THE RELATIONSHIP OF DEVELOPED TENSION TO SOME INDICES OF ISOMETRIC RELAXATION

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ABSTRACT

The relationship of indices of isometric relaxation to peak tension was studied in the kitten papillary muscle preparation.

A linear relationship between peak tension and maximum rate of relaxation (MRR) was observed on examining the interval/strength effect. This relationship was independent of muscle length and thickness.

Increases in calcium concentration produced an absolute increase in MRR. However, when MRR was related to the strength of contraction, the increase in MRR was less than would be expected although the linear relationship to peak tension remained. An additional, frequency-dependent depression of MRR was found at calcium concentrations of 5.0 mM or more.

On the other hand, reductions in calcium concentration produced ambiguous results that also appeared to indicate some retardation of relaxation.

During recovery from short periods of drive, depression of MRR relative to peak tension, and increases in TR/2 were observed. The extent and duration of depression from the interval/strength line was found to be dependent on external calcium concentration in the range from 1.25 to 5.0 mM.

Hypoxia produced an increase of MRR relative to peak tension as contractility declined. This occurred in 2.5 and 5.0 mM calcium, but did not occur in 1.25 mM calcium. Reoxygenation produced depression of MRR relative to peak tension, and increase in TR/2 within 2 minutes. No difference in the extent of depression could be found between 2.5 and 5.0 mM

calcium (in different muscles), but the extent was less in 1.25 mM.

Adrenaline 5 x 10^{-8} g/ml produced a slowing of MRR relative to peak tension at all frequencies, similar to the effects of 5.0 mM calcium. A dose/response determination revealed that adrenaline appeared to increase MRR relative to peak tension at higher doses.

It is suggested that allowance must be made for inotropic effects in examining changes in relaxation. Examination of the relationship of peak tension to maximum rate of relaxation may allow explanation of the effects of interventions in terms of calcium handling by the cell.

The effects of adrenaline, of drive, and of hypoxia might also be interpreted in terms of effects on a particular source of energy that may be involved in calcium sequestration, i.e. glycolytic ATP.

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SECTION I

INTRODUCTION

Muscle behaviour can be described in terms of three mechanical elements : a PARALLEL ELASTIC ELEMENT (PE) which bears tension when the muscle is at rest, a CONTRACTILE ELEMENT (CE) which is freely extensible at rest and is responsible for force development on activation, and an ELASTIC ELEMENT in SERIES (SE) with the CE, which also bears no resting tension. This is the Maxwell model. More complex models have been proposed, but Jewell and Blinks (1, 2) suggest that the simplest model that simulates properties of all cardiac muscle preparations reasonably well is the Maxwell model.

The presence of VISCOUS ELEMENTS have been proposed to account for such properties as stress relaxation, creep, and reductions in resting tension following strong contractions at high frequency (3, 4). They have also been invoked to account for the final phase of isometric relaxation (5). This consists of a slow decline to baseline tension or tensions slightly below baseline, with subsequent slow rise to baseline (creep). This phase will affect the total duration of relaxation.

When the CE is activated, it must progressively cancel the extensibility of the SE in order to develop tension. As elastic elements are considered to slacken on shortening, tension development progressively removes tension from the PE (4).

In the ISOMETRIC preparation muscle shortening is prevented. The CE is considered to shorten and develop tension against a lengthening SE, with PE and overall muscle length remaining unchanged (6), until the stiffness of the SE, increasing in direct proportion to the tension exerted, balances the maximum force exerted by the CE.

Tension decline will occur as the resultant of the opposing effects of SE stiffness tending to pull the CE to its resting length and the resistance of a progressively declining level of activation. Stiffness of the SE has been shown to be directly proportional to the level of tension whether in the contraction or relaxation phase of muscle activity (7, 8). The properties of the SE have been shown not to change with changes in frequency (9), length (10), or up to 60 minutes of hypoxia (11). Only large reductions in temperature (12), or changes in osmolarity of 100 milliosmoles or greater (13) have been shown to affect SE properties. In the absence of the last two conditions, the contribution of SE to relaxation should be a constant direct function of tension. Any changes in the relaxation phase would therefore be due to changes in CE activation.

Controlled stretch applied during isometric contraction to compensate for series elasticity (14), and measurements of the rate of shortening following quick release at various points along the isometric contraction curve (7, 15) indicate that the isometric event may be considered to parallel the course of activation quite well, when corrected for internal shortening (4). For this reason, and because Brady (4) has stated that such techniques as quick stretch (2) and quick releases imposed on isotonic preparations to follow the course of active state have been shown to disrupt the further course of it, the isometric preparation is very useful. The isometric event <u>per se</u> parallels activation with delay, as the SE compliance prevents CE shortening from developing at any instant the tension it could develop against a noncompliant element.

Peak activation measured by quick release is so short that, due

to this delay, the CE is considered not to develop the maximum tension it is capable of.

As the resting tension and consequently the inactivated length of cardiac muscle is increased, peak isometric tension (PT) also increases until a maximum is reached. Further increases cause no change, and then produce a reduction in PT. This is the Length/Tension or Frank/Starling relationship (16).

The muscle behaves as if more tension-producing sites confront one another as muscle length increases on the rising phase of this relationship. Cardiac muscle normally operates on the rising phase, and at the plateau it exerts considerable resting tension (17). As stretch affects the PT similarly whether applied before stimulation or during early tension rise, length at the instant of maximum activation, not that at which it is stimulated, is thought to be the determining factor (1).

Ultrastructural studies of cardiac muscle have shown it to consist of the same basic unit, the SARCOMERE, as skeletal muscle. Cardiac muscle differs from skeletal muscle in proprotion and arrangement of various components, and cardiac muscle differs in different species (17, 36). The following statements apply to cat papillary muscles (19, 36).

