- and d-Tubocurarine-induced neuromuscular blockades on alveolar concentrations of Halothane and Forane. *J. Anesthesiol.* **37**, 573–581.

RANATUNGA, K. W. (1977). Influence of temperature on the characteristics of summation of isometric mechanical responses of mammalian skeletal muscle. *Expl Neurol.* **54**, 513–532.

RITCHIE, J. M. & WILKIE, D. R. (1955). The effect of previous stimulation on the active state of muscle. *J. Physiol.* **130**, 488–496.

ROSENFALCK, P. (1974). Change in active state during the staircase phenomenon of human muscle. *Acta physiol. scand.* **92**, 12–20.

SLOMIĆ, A., ROSENFALCK, A. & BUCHTHAL, F. (1968). Electrical and mechanical responses of normal and myasthenic muscle, with particular reference to the staircase phenomenon. *Brain Res.* **10**, 1–78.

WAUD, B. E., CHENG, M. C. & WAUD, D. R. (1973). Comparison of drug-receptor dissociation constants at the mammalian neuromuscular junction in the presence and absence of halothane. *J. Pharmac. exp. Ther.* **187**, 40–46.

WENDT, I. R. & GIBBS, C. L. (1973). Energy production of rat extensor digitorum longus muscle. *Am. J. Physiol.* **224/5**, 1081–1086.