LATENCY RELAXATION IN FROG SKELETAL MUSCLE

A Dissertation

Presented to

the Faculty of the Department of Biophysical Sciences

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in Biophysical Sciences

---

by

LaNelle Evelyn Geddes

August, 1970

ACKNOWLEDGEMENTS

I wish to express deep appreciation to Dr. David S. Mailman for proposing the problem that is the subject of this dissertation and for his support and suggestions throughout the investigation. A debt of gratitude is owed to my thesis committee, Dr. Allen H. Bartel, Dr. Robert L. Hazelwood, Dr. Addison L. Lawrence, and Dr. John F. Oro, for their counsel and encouragement throughout my educational experience, with special thanks to Dr. Bartel for his role in starting me upon this endeavor.

I am grateful to Mr. Arno Moore and Mr. Tom Coulter for the fabrication of the special equipment used in these experiments.

The encouragement and support so patiently and consistently provided by my husband, Leslie, and without which this goal would never have been realized, is sincerely appreciated and gratefully acknowledged.

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ABSTRACT

Latency relaxation, a reduction in resting tension which immediately precedes the twitch, has been observed in certain muscles. Its basic mechanism and physiological significance are not completely understood, but because it occupies a temporal position intermediate to stimulation and the development of positive tension it has been suggested as a mechanical manifestation of some phase of excitation-contraction coupling.

To more closely investigate the relationship between latency relaxation and the accompanying twitch, frog sartorii were subjected to various procedures and the relative effects on latency relaxation and twitch were compared. The experimental variables included increasing resting tension, immersion into calcium-free Ringer solution with a chelating agent added, the presence of caffeine in the bathing medium, and increased temperature. Except in the presence of caffeine, latency relaxation amplitude was observed to either decrease more slowly than twitch, or increase while twitch decreased. Latency relaxation amplitude reached its maximum at consistently higher resting tensions than did the twitch. In muscles placed into the calcium-free solution, both latency relaxation and twitch decreased with increasing time of exposure, but the former at a slower rate than the latter. At moderate temperatures (25° to 32° C) latency relaxation was potentiated, the twitch less so, or not at all; at higher temperatures (up to 38°C) both events declined, but the twitch fell to a smaller percentage of the control value than did latency relaxa-

tion. With increasing time of exposure to a 1 mM caffeine solution, twitch amplitude increased while latency relaxation amplitude decreased.

The dissimilarities in response of twitch and latency relaxation indicate separate basic mechanisms for the two events and demonstrate that the amplitude of latency relaxation is not indicative of the amplitude of the accompanying twitch. The results of these experiments were consistent with the suggestion that latency relaxation may arise from configurational changes along the thin muscle filaments associated with calcium-troponin interaction. In addition, the recorded latency relaxation amplitude may not only reflect the basic mechanism of the phenomenon itself, but, also, the delay in onset of contraction and the early twitch dynamics. Thus, factors which suppressed or slowed contraction tended to enhance latency relaxation and vice versa.

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CHAPTER I

INTRODUCTION

Latency relaxation is a small, transient reduction in resting tension that has been observed just prior to the contraction of some muscles. Relaxation does not begin immediately on application of a stimulus, but exhibits a latency of its own. Tension declines along a sigmoid course, reaching a maximum decrease, then rapidly reapproaching and recrossing the baseline. Figure 1 is a typical record of latency relaxation.

The mechanical unit of muscular contraction is a twitch, the contractile response to a single stimulus. As shown in Figure 2, a twitch is readily divided into three distinct phases, the latent period, contraction, and relaxation. The latent period is defined as the time between stimulation and the commencement of the development of positive tension, and, as the designation implies, was originally considered to be a mechanically quiescent interval. However, Rauh (58), in his investigation on the duration of the latent period in 1922, reported a small elongation of the muscle prior to the onset of active contraction. The elongation he noted most likely corresponds to the resting tension reduction occurring during the final portion of the latent period and terminated by the onset of contraction. In the frog sartorius this phenomenon exhibits a duration of approximately two msec, or about 1/50 of the duration of the ensuing twitch, and an amplitude on the order of 1/500 of the contractile force. The relative

LATENCY RELAXATION

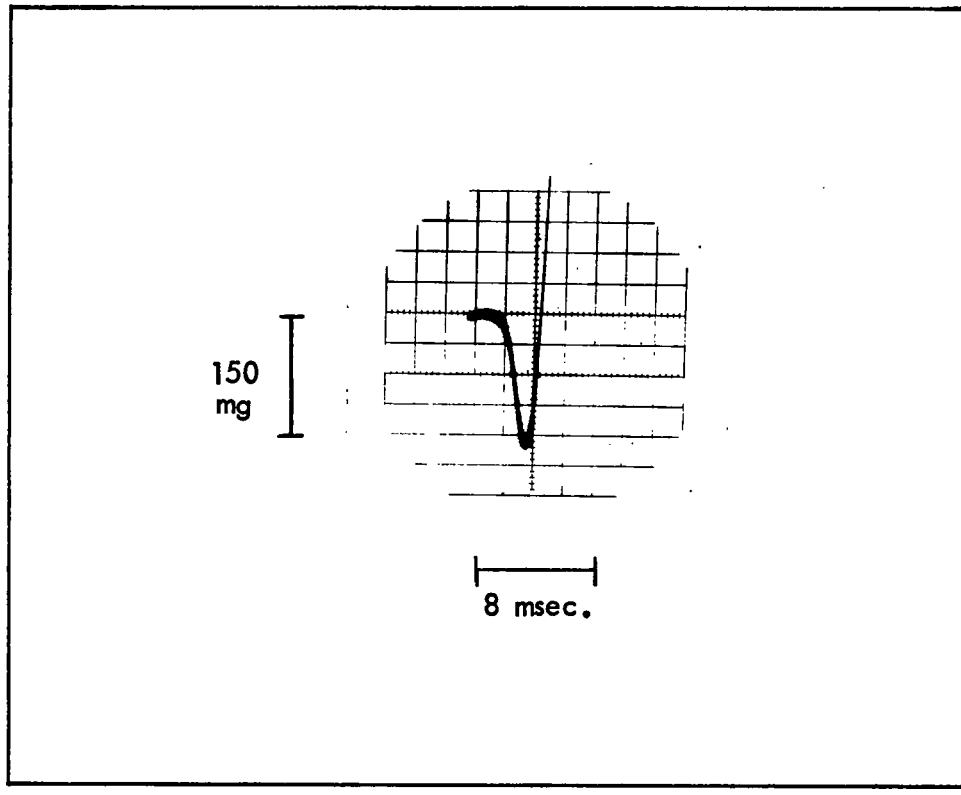


Figure 1. A record of latency relaxation.

The beam was triggered to sweep by the stimulus.

TENSION - TIME PROFILE OF A MUSCLE TWITCH

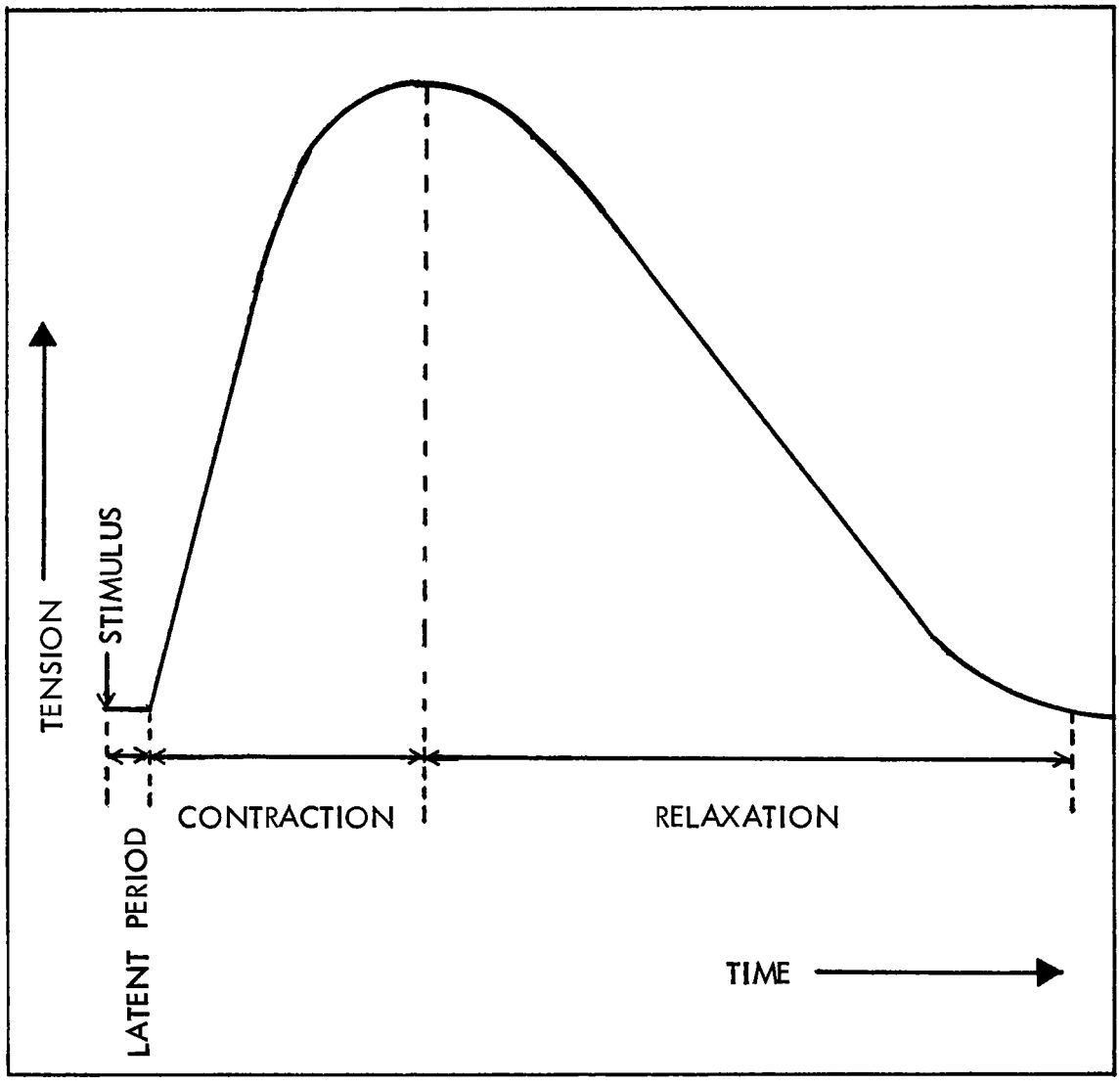


Figure 2. The phases of a typical twitch. In frog sartorius muscle the duration, from stimulus to the end of relaxation, is about 100 msec at room temperature.

amplitudes and durations of twitch and latency relaxation are shown in Figure 3.

Because latency relaxation occupies a temporal position intermediate to the electrical and mechanical events of an activated muscle, it has been suggested to be a mechanical manifestation of some phase of excitation-contraction coupling (64, 65). More specifically, it has been proposed that latency relaxation may be associated in some manner with the mobilization of calcium ions which activate the interaction of actin and myosin and the development of mechanical energy (67). Despite these suggestions, the relationship of latency relaxation to contraction itself is not completely understood. The purpose of these experiments was to compare latency relaxation and twitch in an effort to place the precontractile relaxation in better perspective with active muscular contraction. It was of primary interest to determine whether the phenomenon of the early relaxation is an integral part of the contractile process itself or an independent event. If, indeed, latency relaxation and contractile output are inexorably linked, a change in the former should be associated with a similar change in the latter. However, a demonstration of dissimilar responses between the two mechanical events to an experimental procedure would suggest that they are most likely separate processes and offer some clue as to the basis of latency relaxation and its relationship to the overall process of muscle contraction.

It was also of interest to compare the responses of latency relaxation and twitch under conditions which favored the outward diffusion of calcium from the muscle. There is little doubt that calcium ions play an important role in muscle

DURATION AND AMPLITUDE RELATIONSHIPS
OF TWITCH AND LATENCY RELAXATION

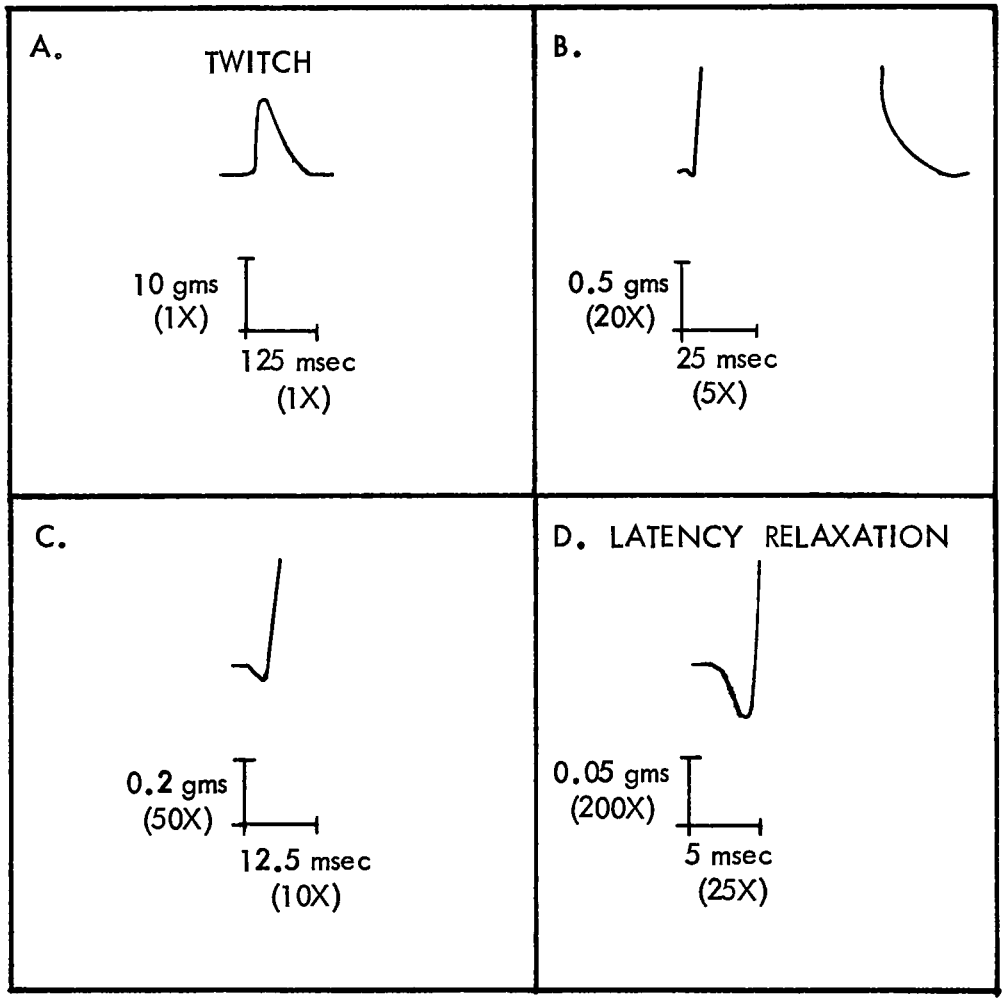


Figure 3. This figure was drawn from a series of oscilloscope records made at increasing sensitivity and sweep speeds. The factors in parentheses refer to an increase above the corresponding values in frame A.

contraction, but their importance to the mechanism of latency relaxation is not known. Observations were made on the time courses of twitch and latency relaxation amplitudes in muscles placed in media to which no calcium was added but which contained a chelating agent to bind any outwardly diffusing calcium.

Although latency relaxation and twitch are displaced from resting tension in opposite directions and to vastly different degrees, the influence of this parameter on the amplitudes of the two events was compared to determine if maximum twitch and maximum latency relaxation occurred at the same value of resting tension. The time courses of twitch and latency relaxation amplitudes at elevated temperatures and in the presence of caffeine were also observed.

The physiological role of muscle tissues is, after all, a mechanical one, and all mechanical events related to muscular contraction should be scrutinized for their role in the overall function. Latency relaxation is a mechanically manifested event, but neither its source nor its importance to muscular contraction is completely understood. It was with a view to a better understanding of this phenomenon that the studies described in this dissertation were undertaken.

CHAPTER II

HISTORY OF LATENCY RELAXATION

Locomotion is one of the most important and noticeable capabilities of man and animals, and an explanation of its physiological basis was offered as early as about 300 B. C. by Erasistratus, who suggested muscular contraction resulted from an influx of animal spirit from the brain which expanded and shortened the muscle (49,17). As unacceptable as this explanation may be when viewed in the light of present knowledge, it was, nevertheless, accepted as valid for centuries (49). The recognition of an electrical component associated with muscular contraction traces its origin to the observations of Galvani (37) and the experiments of Matteucci (38).

Around the mid-nineteenth century scientific instruments became available which permitted visualization of the waveforms of the electrical and mechanical events associated with muscle contraction (38). Use of these devices revealed a latent period existing between the stimulus and onset of contraction (6,7). Most investigators apparently considered the latent period to be an artifact introduced by the inertia of the recording devices (24). Therefore, attention was focussed upon developing recording instruments of low inertia.

In 1922 Rauh published a paper (58) wherein he described a sensitive photographic method of recording muscular contraction through a microscope. This method was essentially inertialess since the muscle contraction itself did not supply the energy to move a recording lever, but only moved a fine, glass fiber in a beam

of light focussed upon it. The movements of the glass fiber were then photographed. Rauh described the care taken to provide adequate resting tension and stimulus strength to have the latent period as short as possible. He was unable to reduce the recorded latent period to zero, but his sensitive recording device revealed latency relaxation.

At first Rauh believed the early relaxation was due to the bending of a small wire loop to which the muscle was attached. He replaced the fragile loop with a heavy, rigid metal plate to which a hook was soldered for affixing the muscle. Still a depression of the tension record preceded the rising phase of contraction. He concluded that since a mechanical event, albeit small, preceded the development of positive contractile tension, the latent period is genuine and contraction is not instantaneous with stimulation, but is preceded by an even earlier event.

Latency relaxation attracted very little attention until the early 1940's when it was "rediscovered" by Sandow (61) who performed the first studies designed to elucidate its underlying mechanism and reveal its physiological significance. Furthermore, he named the phenomenon latency relaxation; in Rauh's paper it had been referred to simply as "die Nase" (the nose), no doubt because of its similarity to a nasal contour. Sandow, like Rauh, unexpectedly recorded latency relaxation during a search for improved instrumentation for use in studies of muscle physiology. He used a piezoelectric crystal phonograph pickup as a myograph and displayed the output on an oscilloscope. The piezoelectric trans-

ducer proved to be an extremely sensitive myograph, so much so that Sandow observed the precontractile relaxation to which he later devoted much attention. The fact that both Rauh and Sandow observed latency relaxation only by the employment of extremely sensitive, rapidly responding transducing and recording devices may give some clue regarding the lack of attention paid to the phenomenon. Its small amplitude and short duration impose stringent requirements on the equipment used to record it.

In 1944 Sandow published a description (61) of the equipment and method he used to record latency relaxation. He carefully measured the duration of certain phases of the phenomenon and associated these with sequential events between the delivery of a stimulus and the contraction itself. The phases of latency relaxation that were measured by Sandow are shown in Figure 4. He equated the time interval L_R with the latency relaxation-induction process. At L_0 tension production was said to begin, but it is overshadowed by the ongoing relaxation process up to the time L when the contractile force overcomes relaxation and the positive twitch tension rapidly develops.

Sandow's first step in proposing a theory to account for latency relaxation was to determine whether the phenomenon belonged to the realm of excitatory or mechanical events. To resolve this question, he arranged a piece of nerve trunk in the recording device in place of a muscle. The nerve was placed under resting tension and treated as if it were muscle. However, stimulation of the nerve produced no response similar to the event of latency relaxation seen in stimulated muscle. This indicated that the basic mechanism underlying latency relaxation

LATENCY RELAXATION PHASES

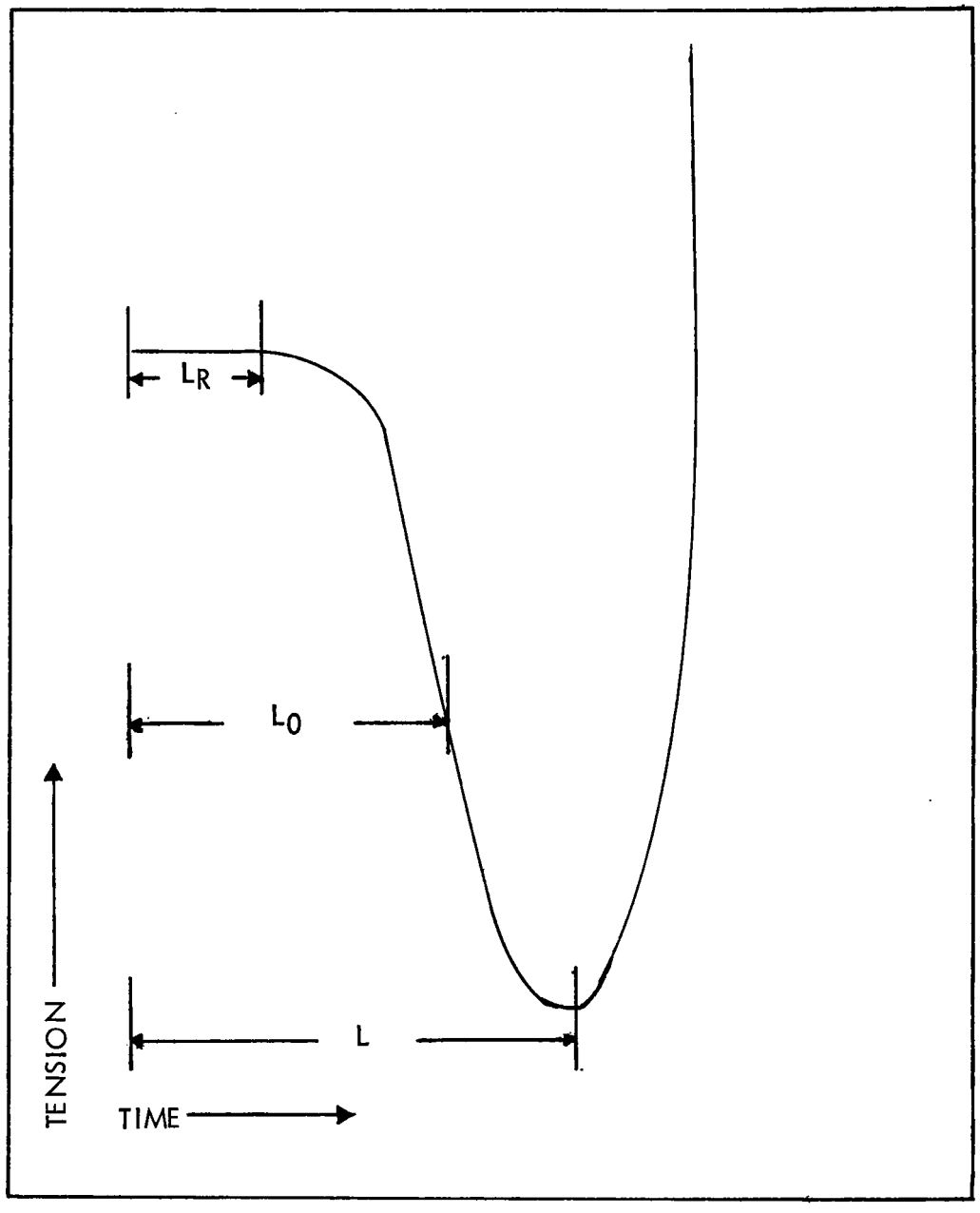


Figure 4. L_R is the latent period of latency relaxation and most likely represents the time required for inward propagation of depolarization. At the point, L_0, onset of contraction begins. Positive tension begins at L.

resided in the contractile portion of the muscle, and suggested to Sandow that it was a function of myosin. Only about five years earlier Engelhardt and Ljubimova (16) had published their important account of the ATPase activity of extracted myosin. With this work in mind Sandow suggested that myosin undergoes sequential changes of form during a contraction cycle and that latency relaxation occurs during the formation of the enzyme-substrate complex, myosin-ATP.

Studies on the effect of increasing stimulus strength (61) showed that the amplitude of latency relaxation increased, decreased, then increased to a maximum value. This behavior of the latency relaxation was explained as follows. At very low stimulus strength (subthreshold for direct stimulation) the muscle fibers were excited only through their motor nerves. With increasing stimulus intensity more nerves were stimulated and hence, more muscle fibers were recruited and contributed to the overall latency relaxation. However, as the stimulus intensity increased further, the threshold value for the most sensitive muscle fibers was reached. Because these directly stimulated fibers contracted sooner after the application of the stimulus than those fibers activated by a nerve impulse with its longer myoneural junction delay, they were already developing positive tension at the time the indirectly stimulated fibers were experiencing latency relaxation. The tension change during contraction is greater than that of the initial relaxation; thus a few contracting fibers can overshadow the tension change in many relaxing fibers. This recession of latency relaxation amplitude (in a whole muscle) continued until a stimulus strength was reached where most of the fibers

were being stimulated directly, and the initial relaxations of many fibers were once again in phase and manifested as a larger latency relaxation. Finally, more and more fibers were stimulated directly and the latency relaxation amplitude increased to its maximum value. Mauro (50) observed latency relaxation in single fibers of frog semitendinosus and tibialis anterior muscles and reported that the amplitude of latency relaxation in multifiber preparations was proportional to the number of fibers present.

Although the underlying mechanism of latency relaxation is not completely understood, a number of suggestions has been offered. In an appendix to Rauh's paper, Garten proposed that the phenomenon was due to a change in the coefficient of elasticity in a stimulated muscle, resulting from the heat associated with muscle activity (58). In 1926 Fischer (19) measured a small increase in the extensibility of muscle during the period of mechanical latency. He equated this to the precontractile elongation reported by Rauh a few years earlier. However, he rejected Garten's hypothesis of a change in the coefficient of elasticity and stated that the process responsible for the elongation was unknown.

No further attempt to explain the mechanism of latency relaxation was made until Sandow began his studies on the subject. He proposed that myosin exists in three forms (60,61), resting, contractile, and a form intermediate to the other two that is responsible for latency relaxation, and that the intermediate form represents an enzyme-substrate complex composed of activated myosin and ATP. That the latency relaxation form of myosin corresponded to an enzymatically

active one was based on several lines of evidence. First, the amplitude of latency relaxation exhibited a temperature dependence (63) similar to that of the primary denaturation of myosin extracted from frog muscle (51). Secondly, Engelhardt (15) found that myosin threads undergo increased extensibility during ATP splitting. He considered the extensibility change the in vitro equivalent of in vivo relaxation and suggested that ATP hydrolysis was catalyzed by myosin ATPase during the postcontractile relaxation. Since myosin ATPase activity appeared to be associated with a relaxation process, Sandow submitted that this could apply to the precontractile relaxation as well as the postcontractile.

At about the same time that Sandow was suggesting a biochemical interpretation of latency relaxation, another group of workers (26,69) was suggesting that the event was an artifact which resulted from a fluid wave, originating with the mechanical response as a local disturbance, exerting a force opposite to that of contraction, and being propagated throughout the muscle at a faster rate than the contractile activity. This explanation of latency relaxation apparently never received very much support.

Hill (33,35) did not examine latency relaxation per se, but he alluded to the work of others on the subject and placed some restrictions on explanations of the phenomenon. From his studies with stretch applied rapidly to muscles early in the latent period, he explained that latency relaxation does not reflect an increased extensibility because muscle is less, not more, extensible during the latent period than at other times in the contraction cycle. He also stated that the

changes occurring during latency relaxation must originate in structures associated with the parallel elastic elements of muscle. As to the exact mechanism of the early relaxation, he commented only that it is "a product of considerable alteration of mechanical state".