These are composed of two types of filament.

Thick MYOSIN filaments (150 Å diameter) consist of approximately 400 myosin molecules. Light meromyosin tails constitute the backbone of the filament, and heavy meromyosin heads with ATPase activity project in a spiral pattern at 140 Å intervals along the length of the filament. Every fourth ATPase site lies in the same plane of the myosin cylinder.

Parallel to and surrounding each myosin filament are 6 ACTIN filaments 100 Å in diameter. Each is equidistant from 3 myosin filaments. Actin filaments are connected at both ends to Z-lines, structures at 90° to the plane of the filament, and consist of 2 strands with a gap in the middle of the sarcomere.

The myosin filament is thickened in the area of the gap.

It is hypothesized that ATPase-activating sites occur on actin filaments in positions corresponding to myosin ATPase sites confronting them. Stretching the muscle causes more sites to confront one another, and this is the basis of the Frank/Starling effect.

A complex of two more proteins, TROPOMYOSIN and TROPONIN, modifies the activity of the actin sites.

The outer membrane or SARCOLEMMA of the cardiac cell invaginates to form T-tubules which encircle the Z-lines, and send small projections longitudinally. These bring extracellular fluid in close proximity to the myofibril.

The SARCOPLASMIC RETICULUM forms a second network around the myofibril, sealed off from the extracellular space and continuous throughout the cardiac muscle cell.

Special branches, the subsarcolemmal cisternae, form close contacts with areas of the sarcolemma and T-tubules.

The active state is triggered by electrical depolarization of the cell membrane. This is considered to be carried to the interior by the T-tubules, and to be conducted directly to the longitudinal reticulum (18).

The cardiac action potential consists of 5 phases, from 0 through 4. The most important from the viewpoint of this thesis is PHASE 2, a plateau of slow decline from a level of considerable intracellular depolarization. This coincides with slow calcium influx (18, 20).

It has been generally accepted that the coupling factor between excitation and contraction is movement of calcium across the sarcolemma, at the T-tubules and possibly elsewhere.

Concentration of free calcium around the myofibrils is less than 1×10^{-7} M in the unactivated state (17). Much more than this is in the cell, but in a bound or compartmentalized form (18).

Changes in Phase 2 calcium influx have been correlated with changes in contractility, but with delay (21, 22). Its effects are thought to be indirect, contributing to an intracellular pool on or near the sarcolemma (23). This pool is thought to be triggered to release calcium into the myofibrils by sodium influx (21, 24), calcium influx (25, 26), by the conductance change itself (27), or by other factors.

The source of activating calcium has been suggested to be the sarcolemma and especially the T-tubules (21, 18) or cisternae of the sarcoplasmic reticulum (2, 18).

Tropomyosin directly inhibits the ATPase-activiting sites on actin when calcium concentration in the sarcomere is low. When this concentration increases, calcium ions bind to sites on troponin which in turn causes conformational or other changes in tropomyosin that disinhibit the actin sites (17). Two types of calcium binding sites are believed to occur on troponin, one of high affinity and the other of low (28). The

high affinity site is believed to control activation (17).

ATP splitting by the myosin ATPase sites results in formation of crossbridges from myosin that swing from site to site on actin, pulling opposing actin strands toward one another. Adjacent Z-lines are pulled closer together, without change in the length of the myosin filament.

Active tension developed is considered to reflect the number of strong actin myosin bonds formed. Shortening velocity is considered to reflect the rate of formation and breakdown thereof (16).

INTENSITY OF ACTIVE STATE, the rate of crossbridge formation, will be determined by :

a. The intrinsic activity of myosin ATPase. Cardiac and red skeletal muscle have lower activity than white skeletal muscle. Maximum shortening velocity is therefore reduced. Lower rate of energy utilization, however, allows prolonged contractile activity without periods of rest and recovery (17).

b. How rapidly the troponin sites are occupied by calcium, in turn dependent on :

I. $(Ca^{++})_i$, as affected by rate of delivery from release sites.

In the ISOTONIC event, shortening velocity at 0 load, V max, is theoretically independent of the number of interacting sites, although inertia in any system that has mass renders this illogical (2).

In practice it cannot be measured directly and is estimated by extrapolation from a force/velocity curve plotted with several loads ranging from that which just prevents shortening, Po, to the smallest

load practical. This curve itself displays the inverse relationship of load, or developed tension, to shortening velocity, and energy release (17).

II. Number of sites interacting per unit time.

In the ISOMETRIC event, maximum rate of tension development $(dT/dt)_{max}$, is dependent on the number of sites interacting per unit time, because the CE is shortening against a resistance consisting of resting tension plus the increasing stiffness of the SE.

MAXIMUM INTENSITY OF ACTIVE STATE, the degree of crossbridge formation when CE is neither shortening nor lengthening, will be determined by whichever is the limiting factor of :

a. Number of actin-myosin sites available for interaction, affected by the position on the Length/Tension curve.

b. Instantaneous $(Ca^{++})_i$: a concentration of 1×10^{-5} M calcium is considered to saturate the troponin sites (17, 29). Calcium concentration around the myofibril is determined by the interaction between :

I. Quantities and rates of delivery from release sites and

II. Reuptake or sequestration of calcium before peak active state (4).

In the ISOMETRIC event, PEAK TENSION is a measure of maximum intensity of active state.

TIME TO MAXIMUM INTENSITY OF ACTIVE STATE is measured as TIME TO PEAK TENSION.

DURATION OF ACTIVITY is measured as TOTAL DURATION OF CONTRACTION, or TIME TO 90% TENSION DECLINE, as the point of return to baseline is difficult to determine (30).

Quick release experiments indicate active state to be terminated before the end of the isometric event (15), and the final phase is probably due to viscous elements (6). Therefore total duration of contraction is probably not a good index of duration of active state.

Time to peak tension has been used by some authors as an index of duration of active state (30). Declining levels of active state persist throughout most of the relaxation phase (15).

Sonnenblick (15) used time to peak tension (TTP) to measure duration of maximum intensity of active state. He considered it to persist during the time period while 95% of shortening velocity as measured by quick release, was maintained, that is, even during the first 5% decline from peak active state.

TTP is a measure not only of the time during which active state is increasing, but also of the point at which deactivation processes, i.e. relaxation, begins (31).

Intervention which produce changes in maximum rate of contraction and peak tension, other than changes in muscle length (the Frank/Starling effect) are said to change muscle CONTRACTILITY. Contractility may be described as the resultant of the interaction of four variables : FORCE, VELOCITY, LENGTH, and TIME (2). At a constant length, the hyperbolic force/velocity curve (see above) is considered to indicate a given level of contractility.

Changes in myocardial contractility have been shown to be paralleled by changes in shortening velocity (V_{max}), in turn associated with the rate at which contractile proteins hydrolyse ATP. The rate

at which myosin ATPase actually hydrolyses ATP is unlikely to be variable in a muscle. Therefore, physiologically important modifications of myocardial contractility are probably due to changes in the extent to which myosin is activated by actin (17), which in turn appears to be governed by $(Ca^{++})_i$ (17, 29).

Increased $(Ca^{++})_o$ is correlated with increases in peak tension and maximum rate of contraction. Increasing $(Ca^{++})_o$ above 4.0 mM does not result in directly proportional increases in $(Ca^{++})_i$, as influx appears to approach saturation at higher concentrations (21).

Increasing frequency of contraction at a constant $(Ca^{++})_o$ causes increases in maximum rate of contraction and peak tension, correlated with increased calcium influx with each action potential (18).

Decline of active state occurs due to sequestration of calcium (2, 17, 29). Less is known about this process than about the contractile portion of the event.

Calcium has been suggested to be sequestered by sarcoplasmic reticulum (21), mitochondria (32, 33), or by reuptake by the sites from which it was released (2).

Studies of calcium sequestration are usually done in isolated microsomal preparations containing membrane of the sarcoplasmic reticulum, and most probably other parts of the cell. Disadvantages of such preparations are :

1. Careful adjustment of concentrations of ions, etc. to yield optimal rates of sequestration may yield conditions, and therefore results, far removed from those <u>in vivo</u>.

2. Such preparations cannot unequivocably implicate a single membrane type as responsible for calcium sequestration.

3. Cell fractionation may disrupt *in vivo* properties, causing such effects as shifts of calcium from one site to another that has been suggested to be responsible for high calcium concentrations in mitochondrial cell fractions (34).

4. Such preparations may give inaccurate values for quantities of calcium handled, as other processes that could contribute *in vivo*, such as reuptake by release sites, are not present.

5. Time constants for calcium sequestration may be inaccurate as possible competition for calcium between release sites, troponin binding sites, and sequestration sites, is not occurring.

The following statements should be considered in the light of these restrictions.

Studies on isolated troponin and on cardiac microsome fractions have shown calcium affinities of the same order of magnitude in both (17, 35).

Uptake of calcium by calcium-sequestering sites is proportional to load delivered. The total amount they can take up is considered to be 40x to 50x the quantity released during activation (21).

It has been stated that, since the course of relaxation is dependent on active uptake and removal of activating calcium, study of the course of relaxation provides a mechanical correlate of this process (37).

Various interventions have been reported in the literature to speed or to prolong relaxation.

Those interventions reported to speed relaxation include :

1. INCREASE IN TEMPERATURE - increase in temperature from 24^{o} to 34^{o} C in cat papillary muscles stimulated at 12 per minute increased maximum rate of contraction and decreased peak tension (PT), time to peak tension (TTP), and time from the peak tension point to 50% decline in peak tension (TR/2) (37).

2. CATECHOLAMINES -

a. Adrenaline 2×10^{-6} g/ml in guinea pig papillary muscles at 36^{o} C stimulated at 180 per minute was reported to decrease duration of the event mainly by increasing maximum rate of relaxation (MRR) (38).

b. Reiter and Schober (39) used adrenaline 7.3×10^{-9} g/ml in guinea pig papillary muscle stimulated at 60/min. At calcium concentrations below 0.4 mM this concentration slowed MRR. At calcium concentrations of 0.8 to 12.8 mM, it speeded MRR.

c. Reiter (40) studied the positive inotropic effects of noradrenaline at concentrations of 1.7×10^{-8} g/ml to 1.7×10^{-3} g/ml in guinea pig papillary muscles at 35^{o} C stimulated at 60 per minute.

Increase in mean velocity of relaxation by maximally effective doses was 60% greater than increase in mean velocity of contraction. Time to peak tension was reduced by 7%, relaxation time reduced by 30% at maximally effective concentration.

Enhancement of relaxation was suggested to limit the positive inotropic effect and flatten the Dose/Response curve.

d. Morad (41) and Morad and Rolett (42) have shown that adrenaline 2×10^{-6} g/ml partially reduces Potassium Chloride contractures

in cat papillary muscles at 25° C.

In reserpinised animals or in the presence of propranolol the level of contracture in these tissues is over 100% of peak isometric tension. Without prior reserpinisation or propranolol, contracture is never greater than 40% of peak isometric tension.