With Hill's stipulation that the structures responsible for latency relaxation must be parallel to the contractile elements of muscle in mind, Abbott and Lowy (1) examined the adductor muscle of Pecten for evidence of latency relaxation. According to these investigators this muscle does not possess a tough sarcolemma analogous to that of frog muscle fibers, and no latency relaxation was recorded. This finding agreed with Hill's association of latency relaxation with parallel elastic structures, for the sarcolemma is in parallel with the contractile fibrils. Furthermore, the view that sarcolemma is the structure mainly responsible for resting tension had been put forth fifteen years earlier by Ramsey and Street (57), and, since latency relaxation represents a change in resting tension, a muscle lacking the anatomical structure supposedly responsible for it would not be expected to show changes in resting tension. However, Lowy and Sten-Knudsen (47) later repeated the experiment on the same invertebrate muscle with an improved procedure and reported finding latency relaxation.

The explanations for the basic mechanism of latency relaxation listed above were given prior to two important developments in muscle physiology, namely introduction of the sliding filament theory of muscle contraction (40,42) and the identification of the sarcoplasmic reticulum as the probable sequestering site of calcium (8). In an attempt to explain latency relaxation in terms com-

patible with the sliding filament theory, Huxley (39) suggested that the actin filaments are joined together in the H zone by thin S filaments. He explained that in a resting muscle the S filaments are under tension, but activation reduces this tension and latency relaxation occurs.

As the process of muscle activation began to be more directly linked with the release of calcium and the importance of the T system in conducting an impulse inward was becoming more fully appreciated, the idea that the latency relaxation might reflect the movement of calcium from the sarcoplasmic reticulum was offered. Sandow (67) suggested that latency relaxation may be due to a change in osmotic pressure in the sarcoplasmic reticulum. He explained that when calcium is released from the sarcoplasmic reticulum, subsequent to excitation, the osmotic pressure within this structure diminishes, and water is lost, relieving some tension within the stretched muscle and producing latency relaxation. Loss of water as the cause of latency relaxation would be expected to lead to a decrease of muscle volume; however, Baskin and Paolini (4) reported a small increase in the volume of muscle coincident with latency relaxation.

Frog sartorius muscles have been the most widely utilized specimens for studies of latency relaxation, but the phenomenon has been observed in a variety of muscles from a number of species. Abbott and Ritchie (2) recorded latency relaxation in the dogfish coraco-hyoid muscle, cat tenuissimus, and rat diaphragm, in addition to the sartorius muscles of frogs and toads. Lowy and Sten-Knudsen (47) observed latency relaxation in the funnel retractor of Loligo and Octopus,

but they reported that it was present over only a small region of muscle lengths. In addition they observed latency relaxation in the anterior byssus retractor muscle (ABRM) of Mytilus. However, two stimuli spaced at an interval of not more than ten seconds were necessary for appearance of the phenomenon; it did not occur in response to a single shock.

It is certain that the classical latent period is not a period of the status quo. In addition to latency relaxation other events occur in the period between stimulation and the onset of contraction; heat production begins before any detectable tension develops (31), and the active state, as evidenced by decreased extensibility, is well underway (33) during the latent period. Other alterations in the muscle during the mechanical latent period include opacity (36) and volume changes (4). Despite continuing studies on the biochemistry of muscular contraction, and ever increasing understanding of the mechanism of muscular contraction and post-contractile relaxation, the basic mechanism underlying latency relaxation and its relationship to the twitch are not completely understood.

CHAPTER III

METHODS AND MATERIALS

An investigation of the relationship of latency relaxation to the associated twitch required a means of simultaneously recording the two events. However, the differences in amplitude, duration, and rate of rise of the twitch and latency relaxation required separate considerations in the choice of equipment for detection and display. Therefore, two separate recording channels were used, one for latency relaxation and the other for the twitch.

Assurance that a recorded output faithfully represents the physiological event requires an understanding of the capabilities and limitations of the instrumentation interposed between the biological preparation and the final display. The characteristics of the twitch and latency relaxation recording channels and their appropriateness for the task are described below.

A. THE TWITCH CHANNEL

Both recording channels consisted of a transducer, a processor, and a display device. A photoelectric myograph was the transducer for resting tension and twitch amplitude. This device, shown in Figure 5, consists of a moveable, opaque shade intervening between a light bulb and a photocell. One end of the muscle was attached to the shade; as the muscle contracted the shade was pulled out of the light path and permitted photons to reach the photocell. The photons dislodged electrons from the cathode of the photocell which were collected by the anode.

PHOTOELECTRIC MYOGRAPH

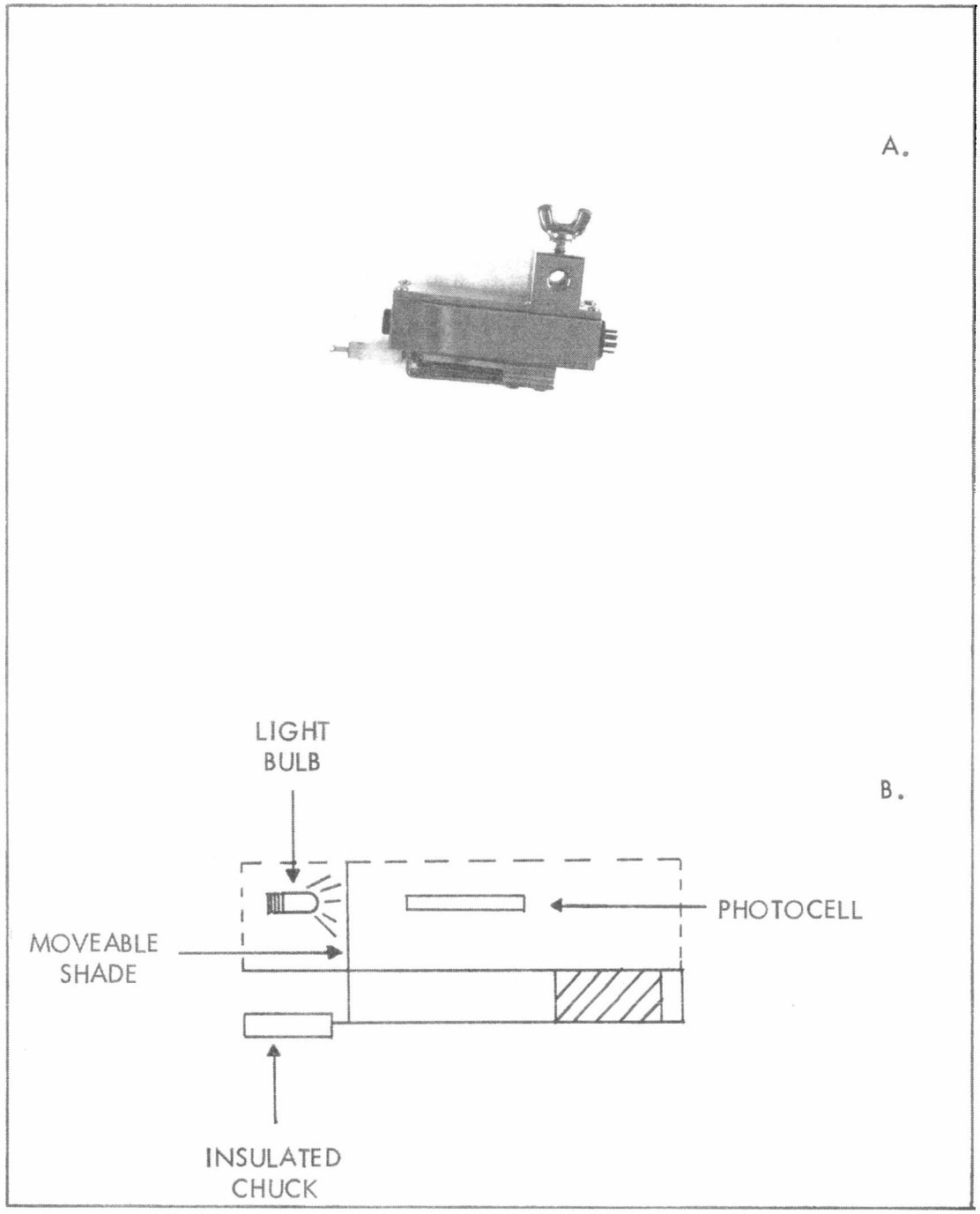


Figure 5. A. A photograph of the photoelectric myograph. B. Schematic figure of the myograph. The dotted lines represent the removed outer case.

The small photocurrent was led through a high resistance; the developed voltage was amplified and used to drive the recording device.

The photoelectric myograph possessed a frequency response extending to zero cycles per second (Hz); this implies that the transducer will produce a sustained output for a sustained input. Alternatively, the device may be described as exhibiting an infinite falling time constant. Only those transducers possessing this characteristic can provide quantitative baseline information. This feature is essential for recording resting tension, which represents a sustained input. The myograph was a commercially available Physiograph "C" myograph (Narco Biosystems, Inc., Houston, Texas) slightly modified by the addition of an insulated chuck extending from the cantilevered spring that carries the shade and to which the muscle was attached. The rationale for this alteration will be discussed later.

The twitch signal was amplified and recorded with a Physiograph power amplifier and ink-writing recorder. The transducer output was direct coupled to the power amplifier. Overall performance of a recording channel is affected by each component comprising it, and in the absence of direct coupling into the amplifier, the low frequency characteristic of the myograph, so necessary to recording resting tension, would be lost in the output.

Accurate recording of resting tension was important for two reasons. First, the amplitudes of twitch and latency relaxation were observed to vary with resting tension; thus it was necessary to perform the studies at a carefully determined, constant resting tension. Resting tension was adjusted before each experiment until a well-defined latency relaxation deflection was obtained, and resting tension

was usually slightly larger than that required for maximum twitch. Frequently, however, during the course of a series of observations the muscle exhibited a small loss of resting tension, even though the external length was not altered. When this occurred, the muscle was stretched slightly to restore the original resting tension. Secondly, an experimental series investigating the amplitude of twitch and latency relaxation, as a function of increasing resting tension was performed, and the accurate measurement of small increments in resting tension was necessary.

A special device, designated a tension meter, was constructed to indicate resting tension values. The tension meter, shown in Figure 6, was connected across the output of the Physiograph channel registering resting and twitch tensions. A sensitivity selection circuit permitted switching in one of four resistors of different values to provide a scale expansion of 1X, 2X, 5X, or 10X. The current flowing through the tension meter deflected the needle of a sensitive galvanometer along a scale that read from 0 to 50 calibratible units. The capacitor and diodes across the galvanometer protected it from damage when the large output associated with the twitch was presented to the tension meter. The deflection selector switch, labelled "Up" and "Down", indicated whether output was registered on the Physiograph pen as an upward or downward deflection, and was included in the device only in the interest of flexibility of use; in these experiments it was always in the "Up" position. Actually, the tension meter was a means of displaying the baseline portion of the tension record on a greatly magnified scale.

TENSION METER

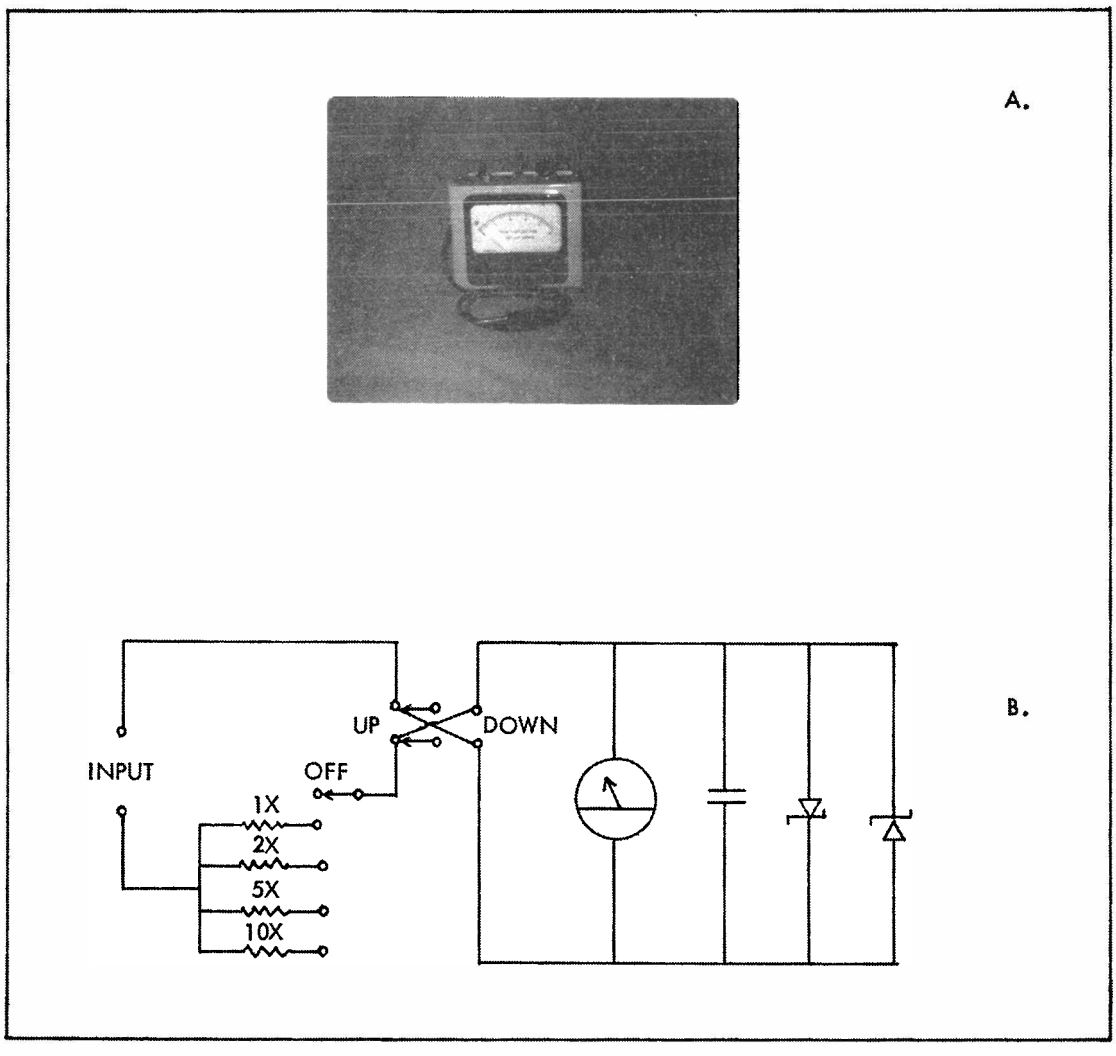


Figure 6. A. Photograph of the tension meter.

B. Simplified circuit diagram.

It was not used to measure twitch tension; the graphic recorder provided this information.

During the course of an experiment, resting tension was logged as so many divisions of galvanometer needle deflection; following completion of an experiment, the muscle was disconnected and the tension meter was calibrated by applying known weights to the myograph. Needle deflection was plotted versus weight to obtain a calibration curve from which resting tension was read directly. At the beginning of each experiment the amplification of the twitch (and resting tension) channel was adjusted to provide a large pen deflection on the twitch record. This setting usually varied from muscle to muscle depending on size and contractile vigor; therefore a calibration curve was made after each experiment. A typical calibration curve for resting tension is shown in Figure 7.

The myograph used in these experiments was designed to record tensions up to 500 grams, therefore, the measurement of resting tension in the lower range of its dynamic capabilities introduced some nonlinearity into the calibration curve, especially at values less than 1000 mg. In order to better visualize this lower portion of the calibration curve, needle deflection was plotted versus weight on three different curves, each covering a different weight range (0 - 1200 mg, 1000 - 5000 mg, and 4000 - 8000 mg). The stiffness of the transducer, which probably introduced the deviation from linearity at very low resting tension, permitted only a small amount of shortening when the muscle contracted, rendering the contraction essentially isometric; it was for this reason the stiff myograph

TENSION METER CALIBRATION CURVES

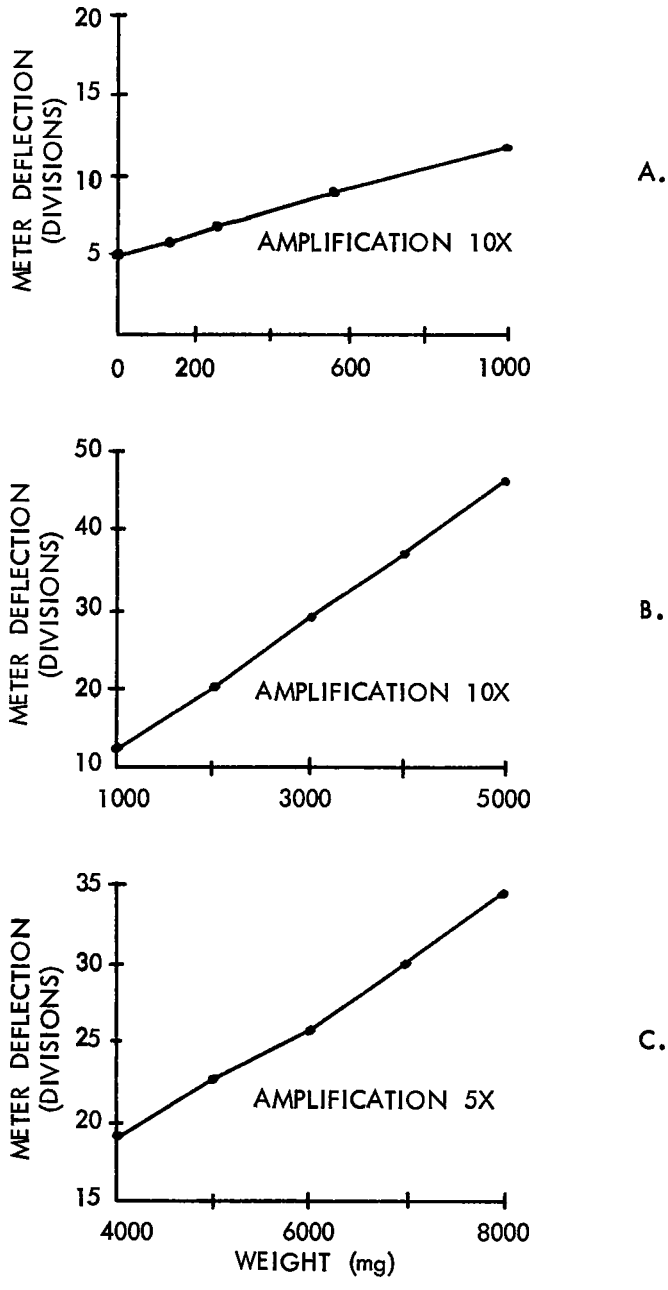


Figure 7. A. 0 - 1200 mg with a scale amplification of 10X. Zero tension was set at 5 divisions on the tension meter scale.

B. 1000 - 5000 mg with scale amplification of 10X.

C. 4000 - 8000 mg with a scale amplification of 5X.

was used. Most of the muscles examined had an in situ length around 30 mm and when stimulated produced about 25 g of twitch tension. For this tension, the myograph lever moved 125 microns, permitting the muscle to shorten by this amount, or about 0.5% of its length at rest. The piezoelectric myograph, to which the other end of the muscle was attached also permitted very little shortening.

The twitch was recorded on one channel of a 3-channel Physiograph recorder. A typical record of a series of twitches with increasing resting tension is shown in Figure 8. The numbers below each twitch are the deflections on the tension meter and represent the resting tension of the muscle. No increase in resting tension is noticeable initially from the position of the baseline, but an increase in resting tension was registered on the tension meter and reflected by increasing force of contraction. The series of twitches at the left of the record is representative of the procedure carried out before each experiment in which resting tension was increased until maximum twitch tension was obtained. The amplification was set high enough for a large twitch display, but not so large that the output exceeded the excursion of the pen. When experimental design called for a constant resting tension, the tension meter needle deflection at which a definite latency relaxation deflection occurred was noted and resting tension was maintained at that value throughout the series of contractions.

On completion of each experiment the muscle was disconnected from the myograph and graduated weights were hung in its place, and a twitch calibration record was constructed. The twitch amplitude was determined from the calibration

TWITCH TENSION WITH INCREASING RESTING TENSION

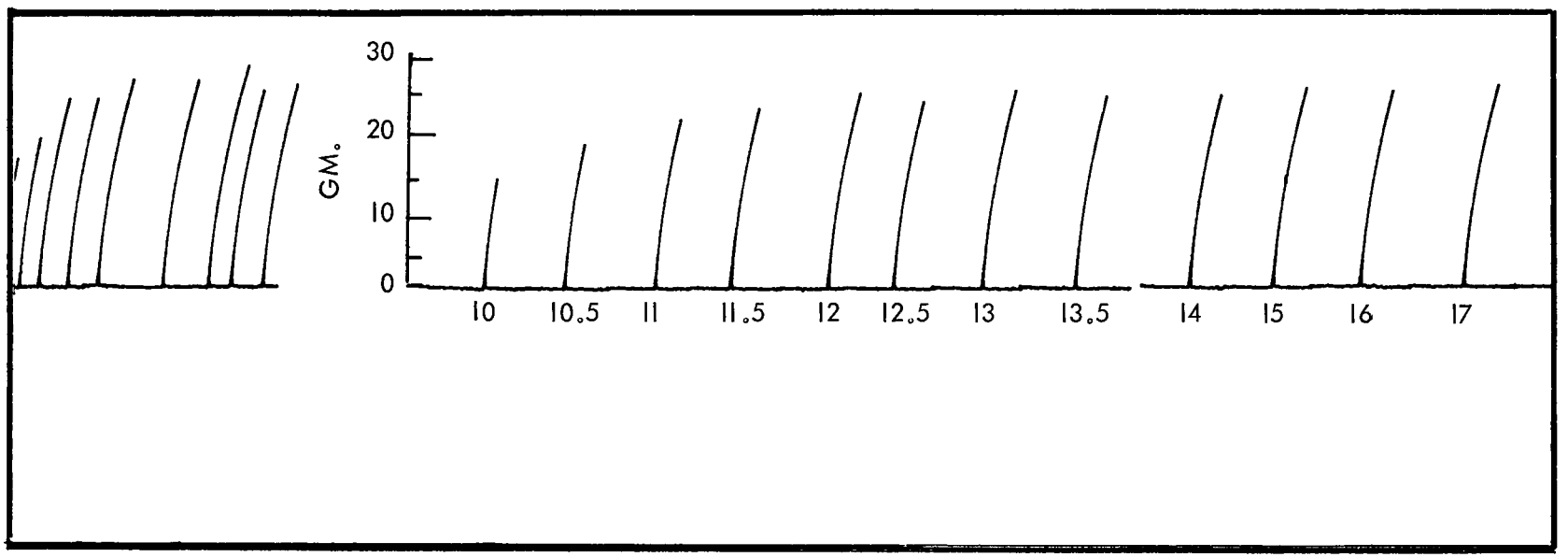


Figure 8. A series of twitches made with increasing resting tension. Each twitch was recorded at a higher resting tension than the preceeding one. The numbers below each twitch are resting tensions expressed as the number of divisions on the tension meter scale. The vertical scale is twitch tension in grams.

record.

In summary, the twitch channel recorded resting and twitch tensions. The twitch tension was detected, processed, and displayed on conventional, commercially available equipment (with the exception of the modification to the myograph hook). The resting tension was similarly detected and processed, but displayed on a specially constructed tension meter. The photoelectric myograph, which possesses a frequency response extending to zero Hz, permitted the acquisition of resting tension values.

B. THE LATENCY RELAXATION CHANNEL

The nature of an event under investigation dictates the characteristics of the instrumentation used to measure it. The equipment used to detect twitch tension was unable to record faithfully the small, rapidly changing latency relaxation.

The transducer for latency relaxation, pictured in Figure 9, is a chip of ceramic piezoelectric material arranged for use as a myograph. A piezoelectric myograph was well suited to the detection of latency relaxation because of its high sensitivity and speed of response.

Piezoelectric crystals are not widely used in myographs, but they do possess several characteristics which make them useful for this purpose. First, a relatively large voltage output (in the millivolt range) is produced for a very small displacement, making them extremely sensitive transducers. Secondly, they move only a few micra for each gram weight applied (45); thus the devices are relatively isometric. Thirdly, their extreme stiffness permits a rapid response to an

PIEZOELECTRIC CRYSTAL MYOGRAPH

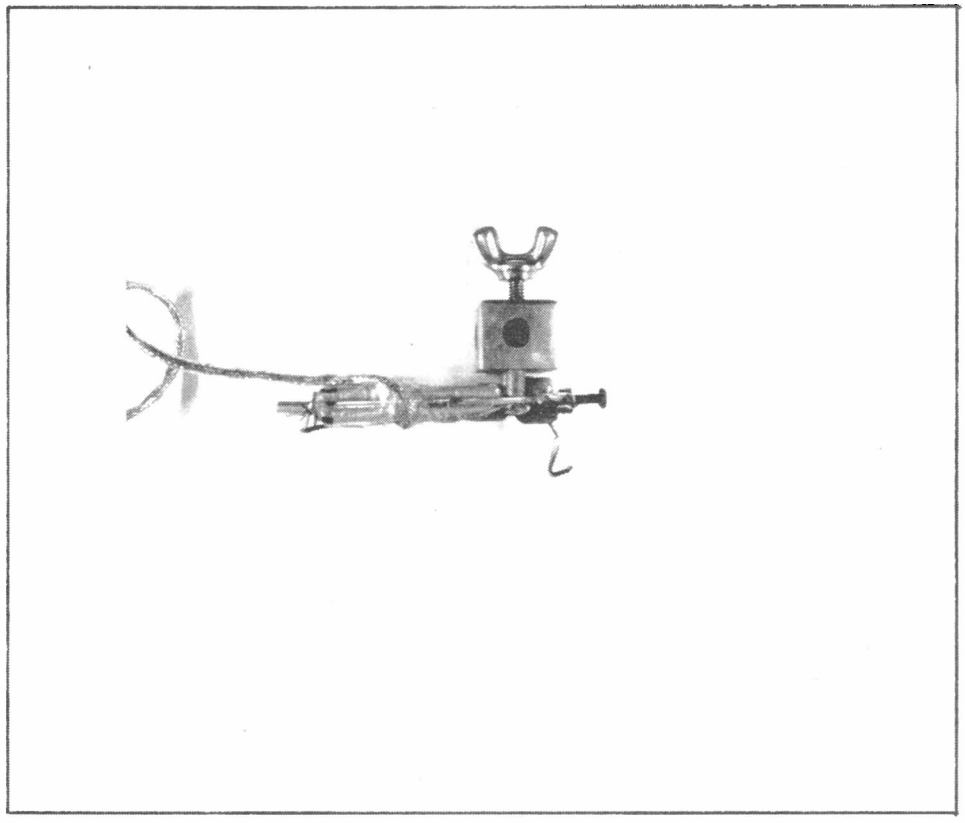


Figure 9. The piezoelectric myograph was connected to the preamplifier through the shielded cable shown at the left of the picture. The insulated hook to which the muscle was attached is seen protruding from the myograph at lower right.

imposed force, i.e., they exhibit good high frequency response. Less desirable, however, is the inability to maintain a sustained output for a sustained input, and, therefore, no information on the baseline (resting tension) can be obtained from the piezoelectric myograph.