Morad and Rolett (42) suggest that speeding of relaxation by endogenous catecholamines may be as important for heart function in high performance states such as high frequency, as the positive inotropic effects.

Adrenaline has been shown to prolong the plateau phase of the cardiac action potential associated with slow calcium influx and its positive inotropic effect (38).

In high calcium concentrations, where peak tension cannot be increased, and in electrophysiological experiments where the plateau phase was terminated prematurely, the speeding of relaxation by adrenaline still occurred (42, 43).

It was concluded that the positive inotropic effects and speeding of relaxation by adrenaline could be separated (42).

A number of drugs can also be used to show this dichotomy.

CAFFEINE 4×10^{-6} M was used by Blinks et al. (22) in cat papillary muscle at 32.5° C stimulated at 12/minute. It was found to prevent active state abbreviation by concentrations of isoproterenol from 2.1×10^{-10} g/ml to 2.1×10^{-5} g/ml, without preventing the increase in maximum rate of contraction and peak tension produced by isoproterenol.

At higher doses of isoproterenol, relaxation was progressively

delayed.

TYRAMINE 3×10^{-3} M, a concentration higher than that used for its indirect sympathomimetic effects, has been shown to exert a direct post-synaptic effect resembling that of stimulated noradrenaline release, except that relaxation is slowed (44).

In guinea pig papillary muscles at 35^{o} C stimulated at 60 per minute, tyramine 3×10^{-3} M enhances the effects of noradrenaline 1.7×10^{-4} g/ml on mean velocity of contraction and DECREASES its effect on relaxation velocity, increasing peak tension by 30% (45).

Interventions reported to slow relaxation include :

1. HYPOXIA

Periods of more than 2 minutes of hypoxia induced with 95% Nitrogen - 5% CO_2 in cat papillary muscles stimulated at 12 per minute at 27^{o} to 34^{o} C caused depression of all parameters of the isometric beat.

Within one to two minutes of reoxygenation, maximum rate of contraction and peak tension begin to recover to prehypoxic levels, and time to peak tension, time to 50% decline in tension (11, 46) and relaxation time (47) are prolonged significantly beyond prehypoxic levels.

Bing et al. (46) further noted that rapid drive (1200 per minute) during recovery from 20 minutes of hypoxia in dog trabeculae carnae at 28^{o} C caused a secondary rise in tension from the previous peak, with no evidence of relaxation.

The same procedure during induction of hypoxia potentiated the peak tension of subsequent beats, with a long refractory period and complete relaxation between beats.

2. REDUCTION IN TEMPERATURE

a. Blinks and Koch-Weser (48) cited a number of papers that reported the prolonging effects of cooling on both phases of the contractile event. The duration of relaxation was increased more than that of shortening or development of tension.

b. Reduction in temperature from 30^{o} to 20^{o} C in rabbit papillary muscles stimulated at 12 per minute was reported to increase peak tension, time to peak tension and maximum rate of contraction while markedly reducing maximum rate of relaxation (49).

3. INCREASED CALCIUM CONCENTRATION

a. Increasing $(Ca^{++})_{o}$ from 1.8 to 5.4 mM in guinea pig papillary muscles at 36^{o} C stimulated at 180 per minute caused no increase in peak tension, but caused relaxation to begin earlier (i.e. reduced time to peak tension), and reduced maximum rate of relaxation (38).

b. Parmley and Sonnenblick (37) reported that increasing $(Ca^{++})_{o}$ from 2.5 to 7.5 mM in cat papillary muscles at 29^{o} C stimulated at 12 to 36 per minute increased peak tension and maximum rate of contraction, reduced time to peak tension and increased time to 50% decline in tension (TR/2).

c. Increasing $(Ca^{++})_{o}$ from 2.5 to 7.5 mM in cat papillary muscles at 29^{o} C stimulated at 12 per minute was reported by Kline and Buckley (50) to increase peak tension and maximum rate of contraction, reduce time to peak tension and prolong the duration of the events, i.e. prolong relaxation time.

4. DRIVE

a. Blinks and Koch-Weser (51) cited several papers that report incomplete relaxation due to drive at high frequency, that persisted for some time after cessation of rapid drive. It was suggested to reflect hypoxia of the central fibers.

b. Bing et al. (46) (see above) have shown drive to produce a further degree of inhibition of relaxation when superimposed on pre-existing hypoxia.

5. DRUGS

Many drugs have been reported to influence relaxation in cardiac muscle. The following is by no means a complete list.

a. 2, 4-DICHLORPHENOXYACETATE (2, 4-D) in a concentration of 2.5 mM in guinea pig papillary muscles stimulated at 40 per minute caused little change in peak tension, reduced time to peak tension, and prolonged relaxation time (52).

b. RYANODINE - Naylor et al. (53) used 5×10^{-6} M to 1×10^{-5} M in dog papillary muscles at 37^{o} C stimulated at 36 per minute. Within 10 minutes of addition of ryanodine, peak tension had declined by 36%, time to peak tension had increased by 45%, and time to 50% decline in tension had increased by 73.9%.

Effects on all parameters were significantly greater at a frequency of 12 per minute. It was therefore concluded that negative inotropic effects of ryanodine are more marked at lower contraction frequencies.

c. TYRAMINE - concentrations greater than 1×10^{-3} M, i.e.

above those which act by stimulating release of catecholamines, were used in guinea pig papillary muscles at 35° C stimulated at 60 per minute. Such concentrations act in reserpinised animals, and in the presence of propranolol.