The inability of a piezoelectric myograph to faithfully reproduce an input which changes slowly or not at all is explained by the equivalent circuit of the device as shown in Figure 10. The force applied to the crystal corresponds to the signal generator; this force produces a voltage arising from a separation of charge and is analogous to the capacitor, C. When an amplifier with a finite input resistance is connected across the piezoelectric crystal, current is drawn through the resistor, R. Therefore, the voltage appearing at the output as the result of a mechanical force applied to the crystal decays with a time constant equal to the product of the capacitance of the crystal and the input resistance of the amplifier (9,54). Not only are sustained inputs distorted in the output, but also events that change slowly with respect to the time constant of the system. Figure 11 shows a twitch recorded from the piezoelectric myograph. The waveform is not a true representation, but more closely resembles the time derivative of the tension. This results from the tension changes of the twitch itself occurring over a span of time that is long with respect to the time constant. Inappropriate as the crystal myograph may be for recording resting and twitch tensions, its sensitivity and speed of response suited it well to detection of the small, rapidly changing tensions of latency relaxation.

EQUIVALENT CIRCUIT OF THE
PIEZOELECTRIC CRYSTAL MYOGRAPH

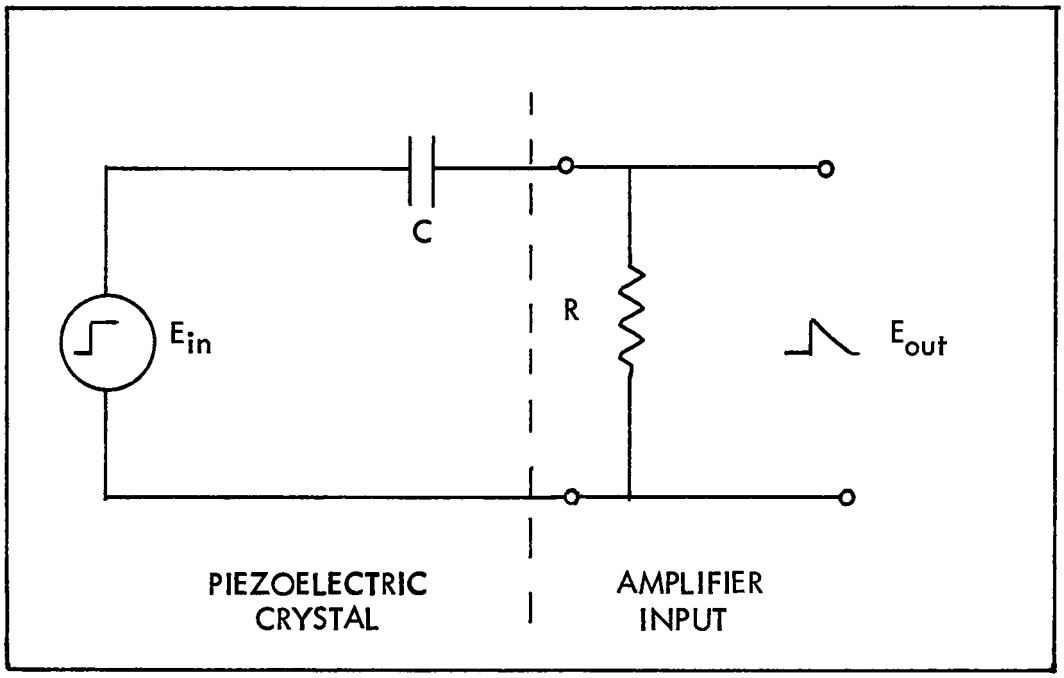


Figure 10. A step function, E_{in}, is pictured as the input to the crystal. The recorded output is shown as E_{out}. The capacitance of the crystal itself is represented by C, and R is the input resistance of the amplifier.

TWITCH TENSION RECORDED FROM

THE PIEZOELECTRIC CRYSTAL MYOGRAPH

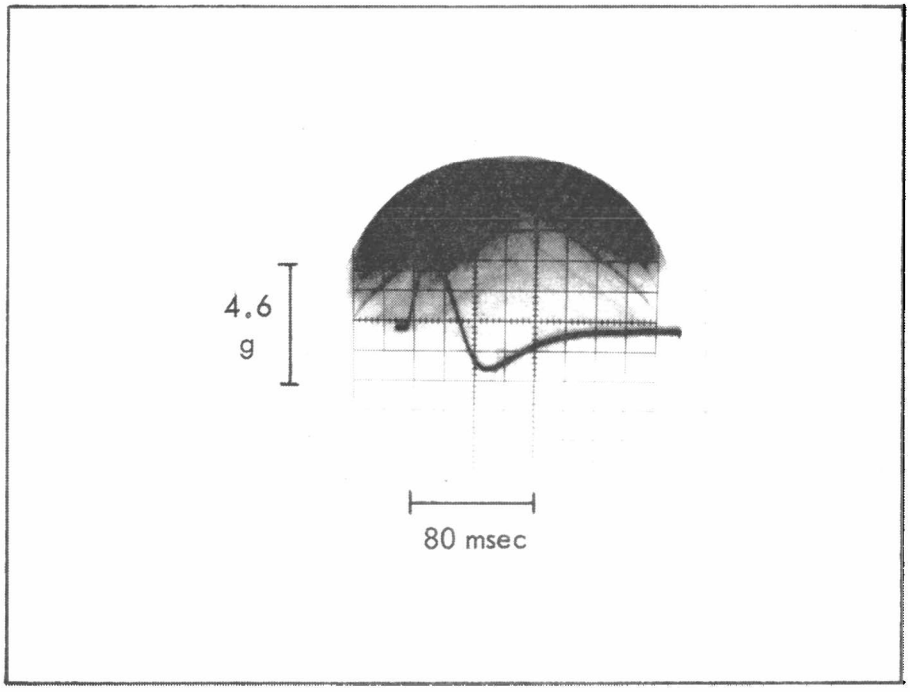


Figure 11. A complete twitch recorded from the crystal myograph. The differentiation of the record is especially noticeable in that portion of the record which goes below the baseline and then slowly reapproaches it.

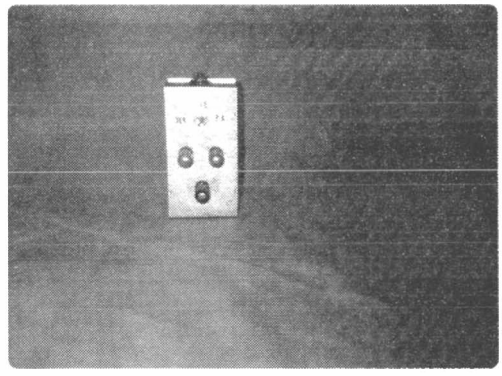
The output voltage from the crystal myograph was amplified by a Physiograph cardiac preamplifier; the variable amplitude selector on the preamplifier was set to maximum gain in all experiments, and provided an amplification of 75X. The output from the preamplifier was designed for connection to the Physiograph pen recorder. However, this recorder is not suitable for latency relaxation display because it lacks sufficient speed of response. Therefore, an oscilloscope was used to display the event. The switching device shown in Figure 12 was constructed to connect the output from the preamplifier to the input of the oscilloscope.

Latency relaxation was displayed on one channel of a 502A dual beam Tektronix oscilloscope (Tektronix, Inc., Portland, Ore.) and photographed on high speed Polaroid film using a Fairchild (DuMont Laboratories, Clifton, N. J.) oscilloscope camera. The latency relaxation signal was applied to both sides of a differential amplifier in the oscilloscope through a pair of probes which attenuated the signal by a factor of 10, but, being shielded cables, they reduced the amplitude of random environmental, electrical noise. The attenuation was easily compensated for by increasing the amplification provided by the amplifiers in the oscilloscope itself.

Retracing the latency relaxation signal from the muscle to the displayed record shows a mechanical distortion imposed on the piezoelectric crystal myograph producing a voltage across the transducer. This voltage was amplified and connected, through a switching box, to the input of an oscilloscope where additional amplification was provided. Records of displayed latency relaxation were made

SWITCHING BOX FOR CONNECTING

CRYSTAL MYOGRAPH TO OSCILLOSCOPE



A.

B.

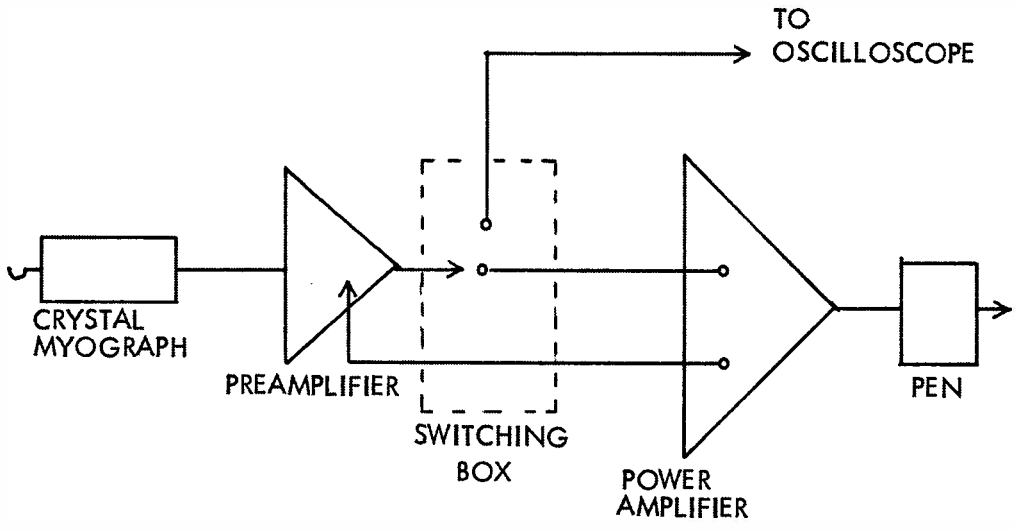


Figure 12. A. Photograph of switching box. B. Schematic diagram of the switching box connected between the preamplifier and the power amplifier. The output from the crystal could be directed to the oscilloscope or the pen.

photographically from the face of the oscilloscope tube.

Having obtained a record of latency relaxation, the question that must be answered is, "Is this record an accurate representation of the event at its source?". It has already been shown that the twitch was not faithfully reproduced by this recording channel. Is this also true for latency relaxation? The adequacy of the instrumentation to accurately present the phenomenon was ascertained in the following manner.

A step function of force was applied to the myograph by the sudden removal of a weight. A 2 g weight was attached to the myograph hook by a single filament of cotton thread which passed through the U-shaped loop of a low resistance heating element connected to a current generator. This device is shown in Figure 13. Placing the weight on the myograph hook deflected the oscilloscope beam which then returned to its baseline because of the electrical characteristics of the piezoelectric crystal. When a stable baseline was reestablished, current was passed through the U-shaped wire heating it to incandescence; the cotton thread burned through and dropped the weight. Before burning the thread, the oscilloscope sweep was set to be triggered by the voltage generated at the crystal when the weight dropped, and the camera shutter was opened. A record produced in this manner is shown in Figure 14. The information necessary to determine the fidelity of reproduction of the latency relaxation records made using this recording channel is contained in that photograph. Determining how rapidly the system responded to the step function was of the utmost importance, for this reveals whether the dis-

CURRENT GENERATOR AND HEATING ELEMENT

USED FOR CRYSTAL MYOGRAPH CALIBRATION

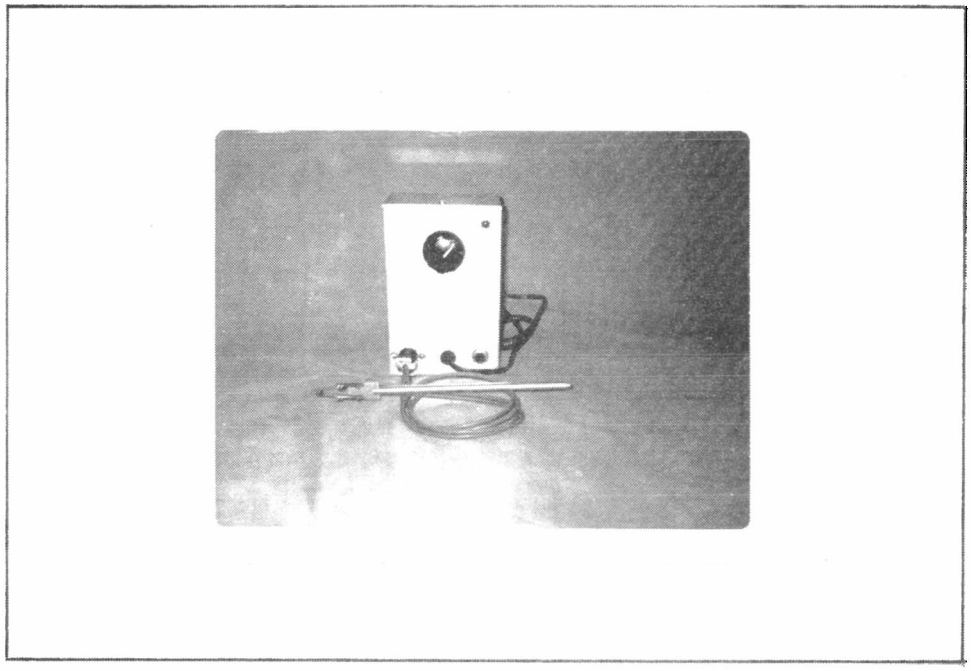


Figure 13. The heating element is attached to the end of the metal rod shown in the lower part of the photograph. The large dark knob on the main assembly adjusts the amount of current delivered to the heating element.

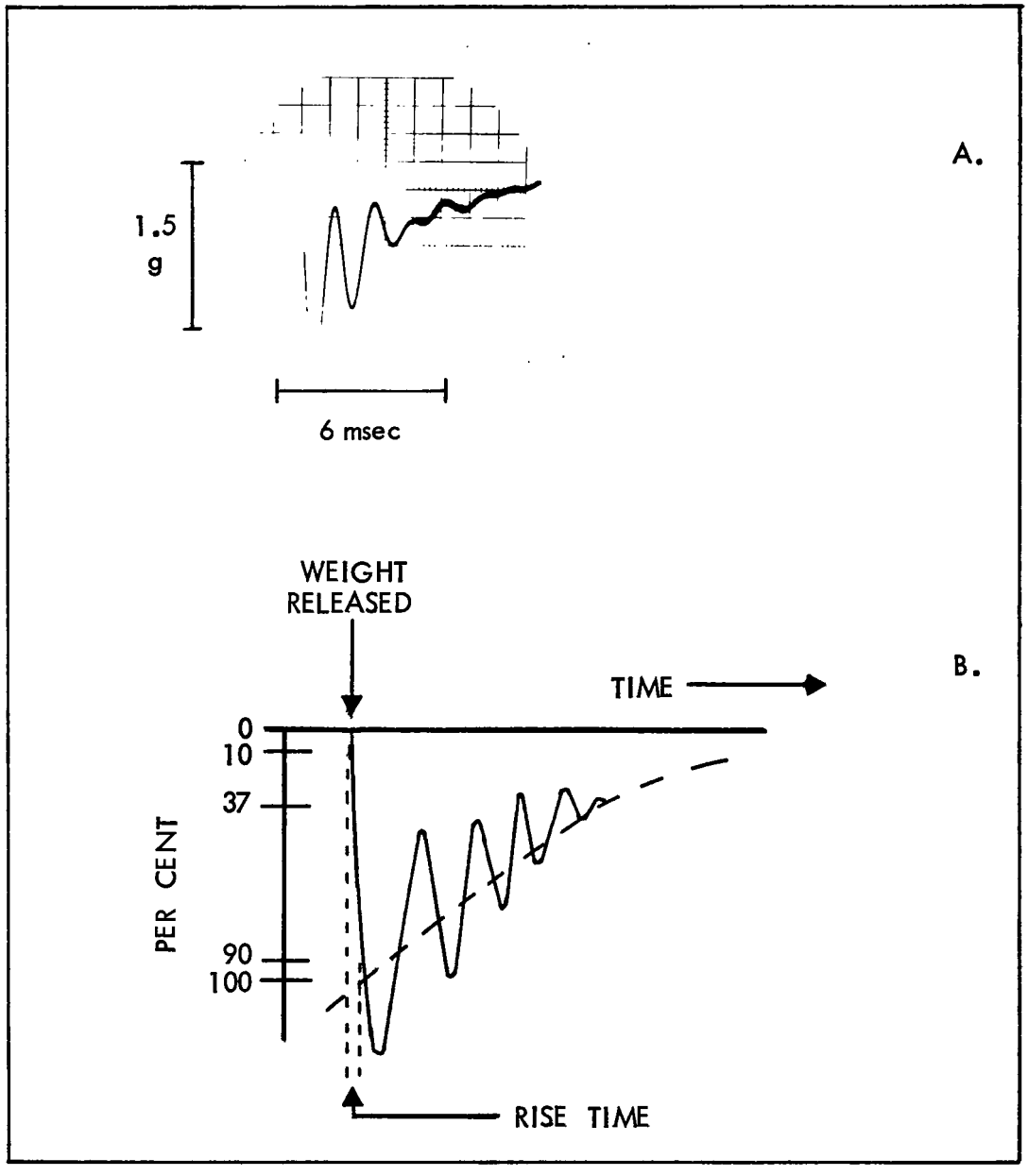


Figure 14. A. Record from the piezoelectric myograph output when a weight is suddenly removed. The sweep began at the third vertical line from the left and the second horizontal line from the top.

B. The record in A redrawn and exponential decay dotted in.

played output can reach its maximum during the time the event (latency relaxation) is present. If the response time of the recording channel is long with respect to the rise time of the event it is to record, the displayed output will not faithfully represent the maximum amplitude of the event at the input, because the output will still be climbing toward maximum, having not yet reached it, at the time the event itself has begun to decline. Therefore, the true amplitude of the event is not recorded.

Rapidity of response is conventionally expressed in terms of "rise time", the time taken for an event to go from 10% to 90% of its maximum value. The rise time measured from the record in Figure 14 is 0.2 msec. The time taken for the latency relaxation to traverse 10% to 90% of the tension change from resting tension to maximum tension decrease was measured at about 1 msec. Therefore, the recording channel possessed a rise time that was shorter than that of latency relaxation. It can be seen in Figure 14 that once the output reached its maximum value it was not maintained, but began to fall toward its original value, even though the input was not changing. The rate of fall is expressed in terms of the decay time constant of the system, the time taken for the displayed output to fall to 1/e, or approximately 37%, of the maximum value. If the time constant of the system is short with respect to the duration of the event measured, a distorted presentation of the input will be displayed, similar to that of the twitch recorded on the crystal myograph. Whereas the rise time, or high frequency response, determines the recording fidelity of the most rapidly changing part of a waveform, the time constant, or low frequency response, governs the fidelity of recording the most slowly changing portion. Both

ends of the frequency spectrum must be considered in ascertaining the overall fidelity of the recording system. The overall time constant of the system used in these studies was approximately 12 msec and the duration of latency relaxation from the time it falls below resting tension to the time it recrosses the baseline is about 2.5 msec. The overall sinusoidal frequency response of this channel was calculated to extend from 13 Hz ($f_L = \frac{1}{2\pi \times \text{time constant}}$) to 2000 Hz ($f_H = \frac{1}{2.5 \times \text{rise time}}$) (25).

The oscillations in the photographed record indicate that this system was underdamped. To remove these from the system would require the addition of damping which would prolong the rise time, and was, therefore, undesirable. However, in an underdamped system such as this one, the 100% point for the output is not at the peak of the first oscillation, but somewhat lower. This is due to the overshoot present in a system with less than critical damping. Determination of the 100% point was accomplished by drawing an exponential curve through the record, and observing where this curve intercepts the vertical axis.

In summary, the adequacy of the system for faithful recording of latency relaxation was examined by presenting a step function at the input and analyzing the output. The system was found to have a rise time shorter than that of the latency relaxation and a fall time, or time constant, longer than the duration of the event. These equipment characteristics indicate that the recorded latency relaxation amplitudes were not distorted by the instruments used to record them.

Having obtained records of latency relaxation, it was necessary to calibrate the crystal myograph in terms of millivolts output per milligram of tension change. Small pieces of aluminum foil of different weights were hung from the crystal stylus with fine cotton thread and suddenly released by burning the thread. The output was photographed and measured in millivolts. Figure 15 illustrates three such records made from increasingly larger weights. The weights used for calibration purposes ranged from 7 mg to 54 mg. The small output from releasing these weights was not sufficient to trigger the sweep of the oscilloscope so it was set to sweep continuously. The thick baseline in the records of Figure 15 is due to multiple exposure of the beam while the camera shutter was open before the string burned through and released the weight. A slight ambiguity in the 100% deflection point was present in all records due to the overshoot discussed above; therefore multiple records were made of the output for each weight and the mean voltage output for a given weight determined. Mean voltage output was plotted versus weight and the regression line of millivolts output on milligrams input was calculated. Figure 16 shows a graph of 26 measurements made with seven different weights, and the calculated regression line. The procedure was later repeated using seven different weights, but only one determination was made with each weight, and the regression line was calculated. The two calculated regression lines are shown in Figure 17 as dashed lines; the solid line has a slope and intercept that are the midvalues of those displayed by the two dashed lines. The slope of the solid line was used as the conversion factor for expressing millivolts output

SUDDEN RELEASE OF THREE DIFFERENT WEIGHTS

FROM PIEZOELECTRIC CRYSTAL MYOGRAPH

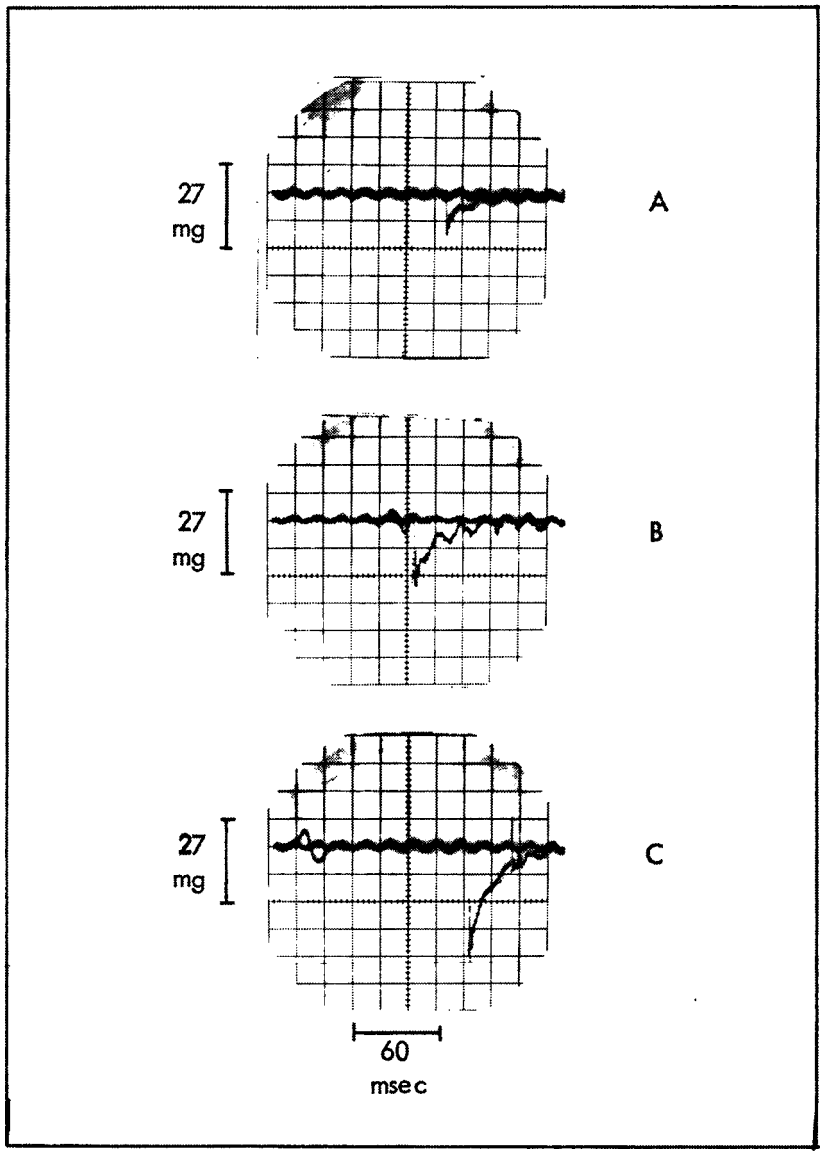


Figure 15. These records were used to calibrate the crystal in terms of mv/gm.

A. Weight released, 6.8 mg.

B. Weight released, 14.0 mg.

C. Weight released, 27.8 mg.

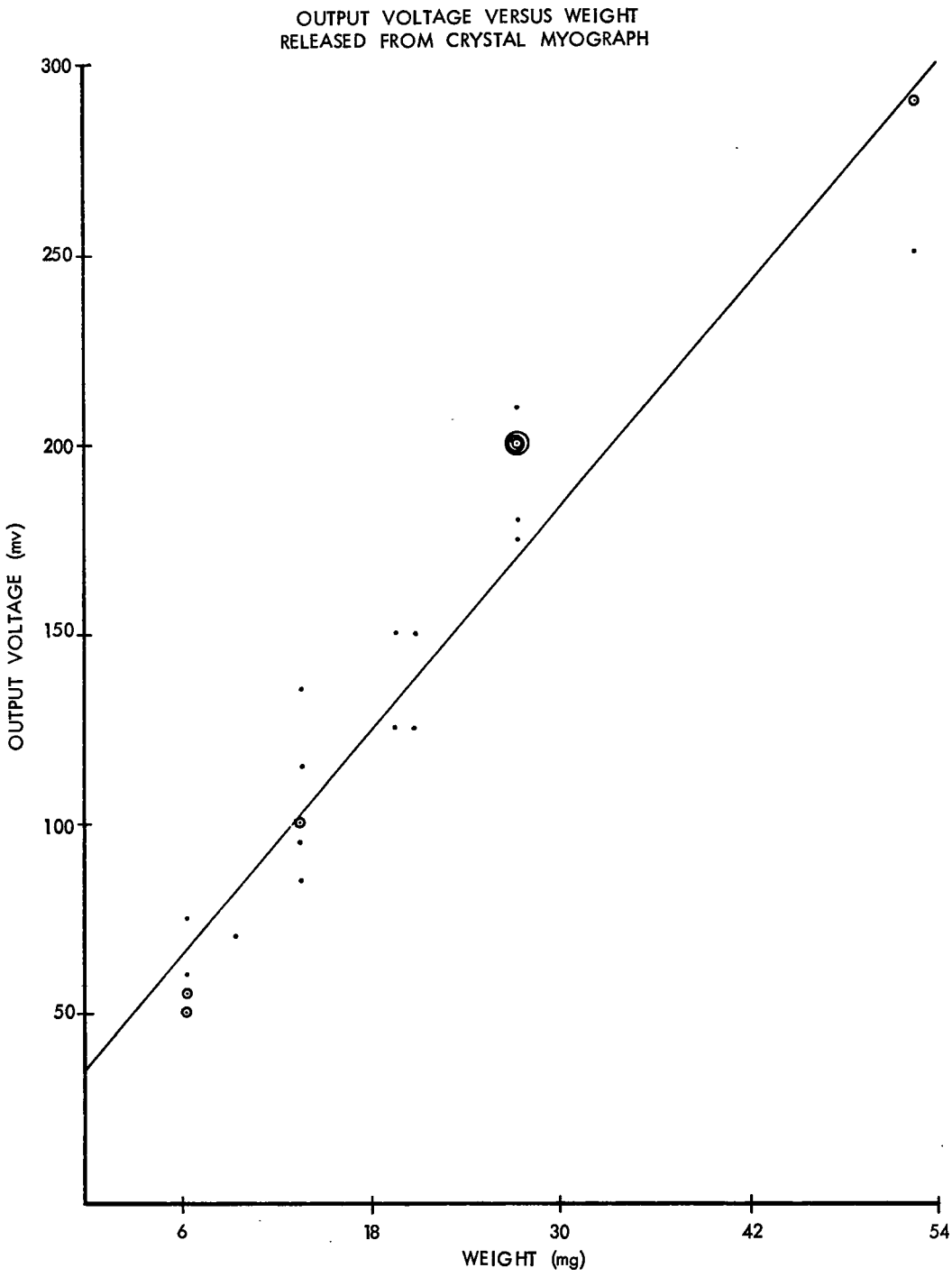


Figure 16. Each point represents a single value. The concentric circles represent multiple similar voltage outputs for the weight indicated. The regression line has a slope of 5.82.