Peak tension is increased, time to peak tension is reduced, relaxation time is prolonged and maximum rate of relaxation reduced (44).

d. DRUGS PRODUCING CONTRACTURE - many drugs produce contracture in high concentrations. Cardiac glycosides (4, 54), nicotine (55), and the drug to be discussed, oligomycin (56), may be mentioned as examples.

OLIGOMYCIN 1 x 10^{-6} g/ml was used in perfused rat hearts at 38° C stimulated at 120 per minute. A gradual increase in diastolic tension began to occur after 20 to 40 minutes. Relaxation between beats gradually diminished until tension in diastole approached that in systole (56).

e. DIHYDROOUABAIN - Reiter (40) used concentrations greater than 2 x 10^{-5} M in guinea pig papillary muscles at 35° C stimulated at 60 per minute.

Maximally effective concentrations caused a 34% greater increase in mean rate of contraction than in mean rate of relaxation. Time to peak tension was decreased 25%, relaxation time increased by 12%.

The maximum inotropic effect was 20% greater than that of noradrenaline in the same preparations. The difference was suggested to be due to at least in part to the opposite effects of the two drugs on relaxation.

f. CAFFEINE - Blinks et al. (22) used 2 x 10^{-6} M to

6×10^{-5} M in kitten papillary muscles at 32.5^{o} C stimulated at 20 per minute.

Lower concentrations, 2×10^{-6} to 6×10^{-6} M, increased peak tension slightly without prolonging the event.

Concentrations of 2×10^{-5} M to 6×10^{-5} M increased peak tension, maximum rate of contraction, time to peak tension, and relaxation time.

Procaine in concentrations of 0.6×10^{-3} M produces increased peak tension accompanied by decrease in time to peak tension. As this effect was prevented by reserpinisation, and blocked by propranolol, it was suggested to be due to release of endogenous noradrenaline.

Procaine 1×10^{-6} M prevents the prolongation of time to peak tension and total duration of contraction without affecting the increase in peak tension and maximum rate of contraction.

Blinks et al. (22) suggest that caffeine has 2 independent actions on contractility. It stimulates release of calcium from release sites to increase maximum contraction rate. This effect is procaine-insensitive.

At higher concentration it impairs calcium sequestration to prolong active state. This effect is procaine sensitive.

The suggestion of 2 independent actions by methylxanthines resembles the case for catecholamines (see above). These two classes of compounds have similar effects on the contractile phase, but opposite effects on the relaxation phase. Each has been shown under specific conditions to inhibit the effects of the other on the relaxation phase, while not affecting the positive inotropic effects. This shall be dealt with further in the Discussion.

STATEMENT OF THE PROBLEM

Previous workers have used several indices of relaxation, usually without experimental verification of any kind. Those indices include time to 50% decline in peak tension (TR/2), time to 90% decline in peak tension (RT), maximum rate of relaxation, $(-dT/dt)_{max}$ (MRR), and average rate of relaxation (ARR).

Different workers use different indices, and it is sometimes difficult to determine how changes in one index in a given experiment relate to changes in a different index in another experiment.

Usually no attempt is made to relate changes in relaxation to changes in muscle strength.

For these reasons, it seemed important to seek answers to the following questions :

1. Is there a regular relationship between muscle strength in terms of peak tension, and any of the measures of relaxation used? Particular reference was made to maximum rate of relaxation and time to 50% tension decline.

2. Do inotropic interventions reported to influence relaxation exert a consistent effect on such a relationship?

3. Can changes in characteristics of relaxation be related to calcium handling by the muscle cell?

SECTION II

METHODS

A. APPARATUS

Isometric tension was measured with a number 903 G10B Statham strain guage. The displacement range was ± 0.015 inches for forces of ± 8.52 grams. Approximate output was ± 20 mV full scale, and maximum nonlinearity and hysteresis was less than 1% of full scale.

The output of the transducer was amplified by a high input impedance 2 stage differential operational amplifier (See Appendix, Diagram 1).

This had a frequency response of 5 KHz and a maximum sensitivity when connected to a No. 903 strain guage, of 10 V out for 0.2 g (0.47 mV) in. This unit contained a bucking voltage adjustment to allow cancellation of resting tension from the baseline tension. The amplified signal was available for recording but was also fed to a differentiator (See Appendix, Diagram 1) with time constant of 40 m sec. This put out the first derivative of the isometric beat.

The isometric event and first derivative were recorded on adjacent channels of a Tandberg-Honeywell Series 100 four channel tape recorder. This had a pulse response, i.e. rise and fall time at 3-3/4 inches per second, of 300 microseconds. Output voltage was ± 5 V full deviation, and linearity in terms of departure from the best straight line through 0, was 0.2%. Drift after 5 minutes warmup was ± 0.01% per Centigrade degree.

The baseline of the channel on which the isometric event was recorded was set far into the negative range with an input offset adjustment on the tape recorder. This allowed recording of the event on the full width

of that track on the magnetic tape, and thus optimum accuracy in subsequent analysis.

The events were observed on a Type 502 Tektronix Oscilloscope after passage through the recorder. Stimuli to the muscle and event-marking trigger signals to the recorder were supplied by a Tektronix type 162 waveform generator.

Output from the type 162 waveform generator to a type 161 pulse generator provided an event-marking trigger signal which passed without delay to channel 3 of the recorder.

Square wave pulses from the type 162 waveform generator were delayed a constant 30 m sec. at all frequences by passage through a Grass SD5 stimulator. This 30 m sec. of resting conditions at the beginning of each event provided a good baseline during data acquisition and analysis (See below).

Stimuli of 5 m sec. duration and amplitude 10% above threshold as determined during equilibration were delivered to the muscle via bipolar punctate platinum electrodes of surface area less than 1 square mm with centers 0.8 mm apart.

B. PREPARATION

The kitten papillary muscle was chosen because its structure (19) and properties (1, 30) have been extensively investigated. Many others have used it and our results could be compared to theirs.

Kittens of 0.7 to 1.4 kg of either sex were killed by a blow to

the neck. The hearts were quickly removed and placed in cold Krebs solution. Papillary muscles 1 mm or less in thickness were removed from the right ventricle.

The lower, nontendinous end was held firmly in a springloaded plastic clip about 1 mm above the junction of the muscle with the ventricular wall, to exclude most Purkinje fibers from the preparation. The upper, muscle-tendon junction was attached with No. 50 silk thread to the strain gauge.

The muscle was immersed in a 50 ml modified Blinks muscle bath (Figure 1) containing Krebs-Henseleit solution of the following composition (mM)-Na^{+} 145.4, K^{+} 4.7, Mg^{++} 1.18, Ca^{++} 2.50, Cl^{-} 125.2, HCO_3^{-} 26.2, $H_2PO_4^{-}$ 1.18, $SO_4^{=}$ 1.18, Glucose 11.20. This was maintained at 31^{o} C by a temperature-controlled waterbath.

Preliminary experiments showed oxygenation from the sidearm alone to be indadequate, in terms of rapid mixing of drugs and dyes added to the sidearm. A second source of oxygenation was therefore added, to deliver gas directly to the bottom of the muscle chamber.

Throughout the experiment the muscle was gassed with 95% Oxygen - 5% CO_2.95% Nitrogen - 5% CO_2 was used for short periods to cause hypoxia.

The muscle was placed in the bath and connected to the transducer. With thread slack, calibration signals for 1 g and 3 g deflections from baseline were recorded with appropriate trigger signals by adding weights to the transducer. 10 V per sec. and 20 V per sec. slope calibrations, delivered by a ramp-generating device, were also recorded with trigger signals. These calibrations were the one ones required for computer-

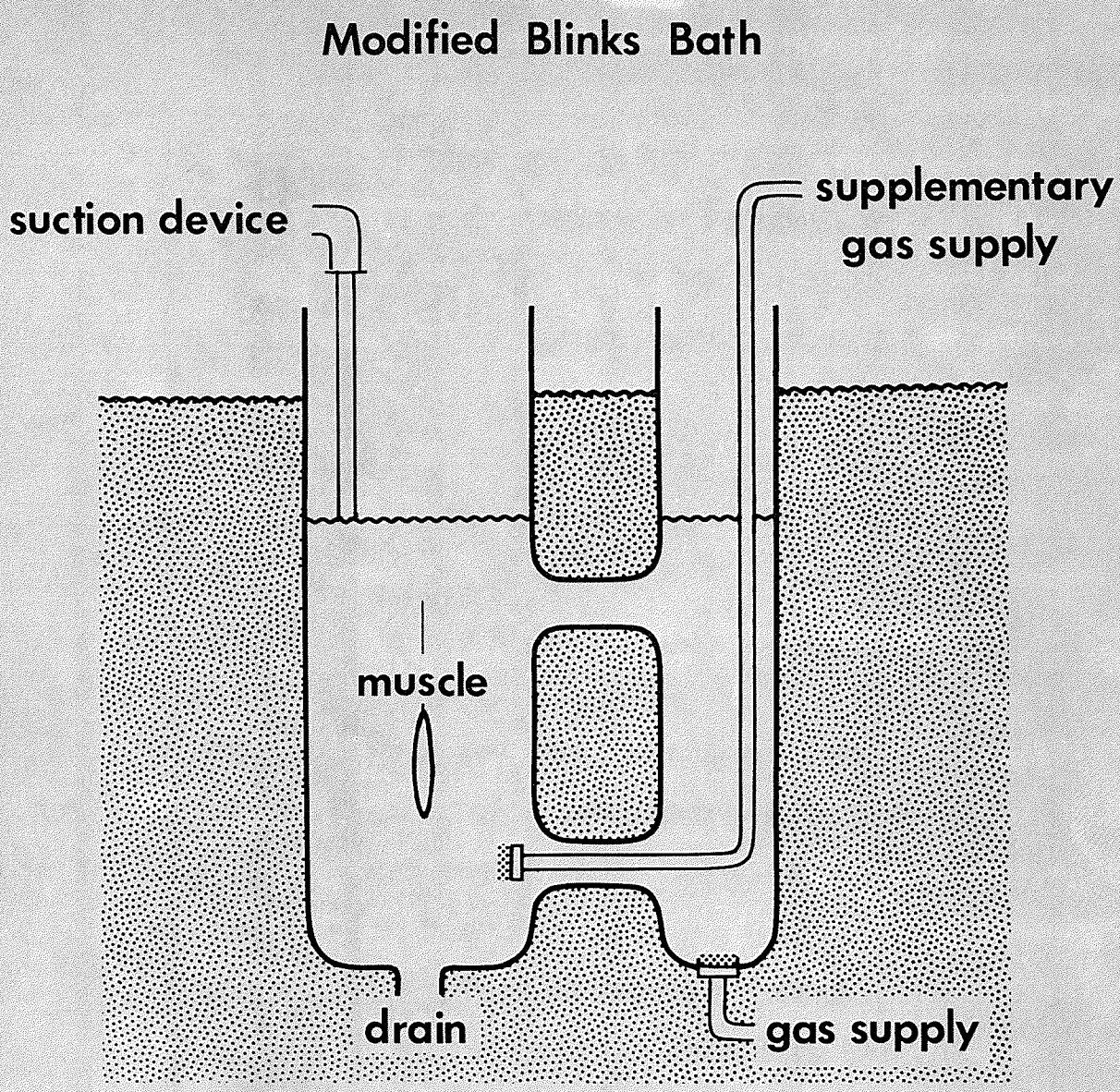


Figure 1

Blinks tissue bath modified to provide additional aeration. For explanation, see text.

assisted analysis of the results.