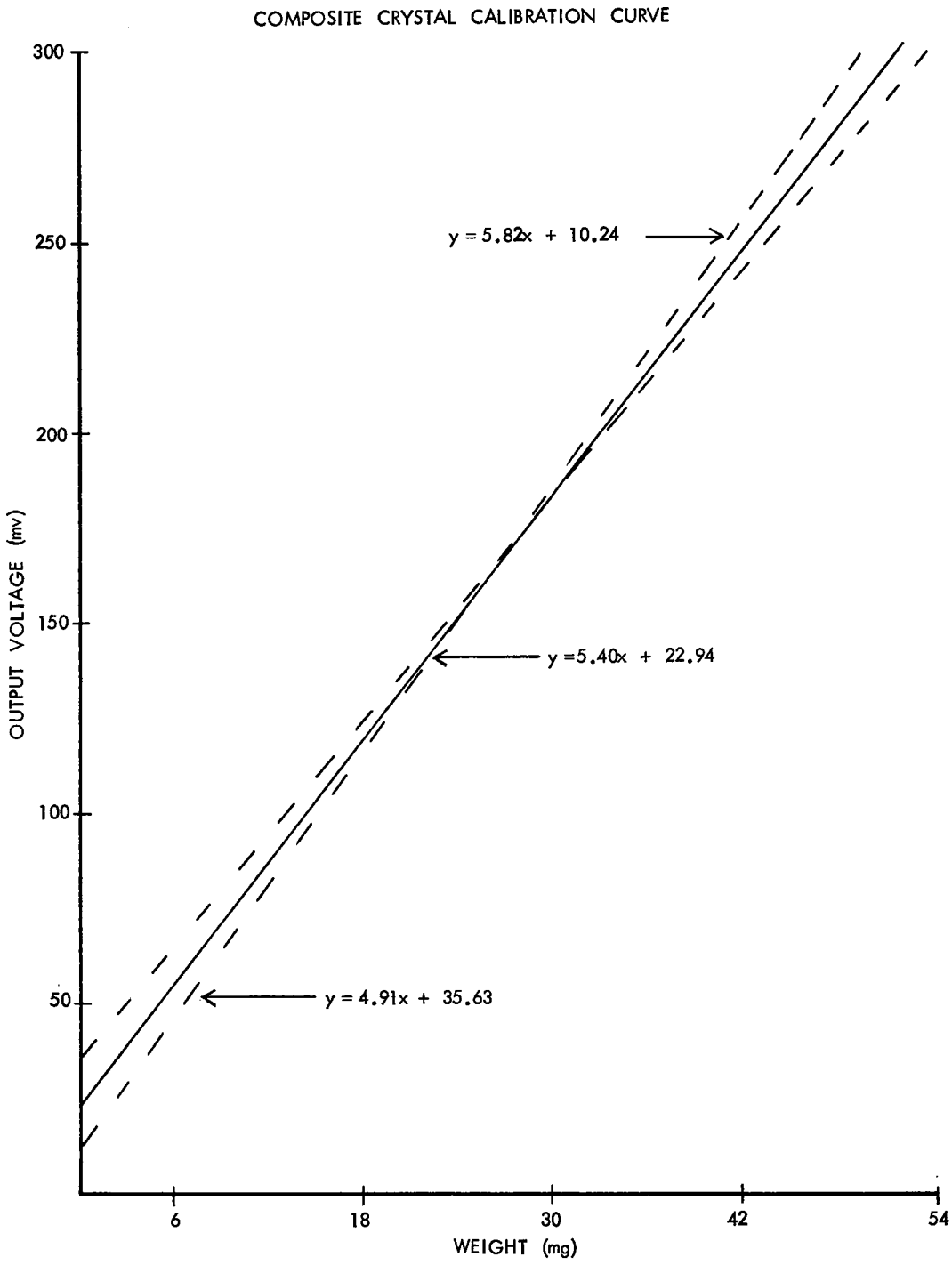


Figure 17. The slope of the solid line is the midvalue of the slopes of the two dotted lines, and was used as the factor for converting millivolts deflection on the oscilloscope to milligrams tension.

in terms of milligrams of tension decrease during latency relaxation. Thus every 5.4 mv output represented a tension change of 1 mg.

In summary, the latency relaxation is a small, rapidly changing event; the device used to record it must be sensitive and able to respond rapidly. A piezoelectric crystal transducer met these requirements and was chosen to detect this event. However, the inherent characteristics of the crystal make it unable to record a sustained input and thus some difficulty was encountered in calibrating it. Calibration was achieved by releasing weights of known values and plotting the voltage they produced versus the weights that produced them. The overall characteristics of the recording channel were determined to be adequate for the faithful reproduction of latency relaxation.

C. EXPERIMENTAL PROCEDURE AND EQUIPMENT CONFIGURATION

Frog sartorius muscles were used in all experiments. One feature of this muscle is its thinness which permits relatively rapid penetration of the bathing medium into the extracellular spaces. In addition its long fibers lie parallel to one another; this arrangement allows the muscles' anatomical and physiological cross-sectional areas to be approximately equal. Muscles which lack this parallel arrangement of fibers would probably produce smaller latency relaxation amplitudes than the sartorius since the recorded precontractile relaxation most likely represents the vectorial sum of tension reduction in all stimulated fibers; non-parallel fibers contribute only the component of their latency relaxation that would be parallel to the major tension axis of the muscle. The animals used in these experiments

were obtained throughout the year and kept in pans of water in the refrigerator until used. Their weights varied from 25 g to 50 g, and the sartorii weighed from approximately 50 mg to 150 mg. Most of the animals weighed around 30 g and the sartorii about 75 mg.

The frogs were pithed in the caudal and cephalad directions, and the skin overlying the muscle was slit. The muscle was dissected free from underlying tissue, starting at the distal end and working toward the pelvic attachment. The lower tendon attachment was cut free, and a loop of fine, copper wire, from which the insulating lacquer layer had been removed by scraping with emery cloth, was placed around the muscle and tied tightly. Lengths of uninsulated wire, extending from the loop around the muscle, were securely fastened to the middle of the lower part of a lightweight metal frame. This frame transmitted the twitch and resting tensions to the photoelectric myograph, and it was one of the pair of stimulating electrodes, usually the anode. (The polarity of the stimulating electrodes did not appear to have any effect on the amplitude of the mechanical response, but for the sake of uniformity an electrode placement convention was chosen and adhered to.) The pelvic end of the muscle was dissected free of its attachment and a similar loop of bared, copper wire was placed around it and tied. Immediately above the muscle another loop was made in the wire. This second loop was fastened to an insulated hook extending from the piezoelectric myograph. The remaining length of wire was connected to the cathode of the stimulator. The muscle was now suspended between the piezoelectric myograph on one end and the metal frame on the

other; Figure 18 illustrates the attachment of the muscle to the two myographs. Short lengths of wire are shown between the muscle and the points of attachment to the frame and the piezoelectric myograph. In practice the lengths of these wires were kept as short as possible since they represent a potential source of damping which could diminish the recorded amplitude of latency relaxation. The upper end of the frame passed through a hole in the insulated chuck of the photoelectric myograph and was secured in place by a screw. A small alligator clamp connected to the stimulator anode was hooked to the frame and completed the stimulating circuit.

With the muscle affixed at both ends the pelvic end was aligned over the tibial end; twisting of the muscle about its long axis was avoided. The photoelectric myograph was attached to a tension-adjusting clamp that could be raised or lowered along a threaded track, and the aligned muscle was stretched just to the point where it was no longer slack. A single stimulus was delivered to test the integrity of the stimulating and recording circuits. About ten minutes were required to dissect the muscle and attach it to the myographs. During this procedure the muscle was kept moist by the application of amphibian Ringer solution. The affixed muscle was placed into a reservoir containing about 20 ml of amphibian Ringer solution composed as follows (in mM); NaCl, 111.2; KCl, 1.8; $CaCl_2$, 1.08; $MgSO_4$, 1.22; $NaHCO_3$, 2.38; NaH_2PO_4, 0.087. The reservoir was brought up from below until the muscle was submerged in the solution where it remained for 15 minutes. Air was bubbled through the bathing solution. All experiments (except those investigating the effect of increased temperature) were performed at room temperature (24°C). Any small

ARRANGEMENT OF EQUIPMENT FOR RECORDING TWITCH AND LATENCY RELAXATION

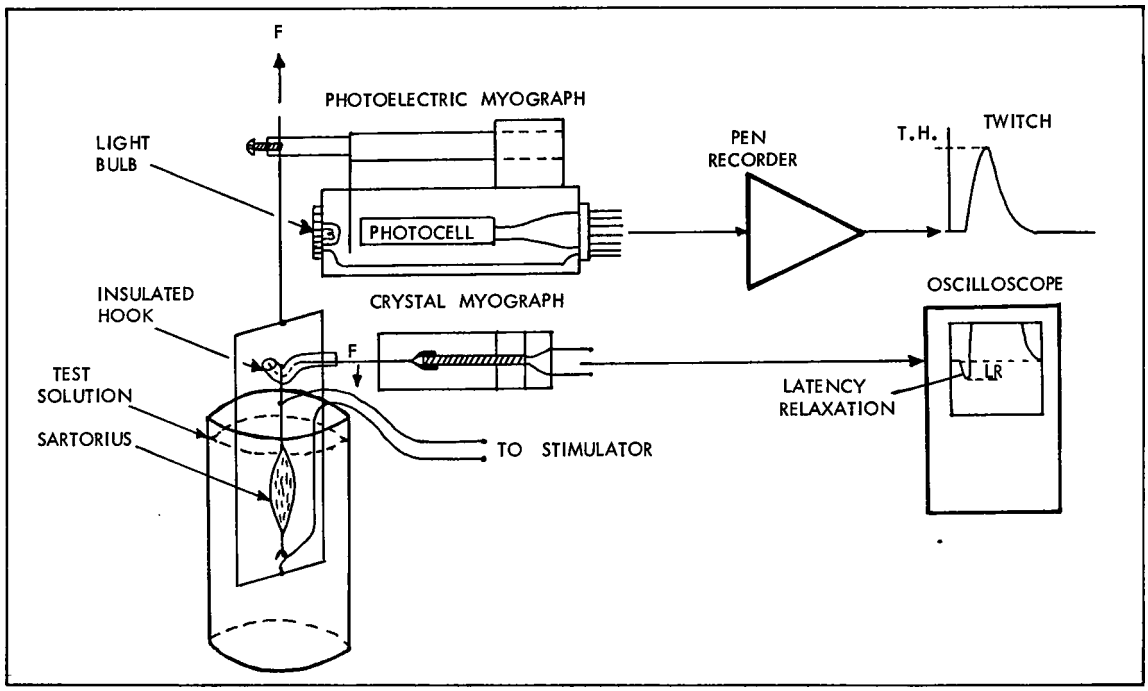


Figure 18. The muscle is shown attached to the piezoelectric myograph on one end and the photoelectric myograph, by means of the metal frame, on the other. The myographs were deflected in the directions indicated by the arrows labelled F when the muscle contracted.

fluctuations in the environmental temperature which may have occurred during the relatively short experimental periods probably had effects that were much smaller than the imposed experimental variable. Furthermore, experiments performed at different times of the day or the year showed no consistent differences that could be attributed to environmental temperature differences.

Up to this point all muscles were similarly treated. The subsequent procedure depended on the particular experiment and is described in a later section with an account of the experimental results. Before any record of twitch and latency relaxation was made the reservoir containing the bathing solution was lowered from around the muscle and excess solution was blotted from the muscle and frame with filter paper. Records of latency relaxation made with the muscle submerged in the solution were greatly attenuated due to the damping imposed by the surrounding liquid.

The muscle was stimulated with a 2 msec rectangular pulse; stimulus intensity was set at 100 v which was supramaximal for both twitch and latency relaxation. Stimuli of shorter duration and lower intensity were not as effective in producing large tension changes. Furthermore, it had been noted that maximum latency relaxation usually occurred at a higher stimulus intensity than twitch. Although the voltage was large, the stimulating electrodes were small; thus delivery of maximal stimulating current required a high voltage.

To record latency relaxation the starting point of the oscilloscope sweep was adjusted, the camera shutter opened, and a single stimulus delivered to the

muscle. The oscilloscope beam was triggered to sweep from the stimulator. A single stimulus gave a single sweep. The starting point of the oscilloscope sweep could be set at any point along the horizontal (as well as the vertical) axis of the tube face; by simply moving the starting point of the sweep it was possible to record four latency relaxation records side by side as shown in Figure 19.

At the conclusion of an experiment the photoelectric myograph was calibrated as described earlier. The twitch records were placed on a back-lighted glass plate and the calibration curve was placed over them with the zero tension line directly over the point from which twitch tension began. Active twitch tension was read directly from the calibration curve. The latency relaxation records were measured in terms of millivolts and then converted to milligrams. To compare twitch to latency relaxation all tensions were expressed in terms of per cent maximum. This normalized all data and permitted comparison of many muscles strictly on the basis of relative mechanical output irrespective of muscle size. Processing the data for numerical information depended on the nature of the experiment and is described under the various experiments.

Both myographs were attached to a weighted stand, cushioned on about four inches of foam rubber. In the first few experiments, the myographs were positioned on separate, upright stands, but the small movements of one stand relative to the other, produced by environmental vibrations, introduced considerable "noise" into the latency relaxation records. Placing both myographs on the same stand provided a differential arrangement for the vibrational signals in that no relative movement

LATENCY RELAXATION RECORDS

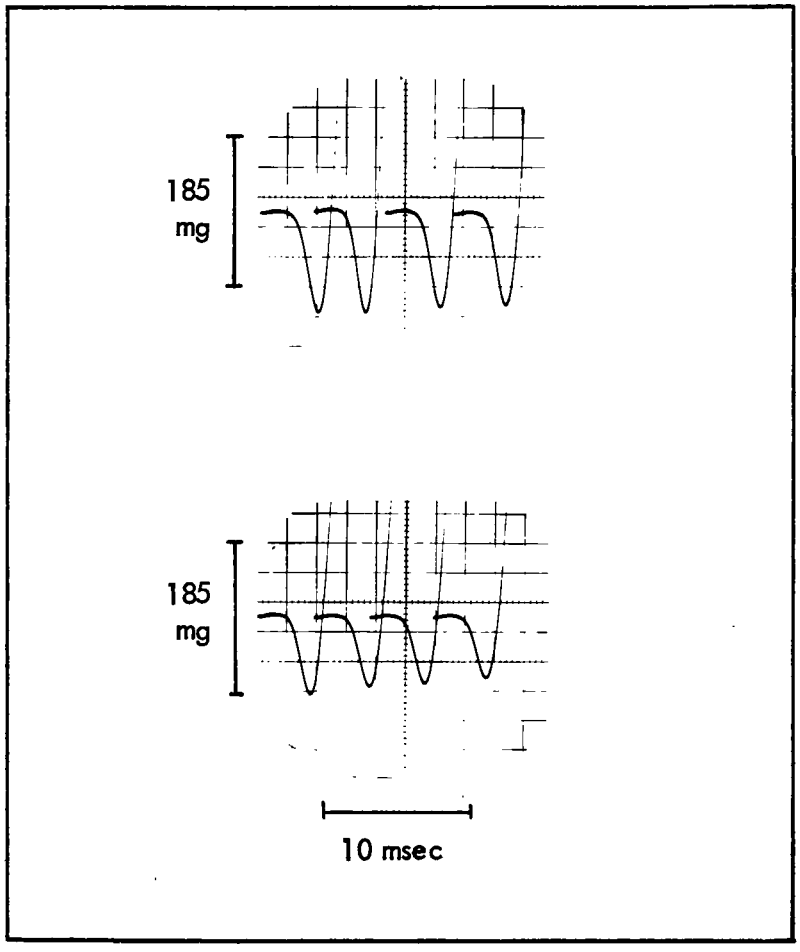


Figure 19. Records of latency relaxation from a muscle stimulated every five minutes. Each sweep was triggered by the stimulus, and the starting position of the beam was moved to the right before each record.

of the two ends of a muscle resulted from vibrations. This arrangement provided almost complete exclusion of mechanical artifacts; the foam rubber pad gave added protection against sudden, vibrational movements.

Although exclusion of mechanical artifacts was readily achieved, another class of undesirable signals required more effort for their identification and elimination. In the early experiments aberrations frequently appeared at the start of the latency relaxation traces. It was verified that these were introduced from the stimulator by altering the intensity, duration, and polarity of the stimulus and producing appropriate changes in the initial part of the records. Stimulus artifact is not always an undesirable signal, but it was in this case because it obscured an appreciable part of the early portion of the latency relaxation signal. Figure 20 shows a series of three latency relaxation records with diminishing amount of stimulus artifact signal in the records, although the intensity of the stimulus applied to the muscle was the same in all three cases.

The procedure for obtaining records consistently free of these artifacts required consideration of two aspects of these signals. First, output from the piezoelectric crystal was in the millivolt range, while the stimulus intensity most frequently applied to the muscle was 100 v. Thus the unwanted signal was much greater than the signal of interest. Secondly, the amount of stimulus artifact that appeared in the records differed from experiment to experiment and depended on such variables as length of stimulator leads, proximity and relative orientation of all electrical conductors in the area of the recording equipment, and to some

REMOVAL OF STIMULUS ARTIFACT

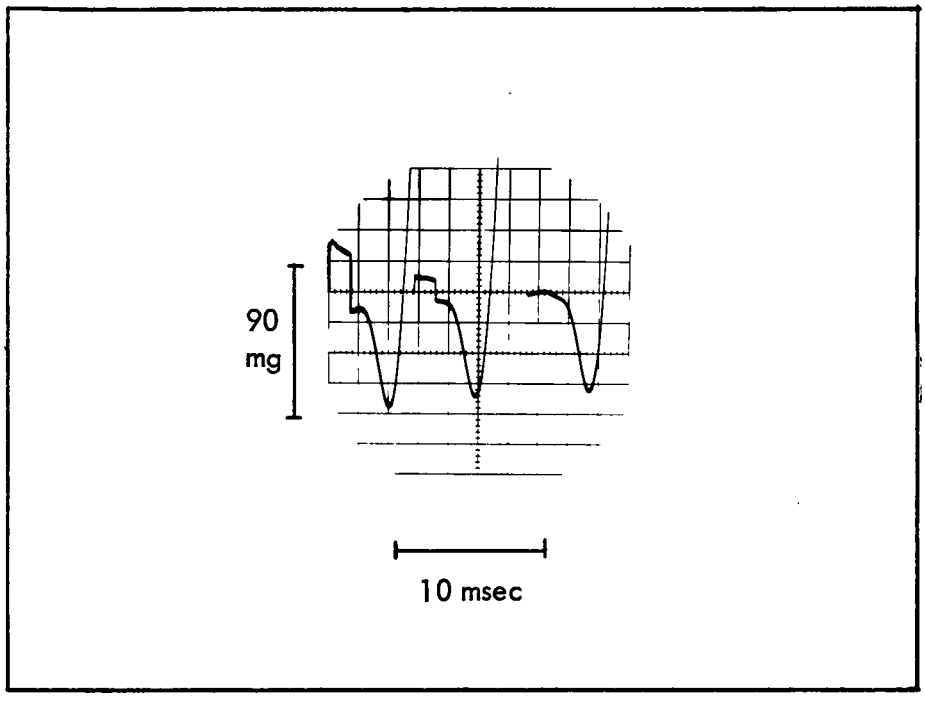


Figure 20. Stimulus artifact is present in the two records on the left. The Wagner earth was adjusted to remove the artifact.

extent, the relative humidity. Therefore, the method for abolishing the artifacts had to be able to exclude a very large signal voltage and yet not interfere with recording the much smaller piezoelectric crystal voltages produced by the latency relaxation. It also had to possess flexibility in the application of varying amounts of correction.

The means by which these signals gained access to the recording channel was indirect. No direct electrical connection existed between the stimulus circuit and the recording channel, but the very nature of the experimental arrangement provided capacitive pathways for entry of unwanted signals. Any two conductors separated by an insulator constitute a physical capacitor. In these experiments the conductors were all leads used to stimulate and record, and the insulating material included air (hence the influence of the relative humidity). The stimulus artifacts were most likely capacitance coupled to the input of the recording channel through these ubiquitous capacitors. The dotted lines in Figure 21 illustrate the lumped capacitances (each capacitor in the figure represents several stray capacitances) that existed between both sides of the stimulator and ground and between both stimulator leads and the two sensing electrodes on the piezoelectric crystal faces. Input to the amplifiers of the latency relaxation channel was derived from these electrodes, and any and all voltages appearing across them will be present in the output.

The amplifiers in the latency relaxation channel were of the differential class. A consequence of this amplifier configuration is that any voltage which is

WAGNER EARTH ACROSS THE STIMULATOR SECONDARY

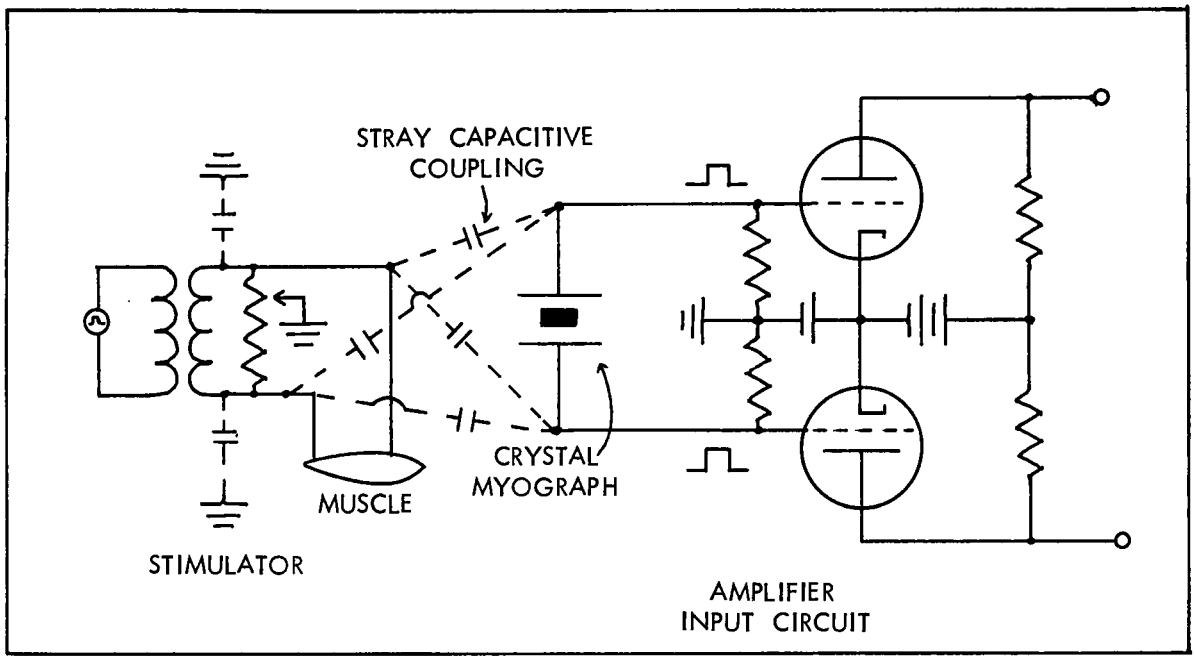


Figure 21. This figure shows only the electrical connections and deletes the mechanical connection of the muscle to the crystal myograph. The dotted lines represent conducting paths that were not physically present. The Wagner earth is comprised of the resistor across the stimulator secondary and the ground-referenced tap.

equally applied between the two grids and common cathode will not appear in the output, which is taken plate to plate (25,45). Therefore, application of the stimulus signal between the two grids and cathode of both amplifier tubes would eliminate it from the output, while the crystal voltage resulting from the latency relaxation applied across the grids of the amplifier tubes would appear, undistorted, in the output.

Removal of stimulus pulses from the records of latency relaxation required that the stimulus artifact be rerouted from a grid-grid application to a bilaterally symmetrical grid-cathode connection. To accomplish this, two criteria had to be fulfilled; 1.) the signal had to be referenced to ground potential (because the cathodes were ground referenced), and 2.) a signal of equal magnitude had to be presented to both sides of the amplifier to attain complete cancellation of that signal in the output.

The first step taken to alleviate the problem was the application of an insulated hook on the photoelectric myograph. Interruption of the direct electrical connection between muscle and myograph case, which had previously existed, reduced the size of the artifact somewhat, but did not completely eliminate it; nor did grounding one side of the stimulator.

The means for removing the stimulus from the output of the latency relaxation channel was obtained by using a modification of the Wagner earth (ground) (73). The ends of a potentiometer were connected to the stimulator terminals and the variable contact was connected to ground. Movement of the variable contact

permitted presentation of the same magnitude of stimulus signal to both sides of the amplifier. (In the absence of the Wagner earth, the two stimulator terminals are referenced to each other, not to a fixed potential, such as ground.) By moving the adjustable contact of the potentiometer the stimulator terminals could be referred to any desired potential with respect to ground. Figure 21 shows the device connected across the secondary of the stimulator, and Figure 20, each trace of which was made at different positions of the potentiometer's tap, demonstrates the effectiveness of this relatively simple device in eliminating a perplexing problem.

In summary, much attention was paid to the equipment used to record the latency relaxation. Although much of it is commercially available its assembly into a smoothly functioning system required special attention in providing the proper interfacing or coupling units. Calibration necessitated special equipment, as did the removal of stimulus artifacts from the records. Two recording channels were used to simultaneously record twitch and latency relaxation; the characteristics of each were dictated by the nature of the event each was required to display. A piece of equipment should not distort the event it measures and in this study serious efforts were made to assure that the instrumentation employed provided data high in accuracy.

CHAPTER IV

EXPERIMENTAL RESULTS

A. THE PHYSIOLOGICAL NATURE OF LATENCY RELAXATION

If latency relaxation is to be accepted as a genuine, physiological phenomenon of stimulated muscle, it must be present in a physiologically activated muscle; if it is not, an investigation of the event is of little value. Sandow (61,63) had reported that latency relaxation could be evoked by indirect (nerve) stimulation as well as direct. However, evidence had been offered that latency relaxation might be attributed to a non-physiological origin (26,29), and it seemed possible that the event may be due to a pulse of heat generated by passage of the electrical stimulating current through an isolated muscle preparation. Although the majority of evidence indicates a physiological basis for the phenomenon, in order to confirm that the phenomenon does indeed occur in neurally activated muscle and to place latency relaxation on as firm a physiological foundation as possible, the first experiments done in this investigation were to verify its presence in a muscle stimulated via its motor nerve.