The bucking voltage necessary to cancel 1 g of resting tension was determined and muscle tension was adjusted to 0.7 to 1 Gm. Stimulation was initiated at a frequency of 30 per minute. Threshold tended to decline to 1/2 to 1/3 of the initial value during the first ten minutes. Stimulation strength was therefore periodically adjusted to maintain it at 10% above threshold to prevent local release of catecholamines (48). Despite the use of punctate electrodes, force/frequency relationships recorded at excessive stimulation voltages differed significantly from those recorded at 10% above threshold.

The length/tension relationship over a range of resting tensions from 0.7 to 1.2 g, in increments of 0.1 g, was then determined.

The muscle was equilibrated at the minimum resting tension resulting in maximum developed tension. This was usually at a value of 1.0 g.

The muscle was equilibrated for one hour at a stimulation frequency of 30 per minute. The bathing solution was changed every 30 minutes throughout the experiment.

The muscle was then stabilized for specific intervals and 5 beats recorded at each frequency.

Stabilization was as follows : 10 min at 3/min; 5 min at 6/min; 5 min at 12/min; 2 min at 30/min. Gas bubbling tended to produce oscillations in the derivative of the event. These could be prevented by occluding the delivery tubing during the time each event was recorded. Frequencies of 1.5 and 60 per minute were used in some experiments.

Prior to interventions, frequency was set to that at which the intervention would be introduced, usually 12 per minute. 5 consecutive signals were then recorded to provide positions on the interval/strength line from which any shift the intervention might cause could be determined. In most experiments those beats fell close to those recorded at that frequency on the interval/strength line.

In changing calcium concentration or washing out a drug, new medium was added from a 50 ml syringe into a sidearm at the bottom of the bath while removing old medium with suction from the top. This prevented exposure of the muscle to air or having it bear any part of its weight in the absence of a buoyant medium. 150 ml of fresh medium was passed into the muscle bath, an amount found by colorimetric techniques to give complete replacement.

C. DATA PROCESSING

The data was analysed in a Digital Lab 8/E computer utilising two programs written in CS-08-LABFOC-V3 overlay to DEC-08-AJAE Focal 1969 computer language.

As the maximum strength of signals the computer could take was 1.5 volts, the recorded signals were attenuated by a factor of 5 by voltage dividers before entering the computer.

PROGRAM 1 - ACQUISITION PROGRAM (See Appendix PED 1).

This program : a) Stored on DEC tape the g deflections and g/sec slopes of the calibration signals in machine units for computations

in PROGRAM 2.

b) Did analogue to digital conversion of each event and stored them on consecutive blocks of DEC tape.

Conversion of the events to numerical values was done in terms of the computer buffer area. Vertically this extended through 1022 points, 511 in each direction (+ above, - below) from baseline. 3 g, the highest peak tension observed, caused a deflection of approximately 320 points, giving a considerable cushion against overflow. Setting the input offset of the channel on which the isometric event was recorded far into the negative range, increased this cushion. More of the buffer area was made available for analysis as the baseline became negative. This widening of the computer field available for analysis was exploited in later experiments by recording events at a greater sensitivity range of the Tandberg.

Horizontally each event occupied a block of 512 points, i.e. 256 points for the isometric signal, and 256 points for the derivative. An internal clock called in by the program set these to m sec intervals at which locations on the event were examined, i.e. the sampling rate. Sampling rates of 3 to 5 m sec (768 to 1280 m sec total duration) were used, depending on the duration of the recorded events. 4 m sec was used most commonly.

c) Displayed the isometric event and first derivative side by side during acquisition and before storage as they came off magnetic tape. This allowed manual rejection of bad events that escaped rejection by criteria built into the program. Each event accepted was indicated by a number on the teletype printout. Given the block number

in which calibrations were stored on the DEC tape as a starting point, any event could be located and examined as often as desired.

PROGRAM 2 - ANALYSIS PROGRAM (See Appendix ANALJ)

This program : a) Displayed the two portions of each event with recorder-imposed noise oscillations and artifacts on the derivative signal due to bubbling, removed by moving-average smoothing. Groups of 5 consecutive horizontal buffer units were dropped 50 vertical buffer units at the positions where this program determined the various indices of the isometric event to be. This allowed a visual check of the accuracy of the program, and rejection of badly-analyzed events.

b) Calculated the values in terms of the calibrations recorded by PROGRAM 1.

c) Printed out deflections in grams, slopes or rates in g/sec, and durations in m sec. of the measures taken from each event.