Figure 22 is a record of three traces of latency relaxation in a sartorius muscle resulting from stimulation of the sciatic nerve just below its point of exit from the spinal cord. These records indicate that the phenomenon is present in neurally activated frog sartorius muscle. The time between stimulus (at the

LATENCY RELAXATION IN FROG SARTORIUS STIMULATED THROUGH ITS MOTOR NERVE

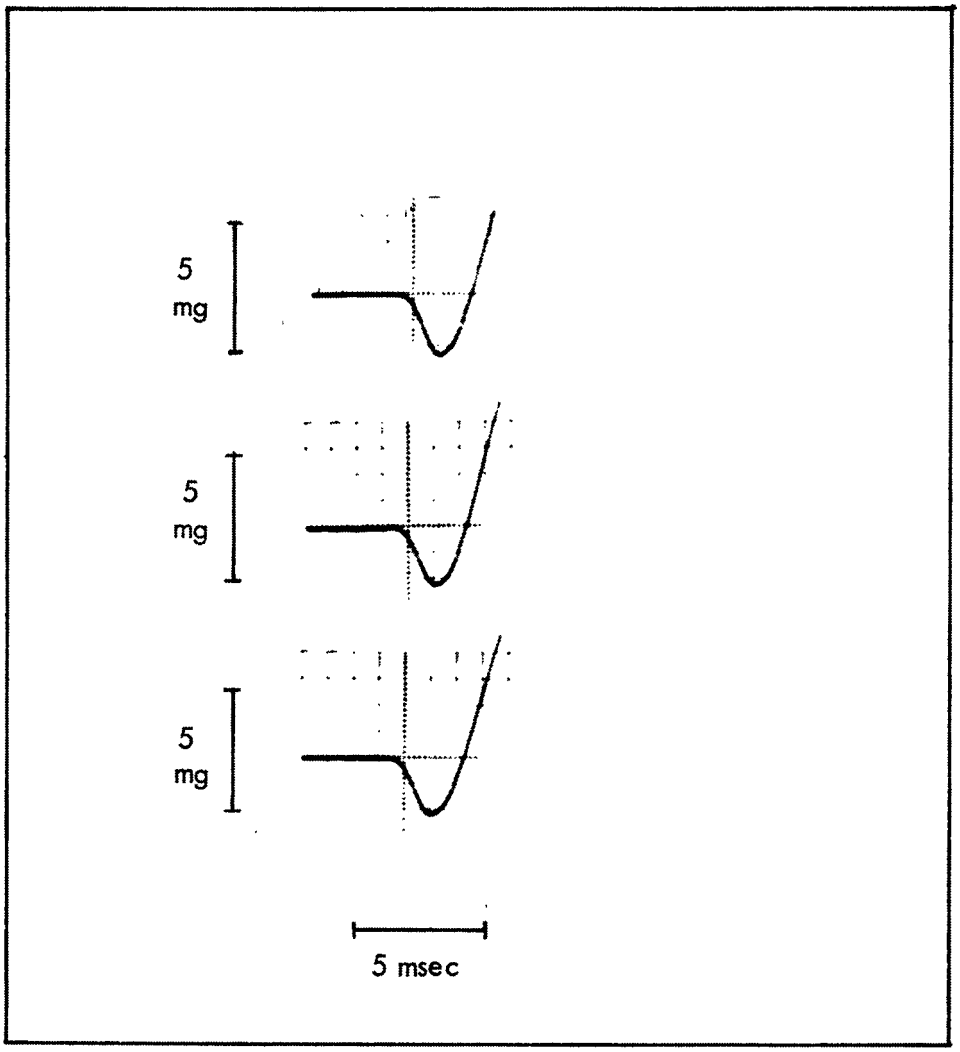


Figure 22. The muscle from which these records were made was activated by a stimulus applied to the sciatic nerve.

beginning of the sweep) and the first sign of relaxation is approximately 3.6 msec in these records. In directly stimulated muscles this period was generally between 2.0 and 2.5 msec. When a muscle is activated through its nerve the excitatory impulse must be transmitted along the nerve and across the myoneural junction before reaching the muscle. The time difference between stimulus and onset of latency relaxation in directly and indirectly stimulated muscle most likely reflects these transmission times.

Both the falling and rising phases of latency relaxation in the records of Figure 22 show diminished rates of tension change as compared to excised muscles (see Figure 1). The recorded amplitude is also smaller than in muscles removed from the animal and securely attached between two myographs. The records were made by freeing the tibial end of the sartorius and attaching it to the piezoelectric myograph; the pelvic attachments were left intact, and as the nerve was stimulated all the muscles supplied contracted. This non-rigid attachment was a probable source of damping which attenuated both the recorded latency relaxation amplitude and the rate of tension development.

Although routine measurement of latency relaxation evoked through nerve stimulation was not used in the later experiments, the evidence that the phenomenon is present in this mode of muscle activation substantiates its physiological genuineness. All further experiments were conducted with excised, directly stimulated muscles.

B. THE INFLUENCE OF INCREASING RESTING TENSION ON TWITCH AND LATENCY RELAXATION

A series of preliminary experiments was concerned with developing experimental protocol, defining necessary equipment alterations and additions, and establishing the consistent appearance of latency relaxation in stimulated muscles. The procedure described in a preceding section represents the evolutionary product of these early experiments. Although the results from these experiments provided information on the behavior of latency relaxation, no data from them were grouped with those from later experiments. The preliminary experiments were performed with a variety of stimulus strengths and durations, modes of attachment for the muscle, types of reservoirs for bathing media, and actual experimental procedure. During the early experiments it was observed that twitch and latency relaxation did not reach a maximum at the same resting tension. This observation prompted a closer investigation of the relationship between twitch and latency relaxation amplitudes at different resting tensions. With the tension meter it was possible to measure accurately small increments in resting tension and the effect of increasing resting tension on the amplitudes of twitch and latency relaxation was examined and compared.

Each muscle was attached between two virtually isometric myographs whose initial vertical separation allowed the muscle to be slightly slack. The muscle was stretched slowly, by raising the photoelectric myograph along a threaded track, to a length at which a resting tension just began to register on the

tension meter. Then the myograph was lowered slightly to the point where no resting tension was registered, and the first determination of twitch and latency relaxation amplitudes was made at zero resting tension. By gradually raising the upper myograph the muscle was stretched as necessary to increase resting tension in definite increments. Usually, 24 measurements were made on each muscle and resting tension was increased by increments of one-half division on the tension meter scale for the first eight measurements, one division for the next eight measurements, and two divisions for the last eight measurements. The meter was calibrated after the experiment was completed in the manner described earlier. The number of milligrams represented by each galvanometer division varied from experiment to experiment, depending upon the amplitude setting of the amplifier in the twitch channel, but usually ranged from about 75 to 150 mg. The records were made as rapidly as possible, and the muscle was resubmerged in aerated Ringer solution only after each four measurements for about one minute (while the camera film was advanced to the next exposure).

A series of twitch and latency relaxation amplitude measurements with increasing resting tension usually required about 25 minutes to complete, therefore the effect of time on the results obtained should be considered. In a separate series of experiments the amplitudes of twitch and latency relaxation as a function of time at constant resting tension were observed (see section F). These data were taken as descriptive of the effects of time on the two events. Since the changes with time were smaller and of a different nature than those with increasing resting tension,

the amplitudes of twitch and latency relaxation observed with increasing resting tension appeared to be influenced primarily by that experimental variable.

Table I lists the maximum twitch and latency relaxation values for nine muscles, the resting tensions at which they occurred, and the corresponding values of latency relaxation and twitch at those resting tensions. Maximum latency relaxation occurred at higher resting tension than maximum twitch in all muscles. Figure 23 shows the amplitudes of twitch and latency relaxation as a function of increasing resting tension. Because the amplification in the twitch channel varied from experiment to experiment, one division on the tension meter scale did not represent the same resting tension in each experiment. Therefore, the increments in resting tension were not the same for all muscles and the data could not be averaged to obtain mean values for the amplitudes at given resting tensions. For this reason, the amplitudes of twitch and latency relaxation in a single muscle, rather than mean values at precise resting tensions, are presented in Figure 23.

The amplitudes of both events always rose more rapidly than they fell. Furthermore, latency relaxation amplitude usually increased slowly in the range of resting tension values where twitch was rapidly increasing. Above the resting tension at which twitch was maximum, latency relaxation amplitude increased more rapidly with increasing resting tension.

TABLE I

RESTING TENSION FOR MAXIMUM TWITCH AND MAXIMUM LATENCY RELAXATION AND THE ASSOCIATED LATENCY RELAXATION AND TWITCH AMPLITUDES IN NINE MUSCLES UNDER INCREASING RESTING TENSION

MUSCLE	MAXIMUM TWITCH (g)	RESTING TENSION (mg)	LATENCY RELAXATION (mg)	MAXIMUM LATENCY RELAXATION (mg)	RESTING TENSION (mg)	TWITCH (g)
117691	24.0	794	51.9	63.0	1375	19.8
117692	26.3	406	37.0	63.0	1919	22.0
122691	19.5	206 244 381	7.4 16.7 19.4	34.3	1913	14.0
122692	23.8	130	2.8	28.7	1300	17.0
124693	24.0	210	11.1	55.6	1500	19.8
127691	27.3	341	18.5	45.4	2000 2330	20.3 19.0
626691	40.5	266	22.2	153.7	3250	32.8
72691	22.0	266	24.1	155.6	3370	18.5
72692	28.5	338	20.4	146.3	3370	21.5
MEAN	26.2	326	21.1	82.8	2233	20.5
S. E.	±2.0	±53	±4.1	±17.7	±259	±1.6

TWITCH AND LATENCY RELAXATION AMPLITUDES IN A SINGLE MUSCLE WITH INCREASING RESTING TENSION

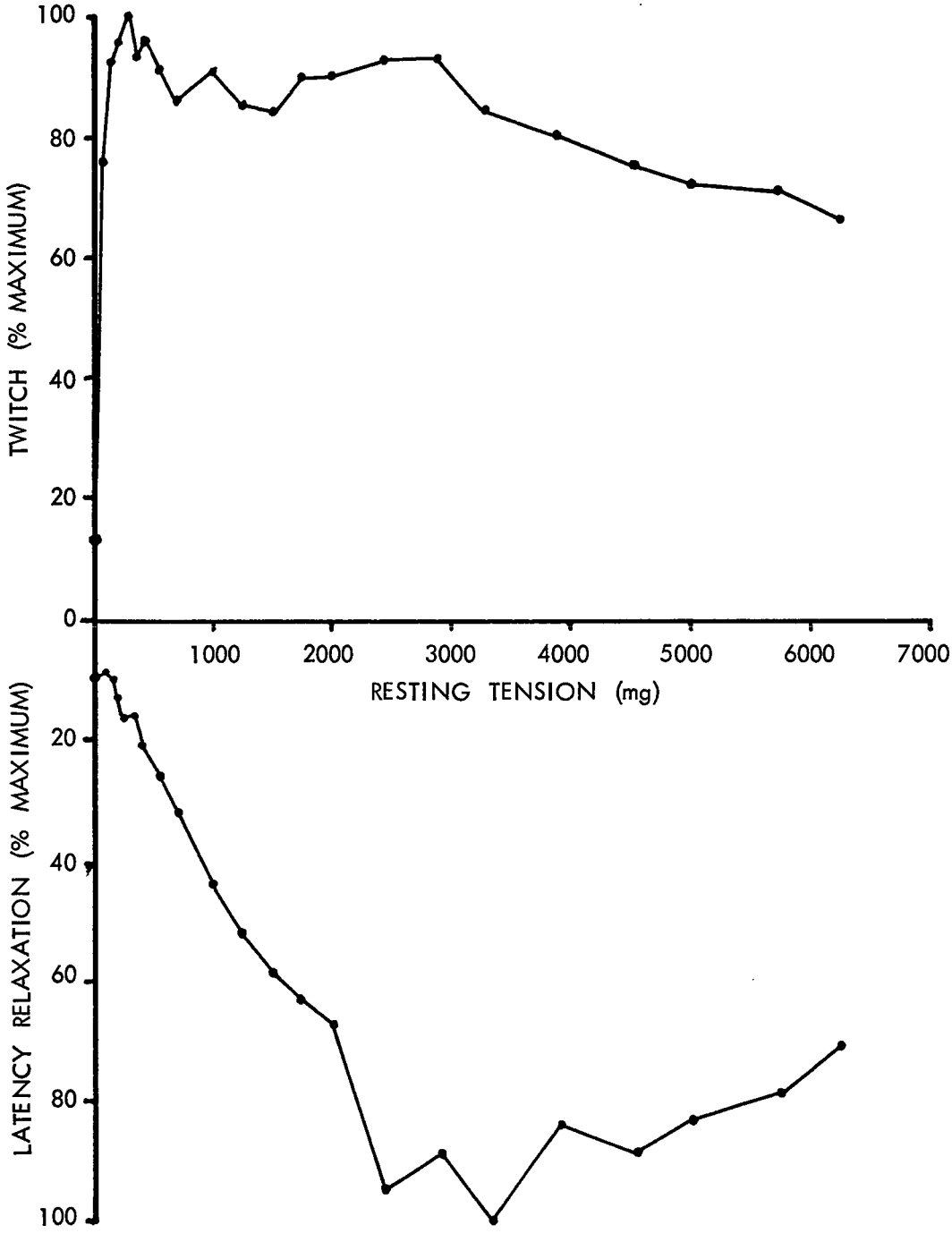


Figure 23. Twitch and latency relaxation amplitudes with increasing resting tension. Resting tension was indicated by deflection of the tension meter.

C. THE AMPLITUDE OF TWITCH AND LATENCY RELAXATION FOLLOWING REPEATED, CLOSELY SPACED STIMULI

Muscles begin to display evidence of fatigue after prolonged or sustained activity. The effects of closely spaced stimuli on twitch and latency relaxation were observed in six muscles. Stimuli, at a frequency of 2/sec, were applied for intervals of different durations. Repetitive stimulation was stopped, a single stimulus applied, and twitch and latency relaxation recorded. This procedure was repeated until twitch amplitude was reduced to a small fraction of its original contractile output. Figure 24 is a record of such a procedure. The latency relaxation records below each isolated twitch are those associated with that particular twitch.

Figure 25 graphically displays the amplitudes of twitch and latency relaxation from the record shown in Figure 24. Twitch and latency relaxation amplitudes decreased by about the same amount at first; however, twitch amplitude fell faster than latency relaxation with continued activity. This behavior was observed in all muscles subjected to closely spaced stimuli. If the origin of latency relaxation is some physical factor, such as elongation due to heating as a result of direct electrical stimulation, latency relaxation would be expected to show an increase under the conditions of these experiments, but it always displayed a progressive diminution, although, not to the same extent as the twitch.

D. COMPARISON OF THE TIME COURSES OF TWITCH AND LATENCY RELAXATION AMPLITUDES IN A MEDIUM WITH NO CALCIUM ADDED

Of all ionic species, calcium ranks high in importance to muscular contraction (11). The effects of increased and decreased calcium in the bathing

TWITCH AND LATENCY RELAXATION FOLLOWING REPETITIVE STIMULI

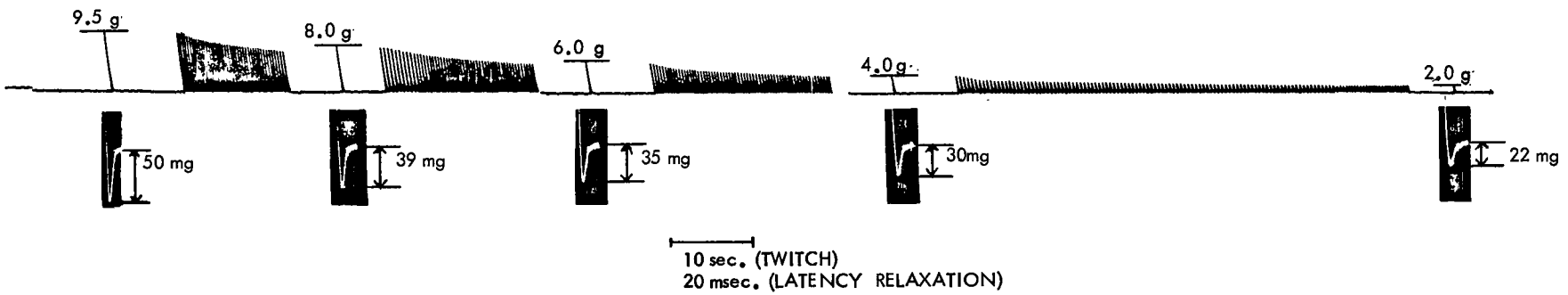


Figure 24. A record of twitch and latency relaxation in a muscle stimulated at a rate of 2 sec. Each latency relaxation is associated with the twitch just above it. The amplitudes of each twitch and latency relaxation are indicated on the record.

TWITCH AND LATENCY RELAXATION AMPLITUDE
AS A FUNCTION OF CLOSELY SPACED STIMULI

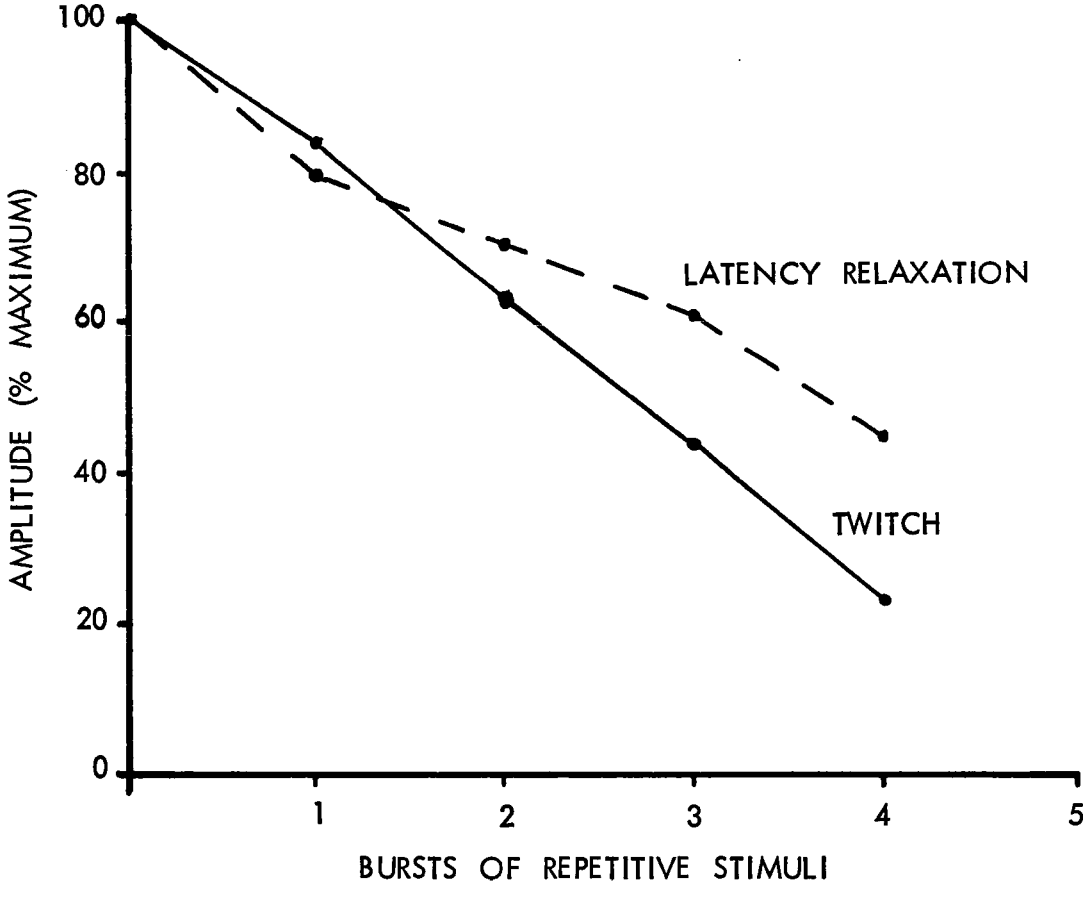


Figure 25. A graph of the data from the records of twitch and latency relaxation in Figure 24.

medium on muscle twitch (12,13,71), contracture (21,48), and membrane potential (5,12,43,46) have been studied, and the efficacy of intracellularly applied calcium in producing contraction is well known (30,53). The experiments described in this section were done to examine the effect of a medium with no added calcium on latency relaxation amplitude as compared to twitch amplitude.

It is generally agreed that calcium ions are released from a sequestering site within the muscle (44), most likely the sarcoplasmic reticulum (8), migrate to the area of actin-myosin overlap (77), and participate in initiation of the contractile process. Sandow (65,67) has proposed that latency relaxation reflects an activation process, and, perhaps, even the actual release of calcium ions. On the basis of these suggestions and the diminished contractile activity observed in muscles placed into calcium-free media, an investigation to determine if latency relaxation was similarly affected by lowered calcium was undertaken. It was reasoned that if latency relaxation reflects a release of sequestered calcium, decrease of the muscle's calcium stores should result in a diminished latency relaxation. Likewise, if latency relaxation reflects an activation of the contractile process, a decrease in latency relaxation would be expected to give rise to a similar decrease in twitch tension proportional to the deterioration of the activation process.

Each muscle was excised, affixed to the myographs, and placed in Ringer solution. After about 15 minutes the first record, identified as time zero, was made. The muscle was then placed into Ringer solution to which no calcium was added but which contained 1 mM CDTA (cyclohexanediaminotetraacetic acid), a chelating

agent, added for the purpose of binding calcium ions diffusing from the muscle, thus maintaining a steep diffusion gradient for calcium, and preventing its reentry into the muscle. Magnesium was added to the bathing medium because it is reported to minimize the fall in membrane potential observed in muscles placed into calcium-free solutions (43). Although CDTA also binds magnesium, the stability of the complex is 150X less than for bound calcium. Every five minutes, the muscle was removed from the solution and stimulated with a single shock to obtain a record of twitch and latency relaxation. Muscles for control experiments were treated similarly except that they were placed into fresh Ringer solution after the time zero record was made. The pH of both CDTA and Ringer solutions was higher at the end of an experiment than at the beginning. Although accumulation of lactic acid in highly fatigued muscles has long been recognized (20) moderate activity is reported to result in increased alkalinity (62).

Figure 26 shows the time course of the twitch and latency relaxation amplitudes as a function of time in the CDTA solution. The graph is plotted with twitch tension extending upward from zero and latency relaxation downward, in the same sense the tension changes were presented by the muscle. The twitch amplitude fell rapidly at first and then more slowly. The final twitch amplitude was only 18% of its initial force after 55 minutes. A diminishing twitch tension with the greatest rate of change early in the experiment was typical behavior for all the muscles observed. The latency relaxation amplitude also decreased, but after 55 minutes it retained 55% of its original value.

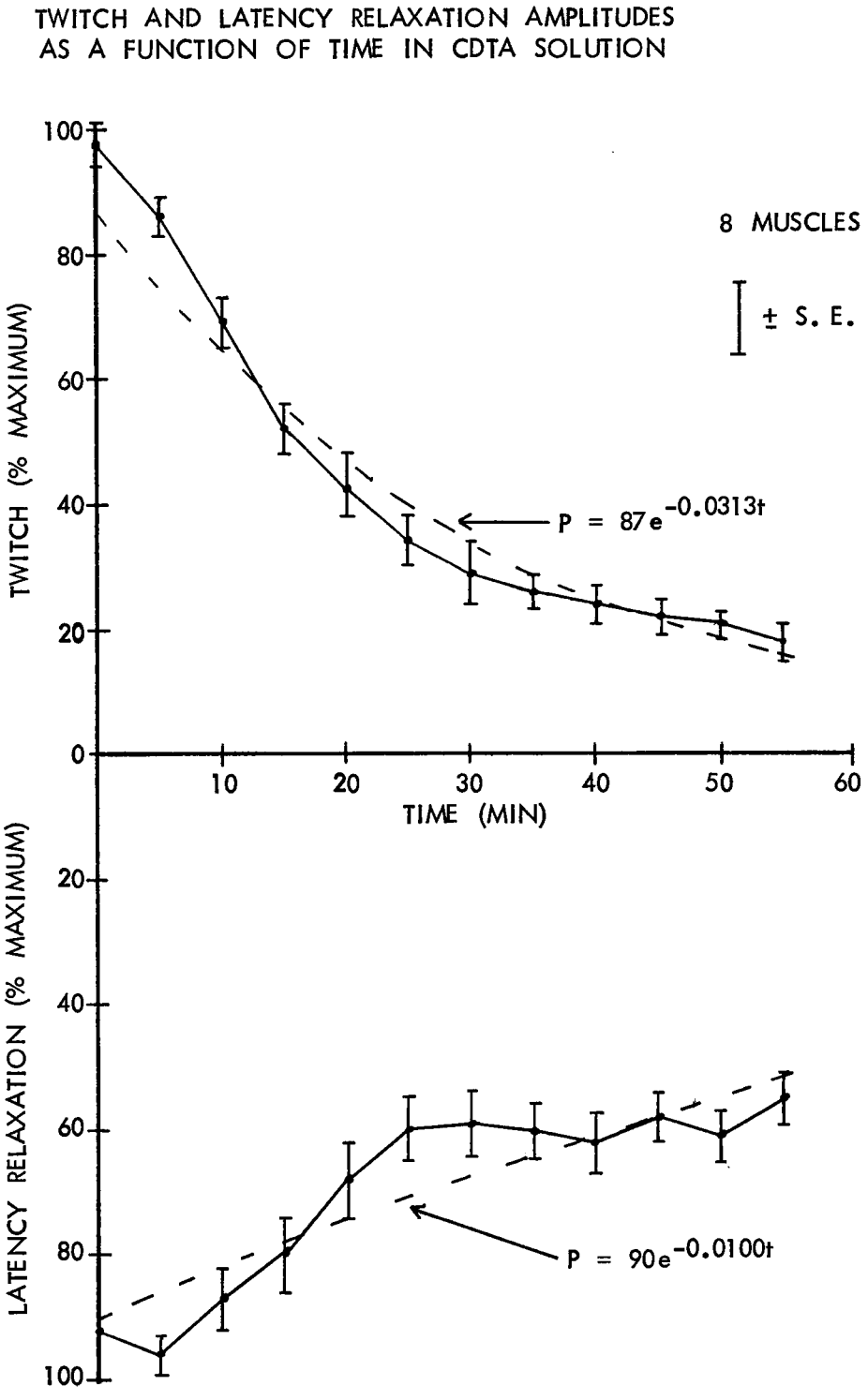


Figure 26. Each point is the mean value of eight muscles. The dashed regression curves are described by the expressions for P.

The time course of latency relaxation amplitude was less well defined than that of the twitch; it displayed an overall decrease, but did not follow the pattern of an early rapid decrease slowing with time. In fact, it was not an infrequent occurrence for an individual record of latency relaxation to show a slight increase over its predecessor. In these experiments resting tension was maintained as close as possible to the pretreatment value which provided maximum twitch. However, when muscles were placed into the CDTA medium they frequently exhibited loss of some resting tension; since it was important to record the amplitudes of twitch and latency relaxation at a constant resting tension to avoid the influence of this parameter, the muscles were slightly stretched to reestablish the initial resting tension.

A quantitative parameter was required for comparison of the overall time courses of twitch and latency relaxation; the quantity chosen was the slope of the regression line, $\log P = At + B$, where P is tension in per cent maximum, t is time in minutes, A is the slope, and B the value of log P at $t = 0$. The values of t and P were read into a programmed PDP-8 computer which calculated A and B. The parameter, A, was the useful one for comparing twitch and latency relaxation because it described the rates of decrease of the two events with time.

The dashed curves in Figure 26 are the calculated regression curves plotted in the equivalent form, $P = Be^{At}$. Since amplitude decreased with time, A was a negative number, and its value for the twitch is -0.0313, and -0.0100 for latency relaxation. The time constants, 1/A, are 32 minutes for the twitch and 100 minutes

for latency relaxation.

Linear, as well as logarithmic, regression lines were calculated for the data from several muscles, but the correlation coefficients were lower for the linear form than for the logarithmic form; therefore, logarithmic regression expressions were used for both twitch and latency relaxation data from all muscles. Correlation coefficients for logarithmic expressions of twitch and latency relaxation are listed in Table II. The correlation of twitch amplitude with the calculated regression curves exceeded the 1% confidence level in all muscles; however, the correlation coefficients for latency relaxation were always less than for twitch, but usually exceeded the 1% level, and in all cases, the 5% level.

Data from nine muscles placed into CDTA solution are presented in Table III. The amplitudes of both events decreased with time in all muscles, but the rate of twitch decay was always larger than the rate of latency relaxation decay.

E. TWITCH AND LATENCY RELAXATION AMPLITUDES IN UNSTRETCHED MUSCLES

The influence of resting tension on twitch and latency relaxation was described earlier. All measurements were made as close to a predetermined value of resting tension as possible because even small changes in resting tension could produce different responses in twitch and latency relaxation amplitudes. Muscles placed into CDTA solution frequently showed some loss of resting tension and had to be stretched slightly to return resting tension to its initial value; therefore, despite the fact that resting tension was maintained constant throughout a given procedure, resting length was increased. The possibility that changes in resting

TABLE II

CORRELATION COEFFICIENTS FOR REGRESSION CURVES OF MUSCLES PLACED IN CDTA SOLUTION

MUSCLE	CORRELATION COEFFICIENT	
	TWITCH	LATENCY RELAXATION
719671	0.894	0.628 (1% = 0.708; 5% = 0.576)
97671	0.874	0.624 (1% = 0.661; 5% = 0.532)
9867	0.895	0.770 (1% = 0.661)
915671	0.771	0.421 (1% = 0.661; 5% = 0.532)
819681	0.889	0.807 (1% = 0.684)
116682	0.932	0.917 (1% = 0.606)
1124682	0.916	0.875 (1% = 0.606)
1216681	0.908	0.611 (1% = 0.606)
612691	0.994	0.686 (1% = 0.684)

TABLE III

FINAL VALUES, DECREMENT CONSTANTS, AND TIME CONSTANTS FOR TWITCH AND LATENCY RELAXATION IN MUSCLES PLACED IN CDTA SOLUTION

MUSCLE	TIME OF EXPOSURE (MIN)	FINAL VALUE (% MAXIMUM)			DECREMENT CONSTANT, A (MIN^{-1})		TIME CONSTANT (MIN)		
		TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH
719671	62	42	35	0.83	-0.0141	-0.0110	71	91	1.28
97671	60	20	44	2.20	-0.0252	-0.0109	40	92	2.31
9867	60	28	59	2.11	-0.0237	-0.0070	42	143	3.39
915671	60	19	69	3.63	-0.0253	-0.0065	40	154	3.90
819681	55	7	45	6.43	-0.0497	-0.0160	20	63	3.15
116682	75	9	50	5.56	-0.0334	-0.0091	30	110	3.67
1124682	75	12	49	4.08	-0.0264	-0.0094	38	106	2.81
1216681	75	8	71	8.88	-0.0341	-0.0050	29	200	6.82
612691	55	26	60	2.31	-0.0250	-0.0108	40	93	2.32
MEAN		19	54	4.00	-0.0286	-0.0095	39	117	3.29
S. E.		±3.9	±4.0	±0.85	±0.0033	±0.0011	±4.7	±14	±0.52

length exerted an influence on the results was not ignored.

The amplitudes of twitch and latency relaxation in three muscles placed into CDTA solution and treated in a manner similar to those described above, with the exception that the muscles were not stretched to regain any loss in resting tension, were examined and compared to those in muscles maintained at a given resting tension. Figure 27 shows the mean amplitudes of twitch and latency relaxation as a function of time. Table IV summarizes the time courses of these muscles; the data therein should be compared to those in Table III which were obtained from muscles also placed into CDTA, but stretched as necessary to maintain a predetermined resting tension. (For ease of comparison the ranges of the respective parameters from Table III are included below Table IV.)

Twitch tension decreased more rapidly in the unstretched muscles than in the muscles maintained at a constant resting tension; the observations were terminated after only 35 minutes, because by that time the twitch amplitude had fallen to a very small value. Accordingly, the decrement constants, A, were larger and the time constants smaller. In fact, the smallest of the decrement constants for the twitch amplitudes of the unstretched muscles was larger than the largest for the muscles maintained at a constant resting tension. The time course of latency relaxation amplitude was similar to that in muscles maintained at a constant resting tension and did not show the faster decline exhibited by the time course of twitch amplitude. Compensation for resting tension loss by slightly stretching the muscles seemed to be justified in preventing a misleadingly large discrepancy between the

TWITCH AND LATENCY RELAXATION AMPLITUDE
WITH NO RESTING TENSION ADJUSTMENT

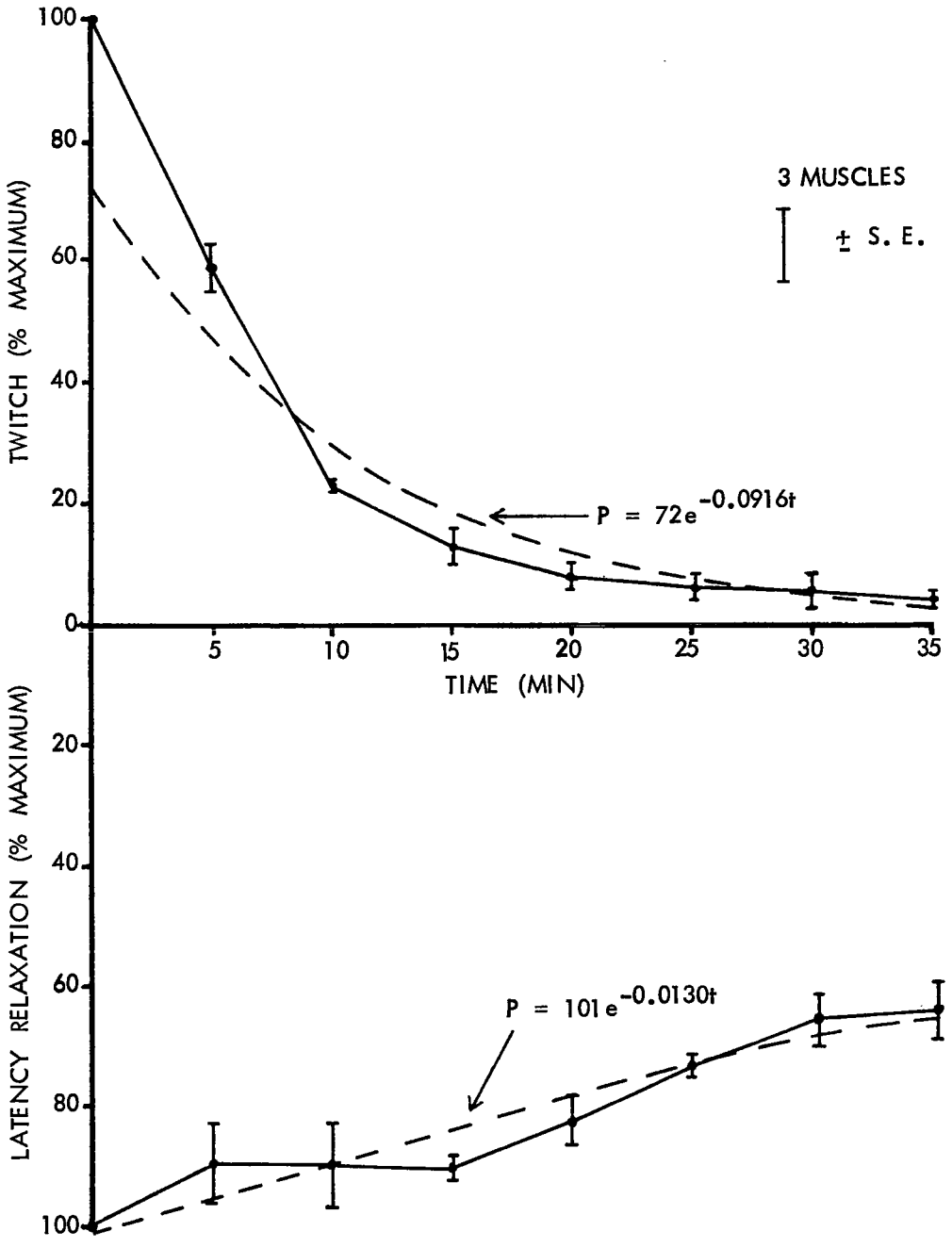


Figure 27. Each point is the mean value of three muscles. The dashed repression curves are described by the expressions for P.

TABLE IV

FINAL VALUES, DECREMENT CONSTANTS, AND TIME CONSTANTS
FOR TWITCH AND LATENCY RELAXATION IN
MUSCLES PLACED IN CDTA SOLUTION
WITH NO ADJUSTMENT OF RESTING TENSION

MUSCLE	TIME OF EXPOSURE (MIN)	FINAL VALUE (% MAXIMUM)			DECREMENT CONSTANT, A (MIN^{-1})		TIME CONSTANT (MIN)		
		TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH
918682	35	8	62	7.78	-0.0707	-0.0145	14	69	4.90
918684	35	3	73	24.33	-0.1105	-0.0048	9	208	23.11
923681	35	1	56	56.00	-0.1310	-0.0129	8	78	9.75
MEAN		4	64	29.37	-0.1041	-0.0107	10	118	12.59
S. E.		±2	±5	±14.15	±0.0177	±0.0030	±1.9	±45	±5.45
RANGE	55 to 75	16 to 62*	42 to 76*	1.00 to 3.44*	-0.0141 to -0.0497	-0.0050 to -0.0160	20 to 71	62 to 200	1.28 to 6.82
TABLE III									

* These values are at 35 minutes, not the final values.

time courses of twitch and latency relaxation.

F. TIME COURSES OF TWITCH AND LATENCY RELAXATION IN MUSCLES PLACED IN RINGER SOLUTION

Twitch amplitude fell more rapidly than latency relaxation amplitude when muscles were exposed to the CDTA solution. Control muscles were placed into Ringer solution and twitch and latency relaxation amplitudes were recorded every five minutes. The control experiments also provided information on the effects of time on the two tension events.

Figure 28 shows the mean amplitudes of twitch and latency relaxation as a function of time in Ringer solution. Twitch amplitude fell, although more slowly than in the muscles placed into CDTA solution, while latency relaxation showed a small increase over the period of observation. The calculated value of A for the twitch regression line is -0.0049, which corresponds to a time constant of 204 minutes, while the value of A for the latency relaxation curve is +0.0003.

Table V summarizes the time courses of these muscles. The final values for latency relaxation were consistently larger than the twitch values, but the ratios, LATENCY RELAXATION: TWITCH, were closer to a value of 1.00 than in the muscles exposed to CDTA solution, indicating a smaller relative change in the two events over a given time period.

In five muscles the regression curve calculated from the latency relaxation data increased over the period of the experiment. The data from the muscles placed into Ringer solution were processed similarly to those from muscles placed in CDTA

TWITCH AND LATENCY RELAXATION AMPLITUDE
AS A FUNCTION OF TIME IN RINGER SOLUTION

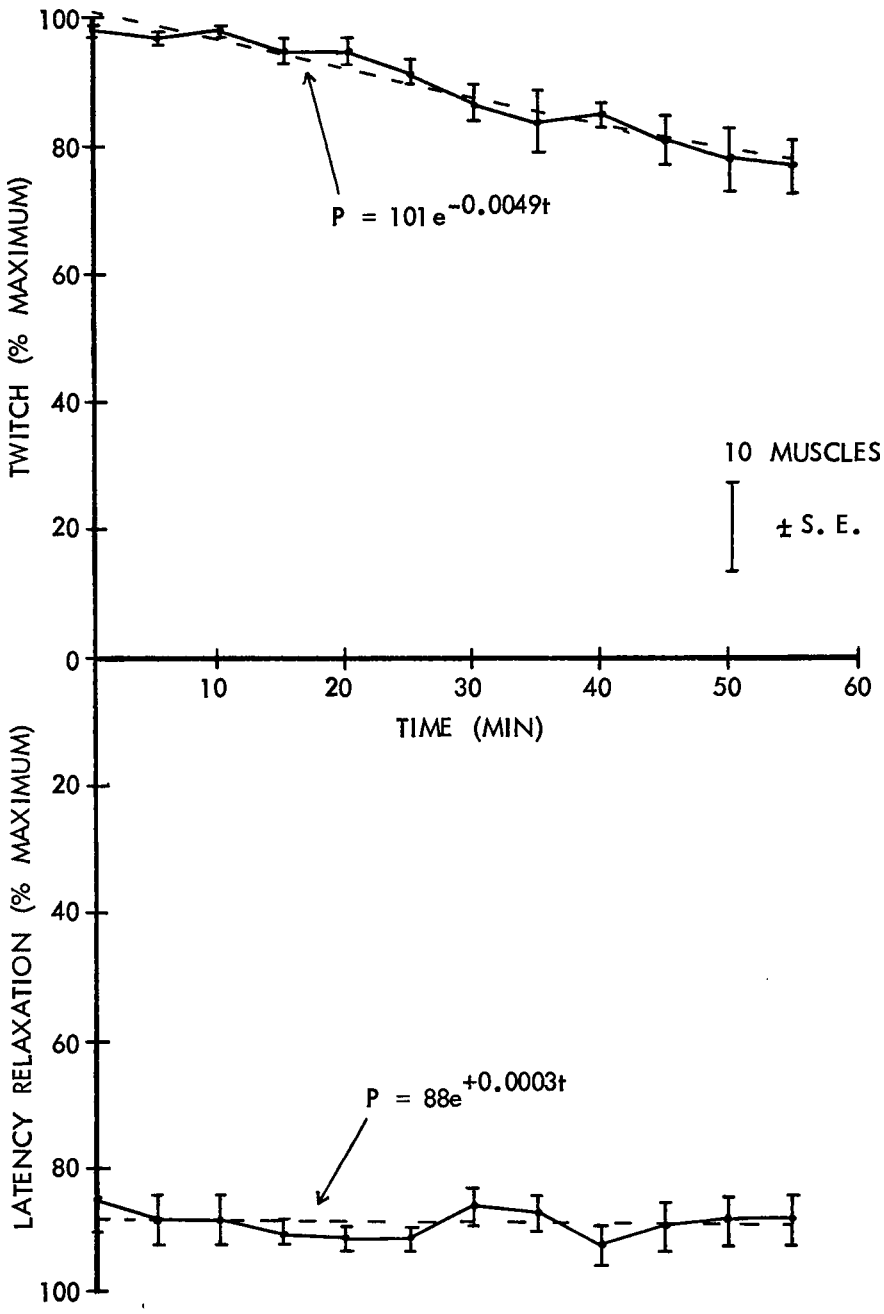


Figure 28. Each point is the mean value of ten muscles. The dashed regression curves are described by the expressions for P.

TABLE V

FINAL VALUES, DECREMENT CONSTANTS, AND TIME CONSTANTS FOR TWITCH AND LATENCY RELAXATION IN MUSCLES PLACED IN RINGER SOLUTION

MUSCLE	TIME OF EXPOSURE (MIN)	FINAL VALUE (% MAXIMUM)			DECREMENT CONSTANT, A (MIN^{-1})		TIME CONSTANT (MIN)		
		TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	LATENCY RELAXATION: TWITCH
719672	63	90	95	1.06	-0.0005	-0.0007	2000	1429	0.71
97672	60	82	91	1.11	-0.0046	-0.0009	217	111	5.11
915672	60	73	88	1.21	-0.0046	-0.0016	217	625	2.88
729681	63	58	89	1.53	-0.0092	+0.0046	109	-	-
812682	63	58	71	1.22	-0.0105	-0.0046	95	217	2.28
1124681	75	71	79	1.11	-0.0045	-0.0037	222	270	1.22
1125682	75	83	94	1.13	-0.0027	+0.0009	370	-	-
1216682	60	82	100	1.22	-0.0042	+0.0019	238	-	-
212692	55	83	91	1.10	-0.0018	+0.0069	556	-	-
612692	55	74	100	1.35	-0.0063	+0.0072	159	-	-
MEAN		75	90	1.20	-0.0049	+0.0010	418		
S. E.		±3	±3	±0.05	±0.0010	±0.0013	±181		

solution; the regression of log P on t was calculated and the value of the slope, A, was used to compare the time courses of twitch and latency relaxation amplitudes.

The correlation coefficients from the regression curves of twitch and latency relaxation amplitudes in Ringer solution are listed in Table VI. They were generally not as large as those from the data on muscles placed into CDTA solution, but there was consistently higher correlation for the data from the twitch than from latency relaxation. The higher correlation coefficients of the twitch regression curves were most likely due to its time course being more monotonic than that of latency relaxation.

G. CHANGES IN TWITCH AND LATENCY RELAXATION IN CDTA SOLUTION AS COMPARED TO RINGER SOLUTION

The preceding material has described the time courses of twitch and latency relaxation amplitudes in muscles placed into either Ringer solution or CDTA solution. Twitch and latency relaxation behaved differently from one another in both environments, and the nature of this dissimilarity varied, depending on the bathing medium. Twitch and latency relaxation amplitudes were compared by examining the data from paired muscles, one of which was placed into Ringer solution and the other into the CDTA solution, to get an idea of the relative susceptibility of twitch and latency relaxation to the CDTA solution. Once again the slope of the regression line was used as the most representative parameter of the time courses of the amplitudes. Table VII compares the values of A for the regression lines of twitch and

TABLE VI

CORRELATION COEFFICIENTS FOR REGRESSION CURVES OF MUSCLES PLACED IN RINGER SOLUTION

MUSCLE	CORRELATION COEFFICIENT	
	TWITCH	LATENCY RELAXATION
719672	0.150	0.064 (5% = 0.553)
97672	0.848	0.260 (1% = 0.661; 5% = 0.532)
915672	0.906	0.378 (1% = 0.661; 5% = 0.532)
729681	0.726	0.626 (1% = 0.684; 5% = 0.553)
812682	0.767	0.716 (1% = 0.641)
1124681	0.891	0.842 (1% = 0.606)
1125682	0.891	0.388 (1% = 0.606)
1216682	0.813	0.652 (1% = 0.661)
212692	0.698	0.779 (1% = 0.684)
612692	0.929	0.926 (1% = 0.684)

TABLE VII

COMPARISON OF THE CHANGES IN DECREMENT CONSTANTS FOR TWITCH AND LATENCY RELAXATION IN RINGER AND CDTA SOLUTIONS

MUSCLE PAIR	$A \times 10^4$				$(A_C - A_R)$		
	TWITCH		LATENCY RELAXATION				
	RINGER, A_R	CDTA, A_C	RINGER, A_R	CDTA, A_C	TWITCH, A_T	LATENCY RELAXATION, A_{LR}	A_T:A_{LR}
71967	-5	-141	-7	-110	-136	-103	1.32
9767	-46	-252	-9	-109	-206	-100	2.06
91567	-46	-253	-16	-65	-207	-49	4.45
112468	-45	-264	-37	-94	-219	-57	3.84
121668	-42	-341	+19	-50	-299	-69	4.33
61269	-63	-250	+72	-108	-187	-180	1.04
MEAN	-41	-250	+4	-89	-209	-93	2.84
S. E.	±8	±26	±16	±11	±22	±20	±0.63

latency relaxation amplitudes in the two solutions, the differences in this value in each pair of muscles, and the ratio of the change in the twitch to the change in latency relaxation. The most informative value is the ratio of the changes. All values were greater than 1.00 which seems to indicate that twitch amplitude was affected to a greater degree than latency relaxation by placing the muscles into CDTA solution.

H. RELATIONSHIP OF LATENCY RELAXATION AMPLITUDE TO ITS DURATION, RATE OF DEVELOPMENT, AND THE EARLY RATE OF CHANGE IN TWITCH TENSION

Contraction most likely begins during the period of latency relaxation, but up to the time of maximum tension decrease relaxation obscures contraction. However, the recorded latency relaxation amplitude is probably reduced by the amount of contractile tension being generated at any time after contraction begins. Therefore, latency relaxation was probably not observed free from the influence of the early part of the twitch.

Some indication of the changes which occur in the latency relaxation mechanism, independent of the contractile process, may be obtained from examining the rate of latency relaxation development, dLR/dt, the rate of rise of twitch tension, dP/dt, and the duration of time net tension was below the baseline. Each of these should exert a distinctive effect on latency relaxation amplitude and contribute to what is actually recorded. The observed latency relaxation amplitude should be proportional to dLR/dt because the more rapidly the tension falls, the

greater the relaxation achieved over a given time period. However, recorded latency relaxation amplitude should be inversely related to dP/dt, since the more rapidly twitch tension develops, the smaller the amount of relaxation manifested before being obscured. The values of dLR/dt and dP/dt were determined from lines drawn tangent to the latency relaxation records as shown in Figure 29. The rate of tension development is most likely not constant throughout the period of latency relaxation (as evidenced by the curved contour of the upward limb of latency relaxation); therefore, there was no better reason for determining dP/dt at the point where tension recrosses the baseline than at any other place, except that it was easily identifiable. However, any overall change in dP/dt should be reflected at the point of baseline recrossing.

The duration and amplitude of latency relaxation should be related in a direct manner, primarily, because a longer duration would suggest a delay in the onset of twitch, but may also indicate an increased value of dLR/dt. Thus, the recorded amplitude of latency relaxation was probably influenced by several factors and did not present a simple picture of the dynamics of the early relaxation.

Accurate measurement of the rates of development of twitch and latency relaxation was difficult because of steepness of the curves and small size of the records. A pronounced change in these values was necessary before it was possible to detect a measurable difference. The changes in dLR/dt and dP/dt were too small to be measured satisfactorily in the muscles placed into Ringer solution; however, the duration of latency relaxation could be determined with more accuracy. The

DETERMINATION POINTS OF dP/dT AND dLR/dT

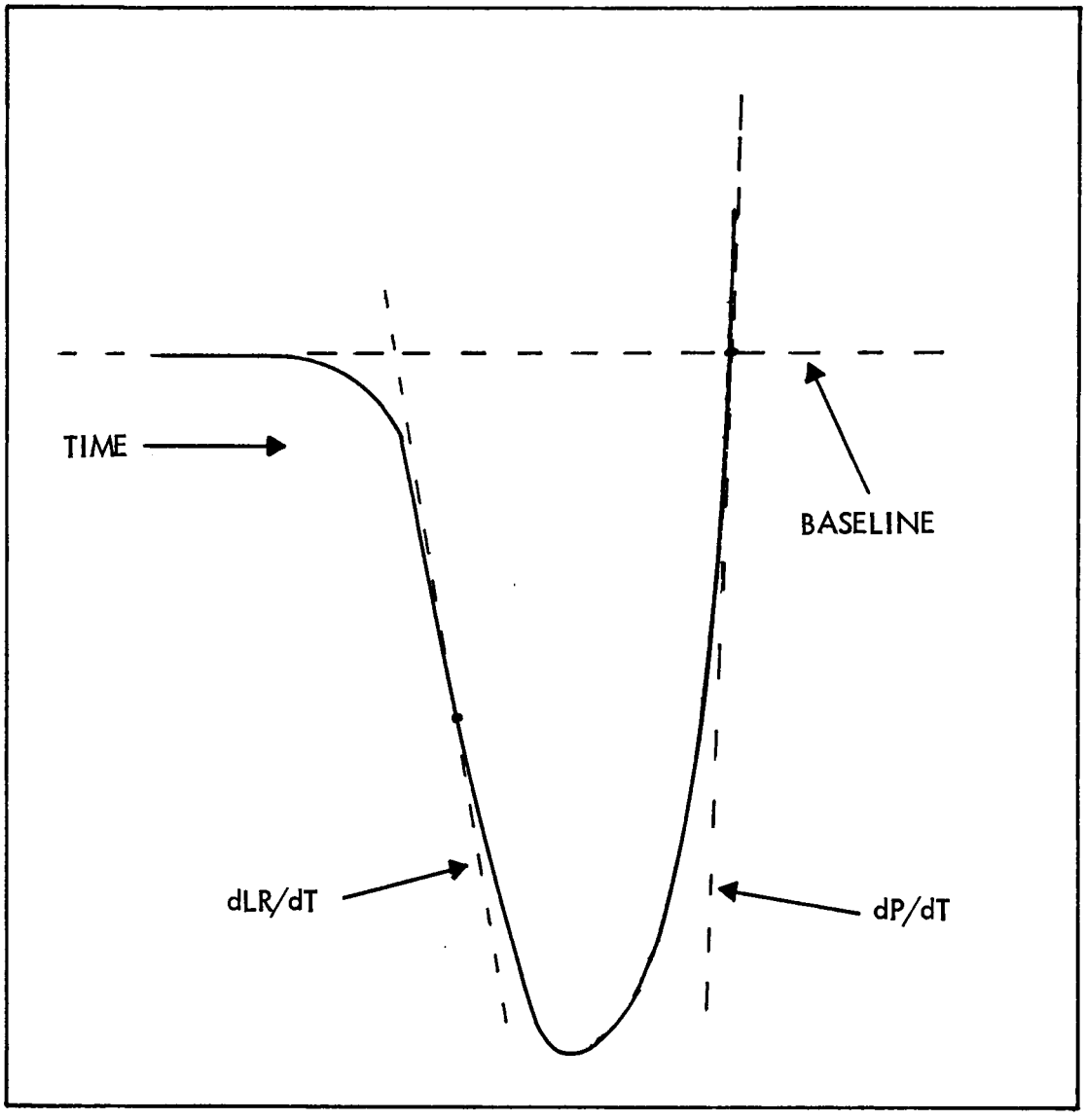


Figure 29. The values of dP/dt and dLR/dt were determined from the angles made with the horizontal baseline by lines drawn tangent to the curve (at the points indicated).

duration of latency relaxation generally increased in those muscles in which latency relaxation amplitude increased during the period of observation. When both twitch and latency relaxation decreased the durations were maximum at the start of the experiment and then shortened. The shortest duration in any muscle placed in Ringer solution was 60% of maximum, and most values were greater than 75%. The largest absolute change in duration was an increase of 1.2 msec, from 2.9 msec, at the start of the experiment, to 4.1 msec after 50 minutes, in a muscle with decreasing twitch and increasing latency relaxation.

Duration, dLR/dt, and dP/dt were measured from the records of muscles placed into CDTA solution. The duration of latency relaxation increased while both dLR/dt and dP/dt decreased. Figure 30 shows the amplitudes and rates of development of twitch and latency relaxation and the duration for which net tension was below the baseline as a function of time in CDTA solution. Each point is the mean value of eight muscles placed into CDTA solution.

I. EFFECTS OF CAFFEINE ON TWITCH AND LATENCY RELAXATION

The alkaloid, caffeine, is a well known potentiator of skeletal muscle activity (3,66,68). When present in low concentrations in the medium, the drug produces an intensified twitch tension, while at higher concentrations it produces contracture. A distinctive feature of caffeine contractures is their occurrence in depolarized muscle (3,21), indicating that this drug affects the contractile, rather than the excitatory, mechanism of muscle. Caffeine contractures appear to require a source of calcium ions for the muscle even though they do not require

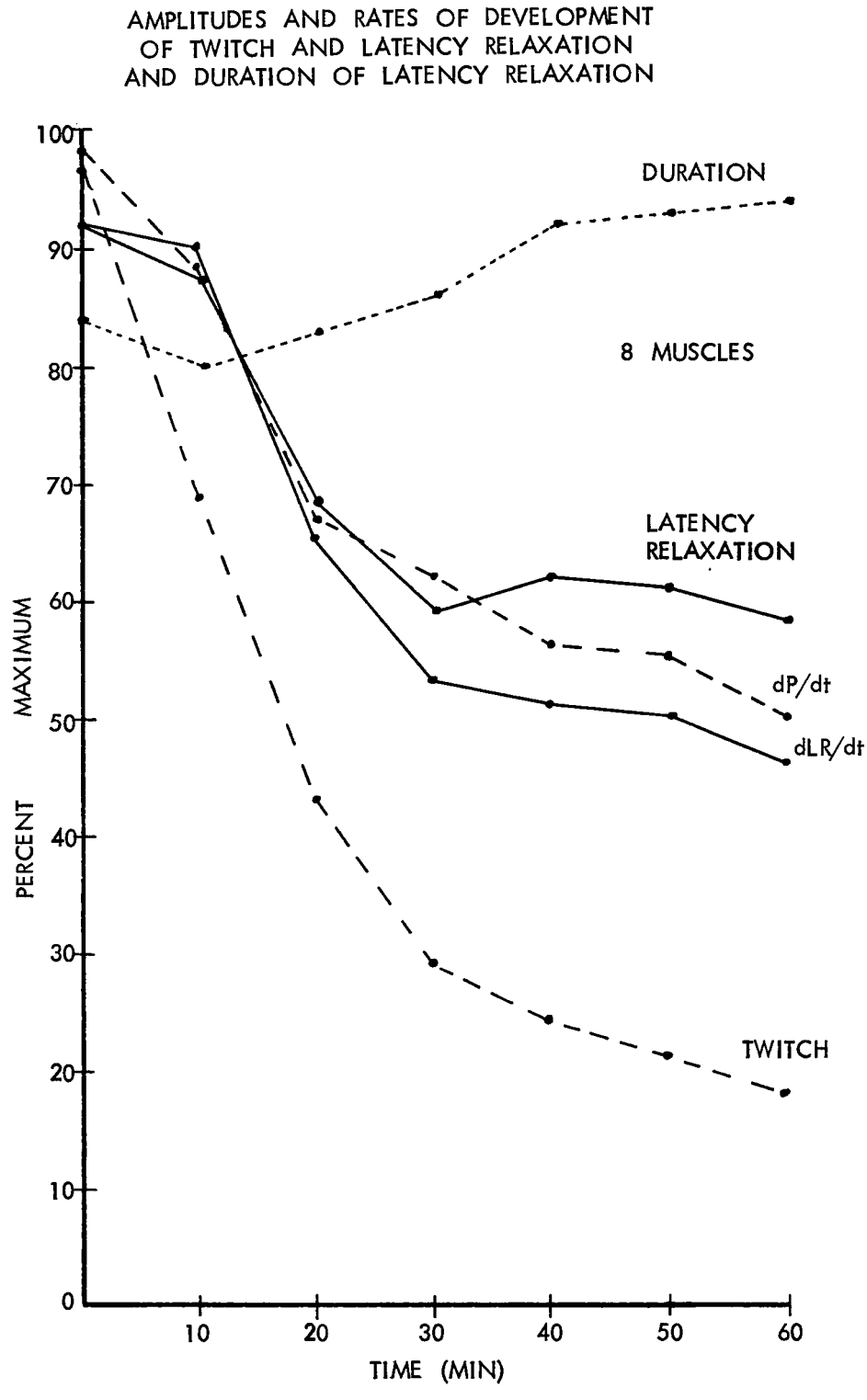


Figure 30. Each point is the mean value of eight muscles. Duration of latency relaxation was measured as the time net tension was below the base line.

the presence of a polarized membrane (22).

The reported results of experiments using preparations of isolated sarcoplasmic reticulum (75) have indicated that the basis of the potentiating action of caffeine is the drug's ability to retard the reaccumulation of calcium into the reticular structures and decrease the affinity of the sarcoplasmic reticulum for calcium.

It was of interest to determine if caffeine potentiates latency relaxation as well as twitch tension, particularly since this drug apparently affects the calcium-sequestering capability of the sarcoplasmic reticulum. Twitch and latency relaxation amplitudes in muscles placed into Ringer solution with 1 mM caffeine were compared. A control record, designated as time zero, was made after a period of 20 minutes in aerated Ringer solution. The muscles were transferred to the caffeine solution, and records were made at 5 minute intervals. Table VIII lists maximum twitch and latency relaxation amplitudes as per cent of control value and the time after exposure to caffeine at which they occurred; Table IX lists similar information for the minimum values. The time courses of twitch and latency relaxation amplitudes in caffeine are shown in Figure 31.

Twitch did not maintain the maximum value reached, but it seldom fell even as low as control value during the experimental period. In contrast, latency relaxation did not increase above control value, but showed a steady decline. The minimum values of latency relaxation were generally less than in the muscles placed into CDTA solution for the same length of time. The duration of latency relaxation was measured in six muscles and observed to decrease with increasing time of

TABLE VIII

TWITCH AND LATENCY RELAXATION MAXIMA
IN CAFFEINE MEDIA

MUSCLE	MAXIMUM TWITCH		MAXIMUM LATENCY RELAXATION	
	% CONTROL	TIME (MIN)	% CONTROL	TIME (MIN)
34701	118	10	100	0
34704	120	15	100	0
34705	131	5	100	0
36701	138	20	100	0
36702	159	30	100	0
36704	125	5	100	0
39701	105	10	103	5
39702	106	10	100	0
39703	121	15	100	0
39704	113	15	100	0
311701	117	20	106	5
311702	115	20	102	5
311704	117	5	100	0
MEAN	122		101	
S. E.	±4		±1	

TABLE IX

TWITCH AND LATENCY RELAXATION MINIMA
IN CAFFEINE MEDIA

MUSCLE	MINIMUM TWITCH		MINIMUM LATENCY RELAXATION	
	% CONTROL	TIME (MIN)	% CONTROL	TIME (MIN)
34701	95	35	39	35
34704	100	0	54	25
34705	100	0	50	35
36701	100	0	50	35
36702	100	0	73	55
36704	100	0 & 35	21	35
39701	84	35	51	35
39702	81	35	30	35
39703	100	0	56	30
39704	100	0	26	35
311701	100	0	70	45
311702	100	0	41	40
311704	100	0	45	45
MEAN	97		47	
S. E.	±2		±4	

TWITCH AND LATENCY RELAXATION AMPLITUDES
AS A FUNCTION OF TIME IN CAFFEINE SOLUTION

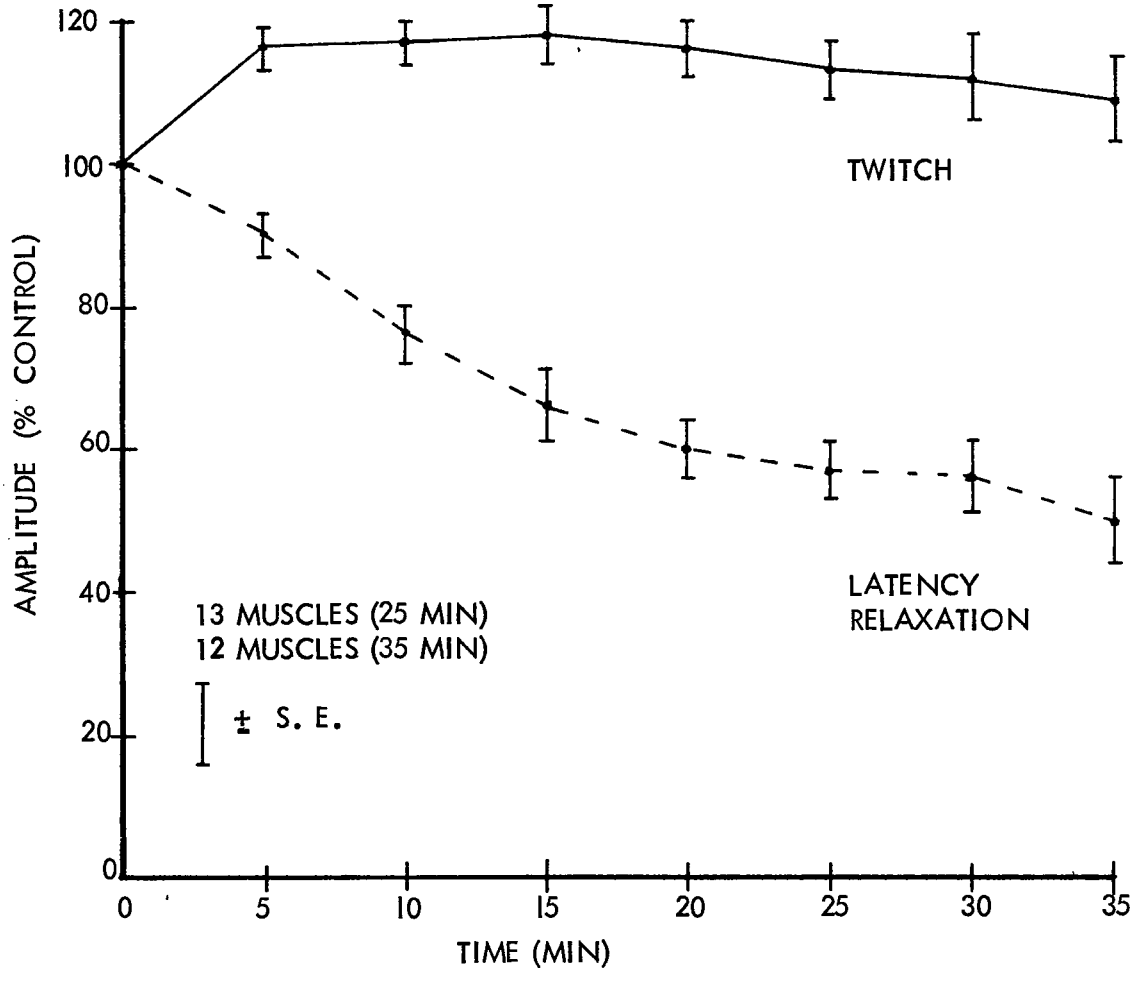


Figure 31. Each point is the mean value of thirteen muscles through twenty-five minutes and twelve muscles through thirty-five minutes.

exposure to caffeine. Furthermore, the rate of tension development, dP/dt, relative to control value, was always larger than dLR/dt in these muscles.

J. TWITCH AND LATENCY RELAXATION AMPLITUDES AT INCREASED TEMPERATURES

Muscles were exposed to increased temperatures to determine if twitch and latency relaxation were similarly affected. A control record was made before the muscles were placed into a reservoir of Ringer solution heated to the desired temperature. The heater is shown in Figure 32 and was specially constructed to fit the dimensions of the reservoir. The temperature of the bath was monitored by an electronic thermometer probe placed in the reservoir in close proximity to the muscle and was maintained to within $\pm$ 0.5°C of the experimental temperature.

The time courses of twitch and latency relaxation at five different temperatures are shown in Figure 33. The amplitudes are expressed as per cent of control value and each point represents the mean value of the muscles examined at the indicated temperature. Twitch amplitude fell below control value over the experimental period at all temperatures; however, latency relaxation amplitude increased and remained above control throughout the experiment at the three lowest temperatures, while at the two highest temperatures it, too, decreased. The data from 26 muscles are summarized in Table X.

The effect of exposure to different temperatures for extended periods of time was observed. Table XI lists the amplitudes of twitch and latency relaxation after periods of time much longer than the usual experimental durations, and Figure 34

ASSEMBLY FOR HEATING BATHING SOLUTION

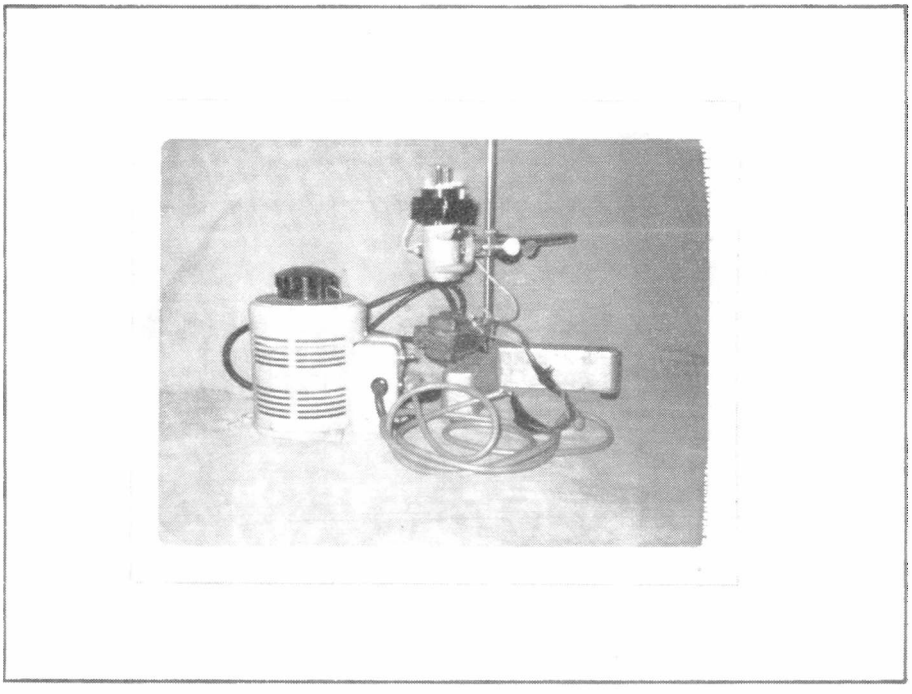


Figure 32. The heating element, molded to fit the reservoir, and a step-down transformer to protect the element are mounted on the stand to the right of the photograph. A variable transformer for adjusting the temperature is to the left of the stand.

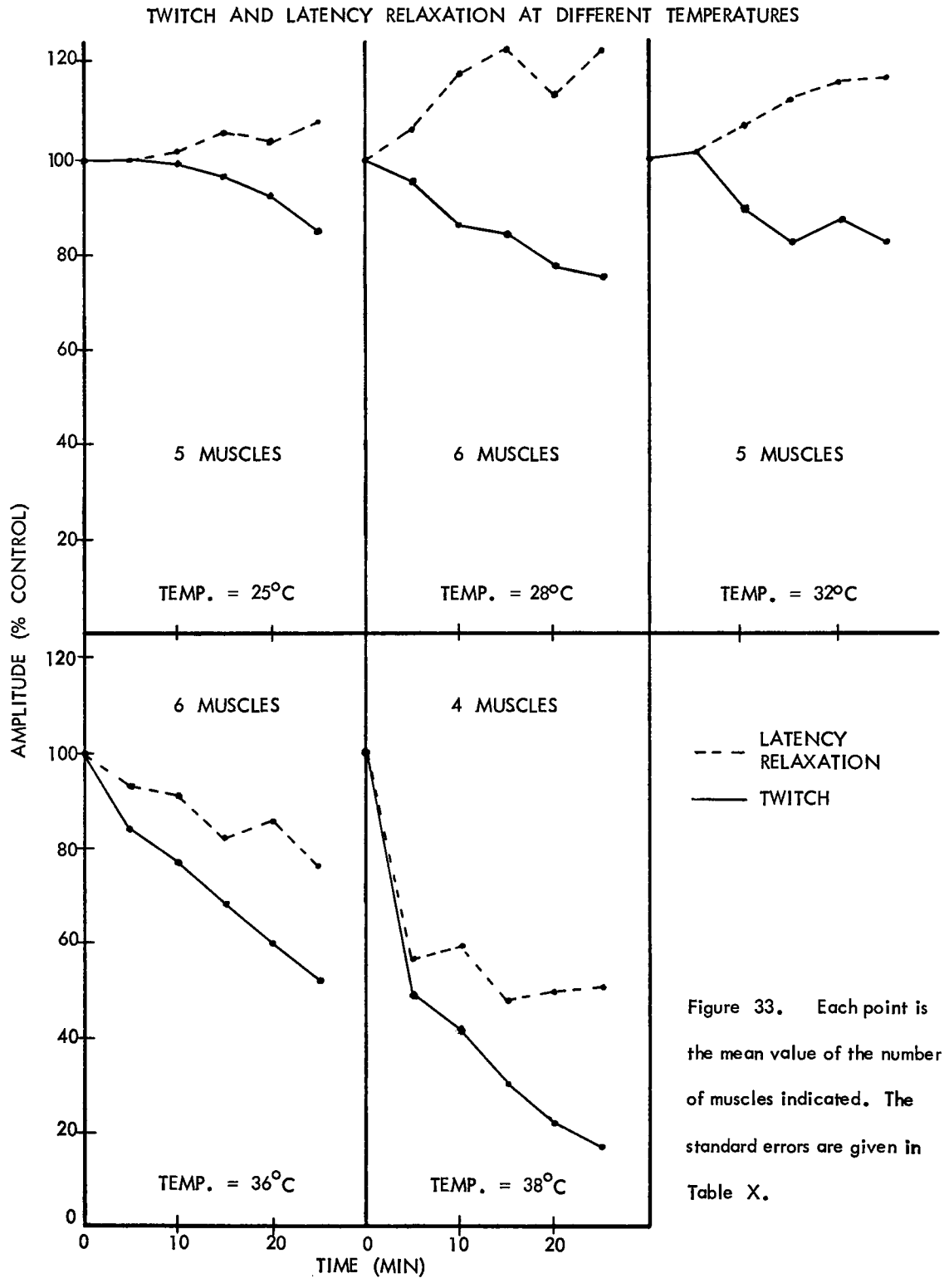


Figure 33. Each point is the mean value of the number of muscles indicated. The standard errors are given in Table X.

TABLE X

TIME COURSE OF TWITCH AND LATENCY RELAXATION
AT DIFFERENT TEMPERATURES

MEAN AMPLITUDE IN PERCENT CONTROL (S. E. IN PARENTHESES)										
TIME (MIN)	25°		28°		32°		36°		38°	
	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION	TWITCH	LATENCY RELAXATION
5	100 (3)	100 (5)	95 (4)	106 (3)	101 (3)	101 (6)	84 (4)	93 (10)	49 (9)	56 (18)
10	99 (4)	102 (7)	86 (4)	118 (3)	89 (8)	107 (7)	77 (6)	91 (7)	42 (11)	59 (18)
15	97 (5)	106 (10)	84 (3)	123 (5)	82 (9)	113 (10)	68 (5)	82 (6)	30 (8)	48 (20)
20	93 (6)	104 (8)	77 (5)	113 (4)	87 (3)	116 (13)	60 (4)	86 (5)	22 (7)	49 (18)
25	85 (5)	108 (12)	75 (5)	123 (5)	82 (4)	117 (14)	52 (5)	74 (10)	17 (5)	50 (16)
NUMBER OF MUSCLES	5	5	6	6	5	5	6	6	4	4

TABLE XI

TWITCH AND LATENCY RELAXATION AMPLITUDES
AFTER EXTENDED TIMES AT ELEVATED TEMPERATURES

MUSCLE	AMPLITUDE (% CONTROL)		CONDITIONS
	TWITCH	LATENCY RELAXATION	
325702	28	124	90 minutes at 32°
	24	148	120 minutes at 32°
46703	57	186	180 minutes at 25°
410703	22	180	120 minutes at 35°
	14	158	180 minutes at 35°
410704	3	45	120 minutes at 38°
	0	15	120 minutes at 38°, then 60 minutes at 32°
32370	0	172	150 minutes at temperatures varying from 24° to 38.5° to 32.5°

LATENCY RELAXATION AFTER 150 MINUTES

AT ELEVATED TEMPERATURES

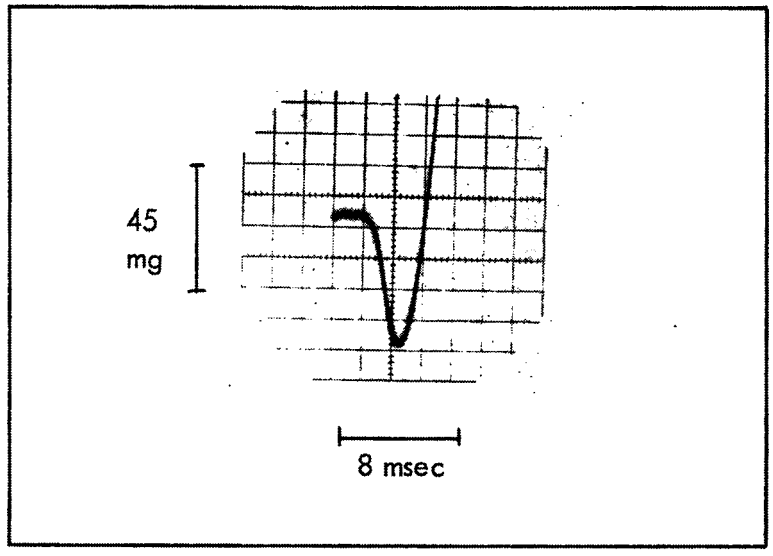


Figure 34. A record of latency relaxation in a muscle after 150 minutes at different temperatures ranging from 25°C to 38.5°C. The associated twitch was too small to be detected on the twitch channel.

is a record of latency relaxation whose accompanying twitch was so small it was not registered on the twitch record at all. Nevertheless, the amplitude of latency relaxation had increased beyond its control value.

CHAPTER V

DISCUSSION

The salient feature of the experiments comparing twitch and latency relaxation was the difference in responses of the two events to experimental variables. The observed dissimilarities suggest 1) that the two events may not be governed by the same factors, 2) that the magnitude of the twitch is not predicted by the magnitude of latency relaxation, and 3) that, in most cases, latency relaxation amplitude is less subject to diminution than twitch amplitude. Despite the differences in response of the two tension events, no evidence emerged from these experiments which would discredit latency relaxation as the reflection of some process in a sequence of events between the stimulation and contraction of muscle. That latency relaxation is a genuine feature of muscle activity and may play some role in coupling excitation and contraction is supported by its presence in neurally activated muscle and its consistent appearance and temporal position between stimulation and tension production.

Sandow has suggested that the calcium ions released upon activation of a muscle are responsible in some manner for latency relaxation (61,67). Other workers (11,23,52) have suggested that the role of calcium ions in initiating muscular contraction may be one of combining with the protein, troponin, and removing an existing inhibitory influence on actin-myosin interaction. Once this inhibition is removed, actin and myosin combine and initiate the contractile

sequence. The troponin moiety is reported to be closely associated with tropomyosin which appears to be located exclusively in the thin filaments (14) and which seems to occur with a periodicity of about 400 Å (55). These findings suggest that potential binding sites may be spaced along the actin filament in much the same manner cross bridges occur along myosin filaments and may offer a chemical identification of the "active sites" of actin. When free calcium concentration falls below 10^{-7} M within the muscle, contraction is inhibited (74); at higher concentrations actin and myosin combine and contraction occurs *in vivo*, and superprecipitation takes place in *in vitro* mixtures of the proteins (76). The combination of calcium with troponin has been suggested to induce conformational changes within the thin filaments conducive to actin-myosin interaction (10); this may be the molecular basis of the activating effect of calcium upon muscle contractile proteins.

The combination of calcium with troponin most likely occurs prior to actin-myosin interaction and contractile activity. The temporal similarity of this process to precontractile relaxation and the proposed conformational changes arising from calcium-troponin interaction (10,11) suggested a relationship between the ion-protein combination and latency relaxation as observed in intact muscle. If the source of latency relaxation is indeed the combination of calcium with troponin, the behavior of the phenomenon observed in the experiments described earlier should be explainable on this basis. The material in this section will offer explanations of the experimental results in terms of this principle.

Calcium ions are probably released from the terminal cisternae of the sarcoplasmic reticulum. Two such terminal cisternae, with a T-tubule (an invagination of sarcolemma) situated between them, are collectively called a triad; these structures are located at the level of the Z line in frog sartorius muscles (56). Autoradiographic studies have indicated a cyclic movement of calcium between the tubular and terminal portions of the sarcoplasmic reticulum and the muscle filaments during activity (78), and the classical experiment of Huxley and Taylor (41) showed that electrical stimuli of very low intensity evoked local contraction only when applied to the triadic region of the sarcomere. This observation suggested an association of this region with the activation of contraction.

Only actin filaments are present in the I bands of muscles; therefore, calcium ions released from their sequestering site into the interfilament spaces of myofibrils on application of a stimulus first encounter only actin filaments not overlapped by myosin. The ions probably combine with troponin sites and produce a configurational change which may be mechanically manifested as latency relaxation. Latency relaxation amplitude should increase rapidly as more ions cover more sites. Some of the released calcium will probably have diffused to the area of overlap of actin and myosin filaments after a short period of time. Contraction would be expected to begin when the first troponin site within combining range of a myosin cross-bridge has been activated by calcium. This suggested sequence is diagrammatically presented in Figure 35.

The contractile tension generated at any single site by an actin-myosin link

ATTACHMENT OF CALCIUM TO ACTIN SITES

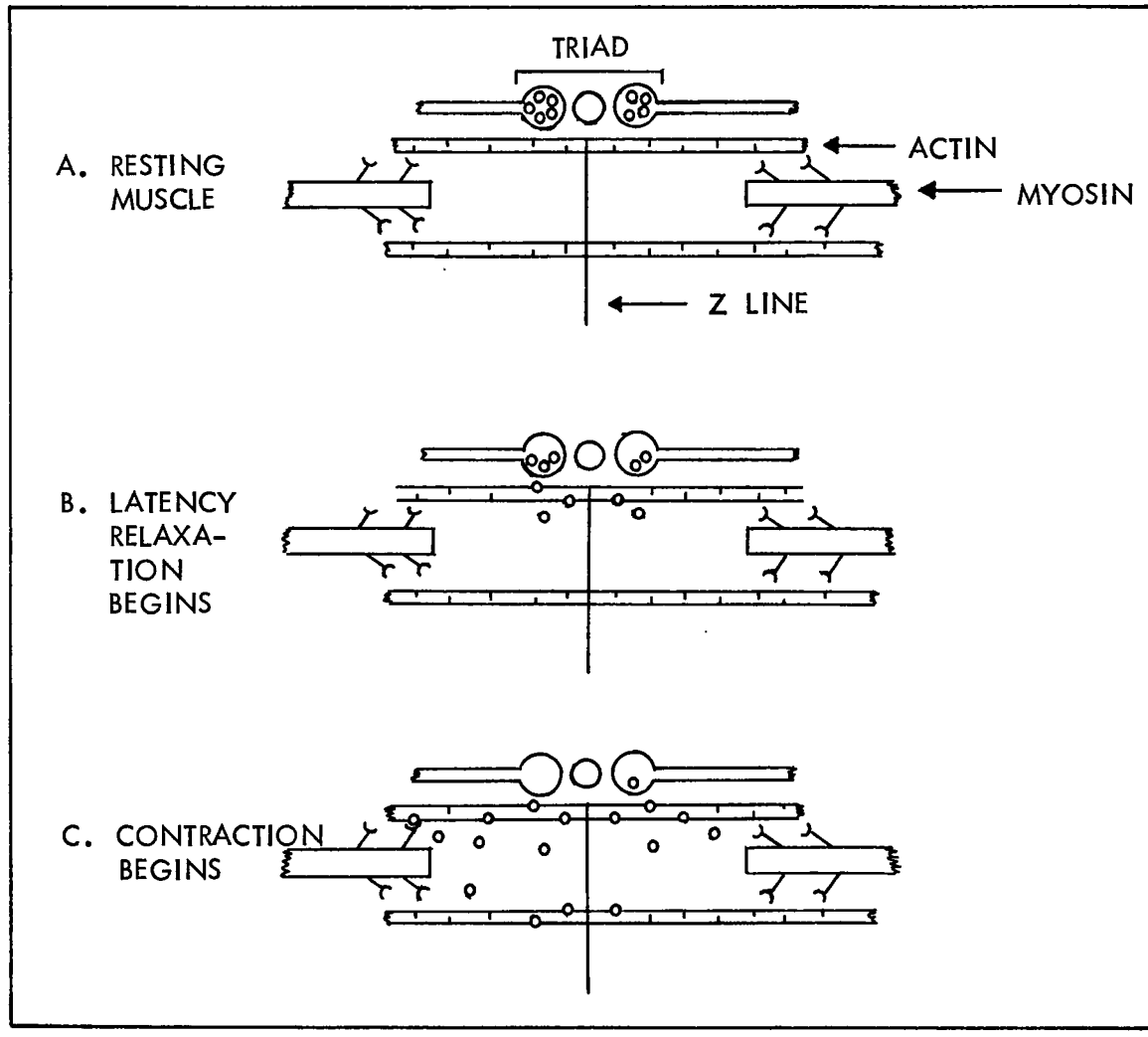


Figure 35. As calcium is released from the terminal cisternae it attaches to sites along the thin filaments. When the first actin-myosin association is formed contraction begins.

is conceivably greater than the relaxation resulting from the combination of calcium and troponin; therefore, the rate of relaxation should begin to slow as the diffusing calcium ions reach the area of actin-myosin overlap and tension production accelerates. Every thin filament site activated by calcium, whether located in the area of overlap or not, may contribute to the amplitude of latency relaxation; however, after contraction begins positive tension probably quickly overshadows latency relaxation. The relaxation process appears to be dominant up to the point of maximum amplitude of latency relaxation, and the net tension decreases; thereafter the contractile process dominates and tension begins to rise. Tension production is said to begin during the downward limb of latency relaxation (61), and at this point the rate of relaxation appears to begin to slow.

The maximum amplitude of latency relaxation is probably determined by several factors and may reflect the net influence of two oppositely directed tension changes as shown in Figure 36. The extent of the precontractile relaxation would probably depend on the amount of calcium released by a stimulus if it is a reflection of the number of troponin sites activated. However, the RECORDED latency relaxation amplitude may also depend on delay in the start of contraction and its rate of development. Thus, the potential amplitude of latency relaxation may be large when a large packet of calcium is released, but under this condition the ions would probably reach the area of overlap more quickly and contraction would begin sooner, thereby obscuring a proportionately larger part of the developing latency relaxation. Conversely, the realizable relaxation would be less if less calcium was available,

TWITCH, LATENCY RELAXATION, AND
NET TENSIONS VERSUS TIME

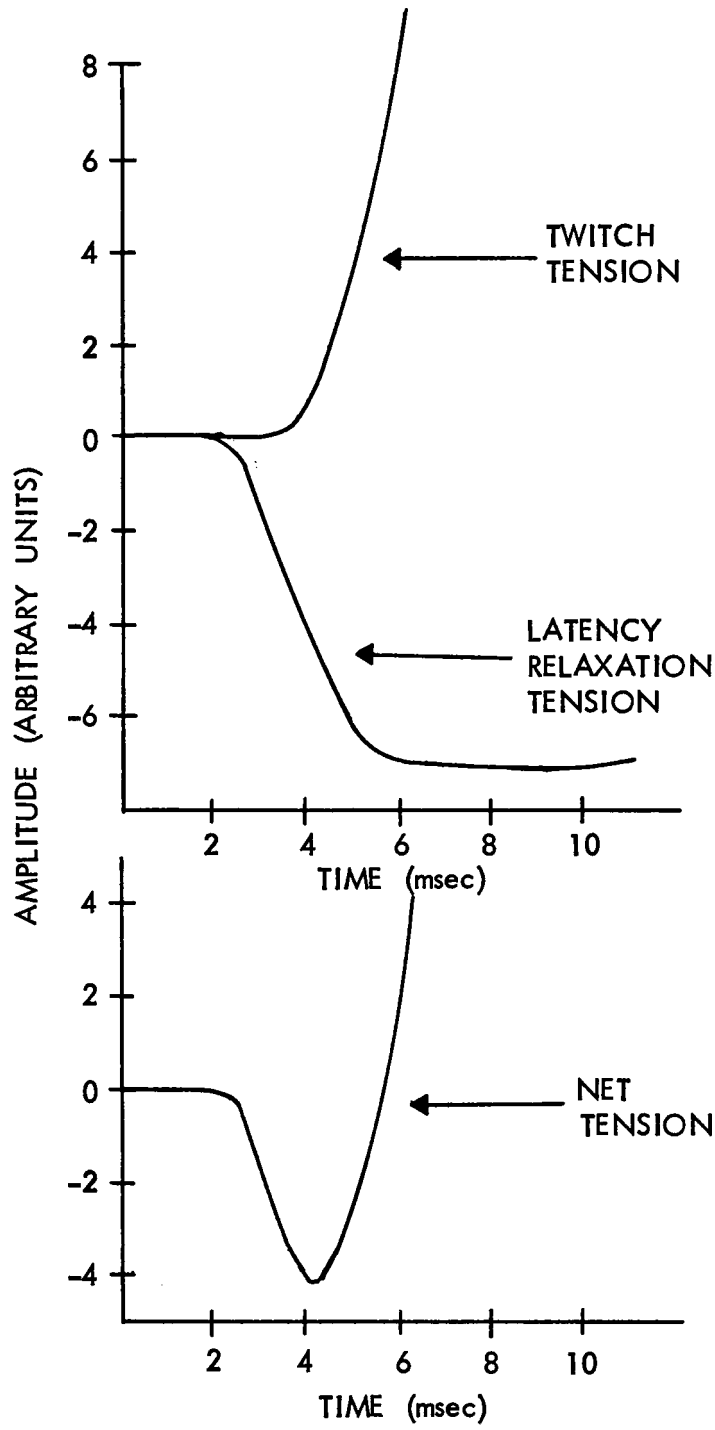


Figure 36. Net tension is shown as the algebraic sum of two oppositely directed processes.

but the rate at which calcium reaches actin sites in the area of overlap would probably be slower, twitch development delayed, and a greater fraction of the total relaxation observed. Thus, recorded latency relaxation amplitude may be determined by at least two processes, release of calcium and onset of contractile tension. If this is so the recorded amplitude of latency relaxation could decrease at a slower rate than latency relaxation itself.

Both direct and indirect (nerve) stimulation should elicit latency relaxation if the phenomenon reflects calcium binding to sites on the thin filaments. In fact, latency relaxation resulting from muscle activation via motor nerve stimulation is basic to this hypothesis, because in situ calcium release is normally initiated only by a neurally delivered stimulus. Latency relaxation was seen to be present in physiologically activated muscle.

Latency relaxation was observed to increase with increasing resting tension more slowly than the twitch. Furthermore, latency relaxation usually showed smaller changes in the range of resting tensions where twitch amplitude was undergoing the most rapid increase than at resting tensions greater than that at which twitch was maximum. An explanation for this observation may be as follows. The I bands are shorter and the amount of actin not overlapped by myosin is probably less at low resting tension; therefore, diffusion distances for calcium ions from the site of release to the area of actin-myosin overlap would probably be shorter, contraction would begin earlier and less latency relaxation would be recorded. Also, there is probably an overlapping of thin filaments at the center of the sarcomeres

at very short muscle lengths (27) as shown in Figure 37. The overlapping thin filaments could reduce the efficiency of the contractile process since cross-bridges in the two halves of sarcomeres most likely move actin filaments in opposite directions. Thus, the three cross-bridges shown in Figure 37A (and the one shown in Figure 37B) that are formed with thin filaments from the opposite half-sarcomere would probably cancel the tension produced at an equal number of sites in the other half-sarcomere. Under this condition contraction probably would be facilitated to a greater degree than latency relaxation as the muscles were initially stretched. The area of overlapping thick and thin filaments probably decreases with further increases in resting tension and muscle length. A larger latency relaxation would be recorded as the number of calcium-troponin associations formed prior to the first actin-myosin complex increases. The duration of latency relaxation was observed to increase as resting tension increased. This might be expected if onset of twitch tension was delayed. Latency relaxation amplitude began to fall at high resting tensions. This loss of latency relaxation amplitude may have been due to changes in the binding sites as the result of forcible stretching, reduction in the release of calcium from the sarcoplasmic reticulum as a result of excessive elongation, or actual damage to some of the overstretched fibers. The observed twitch and latency relaxation amplitudes as a function of resting tension in whole sartorius muscles are in accord with the earlier observations of Guld and Sten-Knudsen (28) who compared isometric twitch tension and latency relaxation to sarcomere lengths in small bundles of frog toe muscles and found that twitch tension declined at sarcomere lengths greater than 2.3 microns,

ACTIN AND MYOSIN OVERLAP WITH INCREASING STRETCH

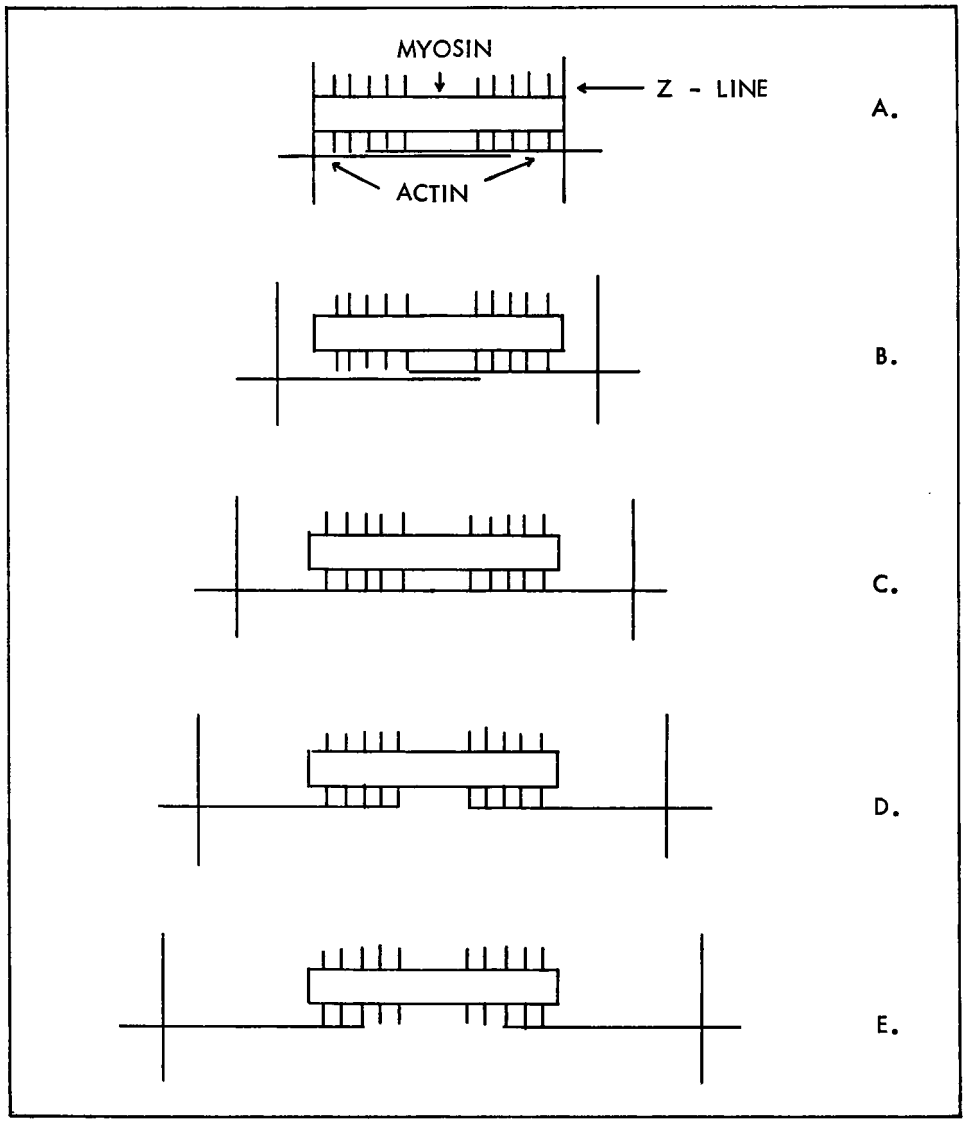


Figure 37. A. Actin filaments from the two half-sarcomeres are overlapped at the center of the A-band.

B. Short lengths of actin filaments adjacent to Z-lines are not overlapped by myosin.

C. No overlap of actin.

D. No abutment of actin filaments, in center of A-band. Twitch height maximum.

E. Longer lengths of actin not overlapped by myosin. Latency relaxation amplitude increasing.

but latency relaxation did not begin to decrease until sarcomere length exceeded 3.1 microns.

The actual configurational changes suggested to be associated with calcium-troponin interaction (10,11) may not depend on resting tension, but their manifestation as recorded latency relaxation probably does. If resting muscle is thought of as an elastic material and is fixed at one end and stretched at the other, the restoring force (resting tension) would be related to displacement from the equilibrium position. The calcium released upon activation may produce changes in the muscle such that a new equilibrium position, at a longer length, is established. This, in effect, would shift the point of equilibrium in the direction of the muscle elongation and reduce the amount of displacement from equilibrium; therefore, the restoring force would be reduced, and latency relaxation would probably increase in amplitude as calcium diffused along the length of the thin filaments covering progressively more sites and moving the equilibrium length closer to the extended length.

Latency relaxation was always smaller than resting tension, therefore part of the resting tension may be exerted by some other element present in muscle. This element is probably the series elastic element described by Hill (32,34). The series elastic element represents extensible tissues in the muscle that must be stretched before the contractile tension can be transmitted to the transducer. Thus, resting tension may influence twitch and latency relaxation amplitudes in the manner described earlier as well as by reducing the compliance introduced by the series elastic element and permitting more rapid transmission of tension changes in the

whole muscle to the recording device.

Twitch appeared to be affected to a greater degree than latency relaxation by exposure to the CDTA solution. If the amplitude losses of both events were due to the outward diffusion of calcium from the muscle, it may be asked why twitch declined at a faster rate than latency relaxation. It was mentioned earlier that the released calcium probably first contacts those segments of thin filaments out of combining range of myosin. All calcium-troponin complexes that may be formed in this area most likely would contribute to latency relaxation amplitude. Activation of sites further along the thin filament and in closer proximity to myosin may be delayed if the availability of calcium within the muscle was lowered. Thus, a larger part of latency relaxation would be observed before being masked by twitch tension. The duration of latency relaxation was observed to increase with time of exposure to the CDTA solution in all muscles which may suggest a delay in onset of twitch.

The rate of rise of twitch tension (dP/dt) was observed to decrease with time of exposure to the CDTA solution and at a slightly more rapid rate than latency relaxation amplitude. If the rate of tension development is governed by the number of tension-exerting actin-myosin links former per unit time, and if these linkages depend on calcium, diminished amounts of free calcium ion may retard their rate of formation and slow tension development. Thin filaments could be drawn into the thick ones once contraction began, and the sites activated early by calcium and contributing to latency relaxation could now be utilized for tension production. However, free calcium probably remains in the sarcoplasm for only a finite period before being

reaccumulated by the sarcoplasmic reticulum; therefore, the slower the rate of tension production, the smaller the tension output would probably be before inactivation begins.

Another factor may be considered to influence the rate of tension development. It has been reported that about four calcium ions will combine with one troponin molecule (11). Possibly actin-myosin interaction, and hence, contraction at a single site, can not occur until a troponin site has its full complement of calcium, but each combined calcium ion may, nevertheless, contribute some configurational change which would enhance latency relaxation amplitude.

Both latency relaxation amplitude and rate of development, dLR/dt,were smaller in the CDTA solution than in Ringer solution. The amplitude of latency relaxation and dLR/dt declined at approximately the same rate during the early part of the experiments; later, dLR/dt decreased more rapidly than amplitude. However, when dLR/dt began to decrease more rapidly, duration of latency relaxation increased with the net result of only slight change in amplitude. The rate of tension development decreased during the time of exposure to CDTA solution and the duration of latency relaxation increased, thereby probably minimizing the effect of a slower rate of latency relaxation development on the measured amplitude of latency relaxation. A smaller value of dP/dt and a longer duration of time for which tension is below the baseline probably reflect a deferred commencement of sufficient contractile tension to overcome the early relaxation. Decreases in rate of development and amplitude of latency relaxation as well as decrease in

the rate of contractile tension development may represent the effects of diminished calcium on the early activation of contraction, while the rapidly decreasing twitch tension amplitude may indicate not only the slower activation, but also a later, more direct, influence of diminished calcium on the contractile mechanism itself.

When the differences in the twitch and latency relaxation amplitudes of paired muscles, one of which was placed into Ringer solution and the other into CDTA solution, were compared, it was observed that the decline of amplitude was greater for twitch than for latency relaxation. Latency relaxation may have displayed less of a difference between the two solutions because the obliterating effects of intact twitch dynamics, which may obscure a large part of the early relaxation in Ringer solution, were less pronounced in CDTA solution.

The importance of twitch dynamics in determining the recorded latency relaxation amplitude was further indicated in the muscles placed in Ringer solution. Latency relaxation amplitude increased in several of these muscles while twitch decreased. It is probably unlikely that the basic mechanism of latency relaxation would become more vigorous with time in an excised muscle, but it is possible that the relative decay of contractile activity was more rapid in these muscles and exposed a larger portion of the existing latency relaxation.

Twitch amplitude in muscles placed into CDTA solution and maintained at a constant external length, despite some loss in resting tension, fell at an accelerated rate although the time course of latency relaxation amplitude was not appreciably different from those muscles maintained at a constant resting tension. The tension

production process probably did not deteriorate any faster in those muscles than in any other muscles placed into CDTA solution, but the amount of twitch tension transmitted to the transducer may have been seriously compromised by some structural change in that part of the muscle comprising the series elastic element, resulting in a "softer", more compliant material. Therefore a larger part of the contractile tension may have been wasted in stretching this material prior to any displacement of the tension transducer. The muscles were always under some tension at rest although resting tension decreased below the initial value; therefore latency relaxation was apparently recorded as usual, but a portion of the twitch amplitude may have been damped out by a more compliant series elastic element. Furthermore, latency relaxation had been observed to change more slowly at low resting tension than did twitch amplitude; thus a loss of resting tension would probably affect twitch amplitude more than latency relaxation amplitude.

Twitch and latency relaxation amplitudes declined when the muscle was stimulated with repetitive, closely spaced stimuli. The decreased amplitudes may reflect the depletion of immediately available energy stores. Sandow (59) reported that the amplitude of latency relaxation was smaller following tetanic stimulation and that the magnitude of the change was related to the duration of the tetanus and the frequency at which the muscle was stimulated. The amplitude loss was reported to be regained after 15 to 30 minutes of rest; however, no such reversal was observed in iodoacetate-poisoned muscles. The muscles in the experiments reported here were not stimulated at a tetanic rate, but the activity was apparently sufficient to produce

early fatigue of the twitch; effects of fatigue would be apparent in latency relaxation also if the phenomenon possesses an energy-requiring component. Experiments with isolated sarcoplasmic reticulum have reportedly indicated that the accumulation of calcium ions by the vesicles requires a source of energy (72) and that a variety of nucleoside triphosphates can supply this requirement (29). If these observations are extended to intact muscle the observed decreases in latency relaxation with repetitive stimulation may be explained as a reflection of depletion of releasable calcium. Support of this explanation is provided by Winegrad's observation that the amount of calcium in the terminal cisternae of the sarcoplasmic reticulum is diminished during and immediately following a tetanus and that a period of several seconds is required for its reaccumulation in this region (79).

Caffeine was unique in that it produced twitches which were larger than the associated latency relaxations. Studies on the effect of caffeine on preparations of isolated sarcoplasmic reticulum have indicated that the drug reduces the uptake of calcium by this structure (75). Furthermore, an increase in the early rate of twitch development has been reported in intact, caffeine-treated muscles (68). Latency relaxation amplitude was observed to decrease in the experiments with caffeine-treated muscles described earlier in this dissertation while twitch increased. The decreased latency relaxation amplitude may be the result of 1.) less calcium in the terminal cisternae available for release and the early, exclusive effect on thin filament sites, and 2.) a more rapid onset of contraction. Latency relaxation amplitude appeared to decrease at an even faster rate in the caffeine solution than it

did in the CDTA solution.

The time courses of twitch and latency relaxation were different at elevated temperatures, especially below 36°C where latency relaxation amplitude increased and twitch amplitude decreased. The increasing latency relaxation may have been due to depressed twitch dynamics at these temperatures; however, at higher temperatures latency relaxation also diminished.

It has been suggested that one of the important factors determining the amplitude of latency relaxation may be the interval between release of calcium and the onset of twitch. It follows that if this interval was reduced little or no latency relaxation would be observed. The triads are located at the level of the Z line in frog sartorius muscles (56). Thus, calcium released from the terminal cisternae first encounters only actin filaments, not overlapped by myosin. Onset of contraction is probably delayed until calcium reaches and activates sites on actin that lie within combining range of myosin.

The anatomical location of triadic structures at the level of the A-I junction in certain muscles probably results in calcium being released at the area of actin-myosin overlap. Distance between the site of calcium release and the area of overlap, as well as the number of troponin sites combining with calcium before tension begins, probably would be reduced in these muscles. One such class of muscles with the triads located at the level of the A-I junction, rather than at the Z line, is the sound-producing muscles of the swim bladder in toadfish (18). Electron micrographs show an extensive, highly developed sarcoplasmic reticulum in these

muscles. Physiologically, these muscles are of interest because they exhibit contraction times that rank with the fastest muscles known (70).

More germane to the question of the underlying mechanism of latency relaxation is the observation that no equivalent event has been observed in the toadfish swim bladder muscles. Skoglund (70) called attention to its absence, but suggested the possibility that his recording method lacked sensitivity; this point merits evaluation. Skoglund employed the RCA 5734 mechano-electronic transducer tube (RCA, Harrison, N. J.) firmly applied to the belly of the bladder muscles; he also measured the pressure in the bladder with a high fidelity capacitance pressure transducer. Latency relaxation was not seen in the 5734 myograms or the bladder-pressure recordings. The 5734 has been used to record latency relaxation in frog muscle (71) as has the method of laying a recording stylus on the surface of the muscle (63). The 5734 is a sensitive, rapidly responding transducer with a rise time of 0.04 msec. Skoglund stated that the total time for contraction of toadfish bladder muscles is about 10 times shorter than in the frog sartorius muscle. With a typical latency relaxation rise time of 1 msec for frog muscle, the toadfish bladder muscle may be expected to have a latency relaxation rise time of about 0.1 msec. The 5734 transducer is capable of responding this rapidly.

The latent period and the early part of the twitch in excised toadfish swim bladder muscles were recorded in this laboratory using the piezoelectric crystal myograph. Figure 38 is a record of six sequential twitches in this muscle made at increasing resting tension. No deflection such as that seen preceding the twitch in

ONSET OF TWITCH IN TOAD FISH

SWIM BLADDER MUSCLE

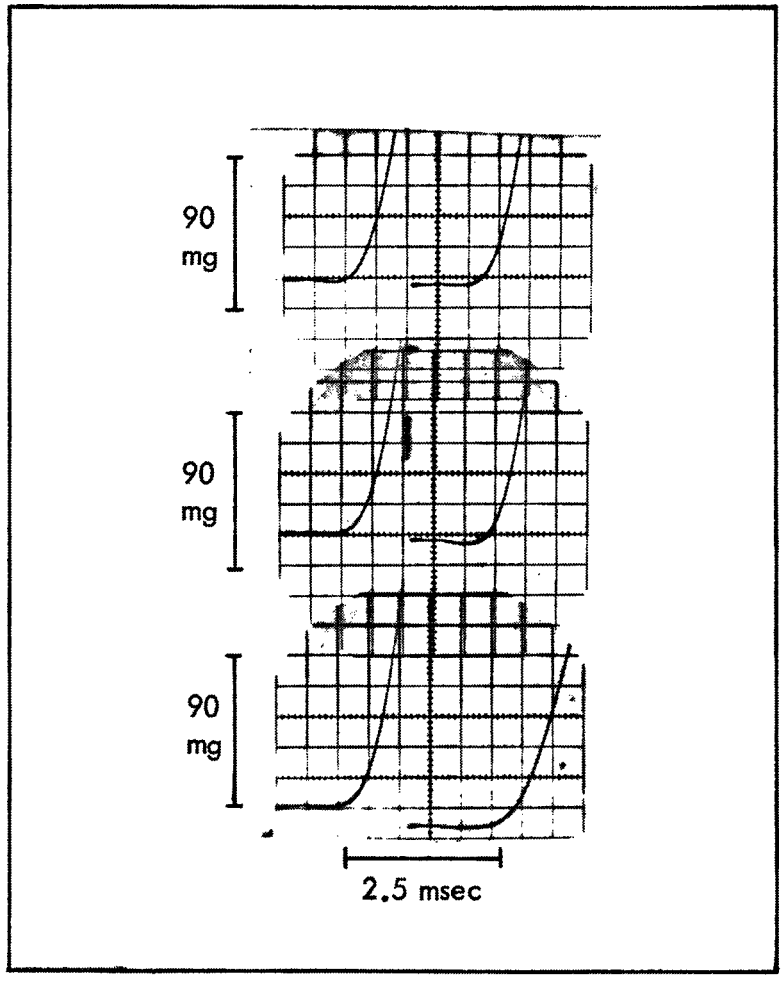


Figure 38. These records were made from an excised muscle attached to the myographs in a manner similar to the attachment of the frog muscles.

frog sartorius muscle is apparent in these records.

The discussion has centered primarily on an explanation of the mechanism underlying latency relaxation and the effective interaction of its suggested mechanism and twitch development in determining the observed amplitude of the early relaxation. Mention should be made of the probable relationship of the latency relaxation process to the contraction itself. Twitch amplitude may be related to latency relaxation in the sense, that if latency relaxation is a reflection of calcium-troponin combination, it most likely must occur before any active tension can be exerted. The potential twitch amplitude may, therefore, depend on the amount of calcium released and inhibition to actin-myosin interaction removed. The actual twitch amplitude probably will be determined also by resting tension, area of actin-myosin overlap and sarcomere length, the period of time calcium is free in the sarcoplasm, and the number of actin-myosin linkages formed. If calcium reaccumulation begins before an appreciable number of tension-generating links are made, twitch amplitude may be small despite the presence of many actin sites activated by calcium, but not utilized for contraction. While latency relaxation amplitude may be a function of the number of calcium-troponin complexes formed along the length of the actin filaments and the delay in onset of positive tension, twitch amplitude may be a function of the number of activated sites that come into combining range of myosin cross bridges and the length of time ionized calcium is present in the interfilament spaces.

Reiterating the main points of this section, the observed time course of

latency relaxation amplitude as compared to twitch was discussed in terms of the relaxation as a possible mechanical manifestation of configurational changes occurring at sites along the actin filaments. It was also suggested that the recorded amplitude of latency relaxation may be determined, at least in part, by the onset of the twitch itself.

SUMMARY

The results of this investigation showed that latency relaxation amplitude generally declined more slowly than twitch amplitude. It was suggested that part of the slower latency relaxation decline may be explained in terms of a more rapidly failing twitch exposing more of the existing relaxation. A larger part of latency relaxation was probably exposed when the onset of twitch tension was delayed. This was observed in the resting tension experiments where latency relaxation amplitude continued to increase even after twitch had reached and exceeded its maximum value.

It was suggested that latency relaxation was possibly the physiological representation of calcium binding to sites distributed along the thin muscle filaments. If such a calcium dependency is valid, latency relaxation should be adversely affected by depletion of available calcium. A steep efflux gradient for intracellular calcium was provided by placing muscles into amphibian Ringer solution to which no calcium had been added, but which contained a chelating agent to bind any calcium diffusing from the muscle and prevent its re-entry into the muscle. Latency relaxation amplitude decreased when muscles were placed into this medium, but it did not decrease as rapidly as twitch amplitude. This may suggest that when the calcium stores of a muscle are reduced the latency relaxation effect of the remaining ions is preferentially exerted over the twitch effect due to the closer proximity of latency relaxation sites to the point of calcium release.

Latency relaxation precedes the onset of contraction and may represent some process necessary for the initiation of contractile activity. However, the amplitude of latency relaxation does not appear to predict the contractile force of the ensuing twitch, partly because the recorded latency relaxation may not represent the basic phenomenon by itself, but rather the net tension resulting from two oppositely directed processes, displaced slightly in time, and of different magnitudes. Furthermore, twitch tension may depend on many factors other than the amount of calcium released. Nevertheless, if latency relaxation is a reflection of calcium binding and the removal of inhibition to actin-myosin interaction, its physiological significance may be that, when all other parameters influencing twitch tension are optimum, it is the limiting factor for muscle contraction.

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