Figure 2 illustrates the isometric event and first derivative thereof. The following measures were determined :

PEAK TENSION (PT) in grams at the point of maximum deflection from baseline on the isometric curve; TIME TO PEAK TENSION (TTP) in m sec, from the 0-crossing point on the derivative curve; MAXIMUM RATE OF CONTRACTION, $(dT/dt)_{max}$ (MRC) in g/sec, from the trough of the derivative curve; MAXIMUM RATE OF RELAXATION, $(-dT/dt)_{max}$ (MRR) in g/sec, from the peak of the derivative curve; TIME FOR PEAK TENSION TO DECLINE BY 50% (TR/2) in m sec, measured from the 0-cross-projected Peak Tension point on the Isometric curve; AVERAGE RATE OF RELAXATION TO 10% OF PEAK TENSION (ARR),

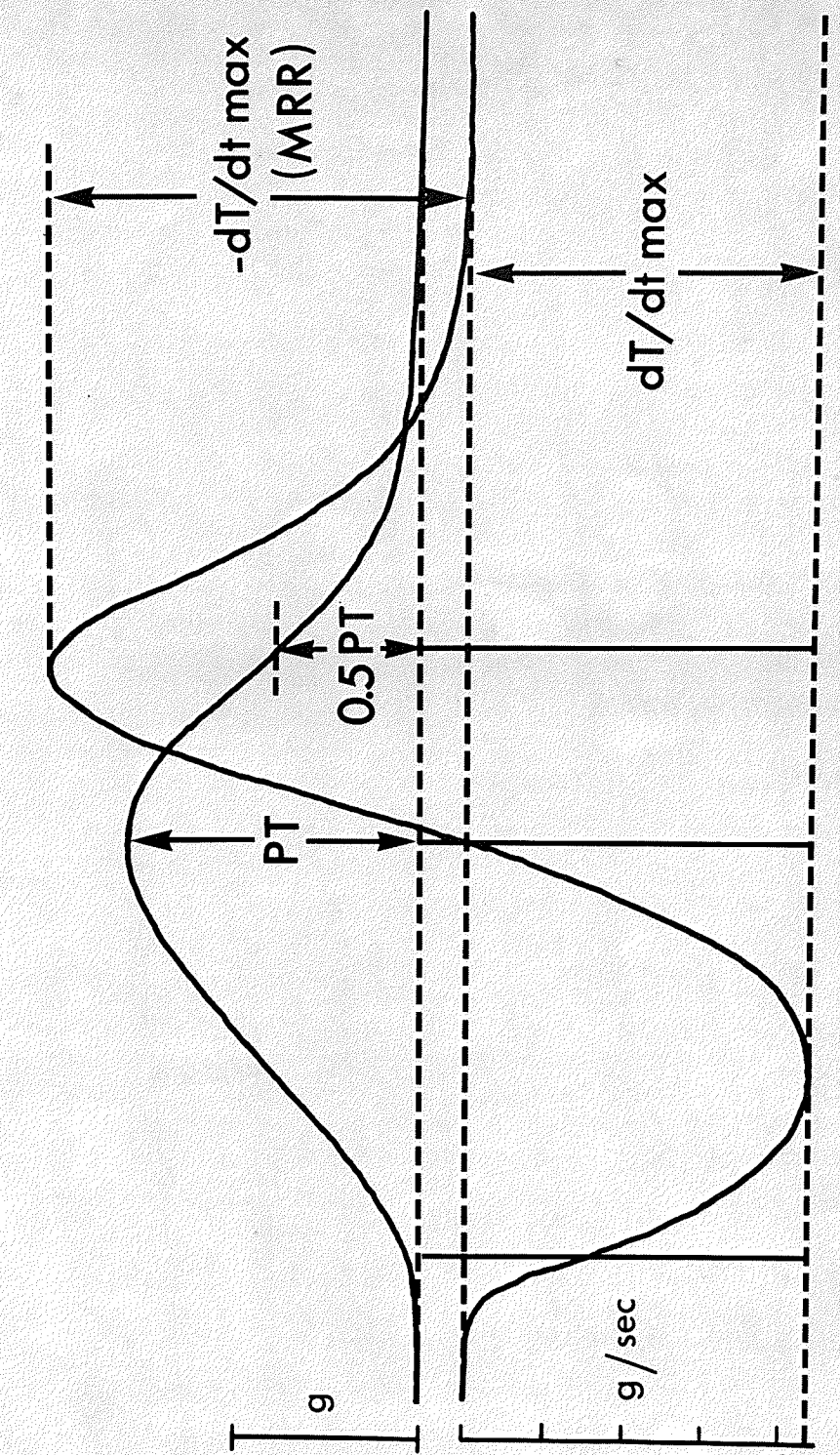


Figure 2

The isometric contraction and first derivative thereof, with measurements used in analysis. For explanation, see text.

in g/sec, by dividing 90% of Peak Tension by the time from 0-cross Peak Tension to 90% tension decline.

Due to slight oscillations in the baseline, determinations of the exact points at which tension rose above 0 g, and of return to 0 tension at the end, were found to be very inaccurate. This caused considerable variability in measures involving these points, particularly measures of time.

Therefore the beginning of the event was taken as 2 vertical buffer units above baseline on the isometric curve. The point at which tension had declined by 90% was used to determine ARR.

Time to peak tension determined from the isometric curve was also quite variable, as the actual tension plateau was very broad in terms of the sampling rate, and the program could take the peak tension point from any location along this plateau.

However, by determining the time to 0-crossing on the derivative curve, a much more limited span, a good deal of variability was eliminated. This was verified by determining PT at a point 256 horizontal buffer units to the left of this point, i.e. the corresponding location on the isometric curve. PT here was virtually identical to that determined on the isometric curve itself.

Use of this point from which to locate the position of 50% and 90% tension decline considerably reduced the variability of TR/2 and ARR, respectively.

SECTION III

RESULTS

RESULTS

A. RELATIONSHIP OF INDICES OF RELAXATION TO DEVELOPED TENSION

In order to determine the relationship of measures of relaxation to peak tension developed, an intervention was required which would alter peak tension without changing conditions external to the muscle.

It is well known that changes in frequency affect contraction strength; this is the interval/strength relationship (51). Koch-Weser (30) used frequencies from 0.2 per minute, the rested state interval, up to 300 per minute in cat papillary muscle. He found the following :

1. Peak tension increased with increases in frequency. It reached a maximum at frequencies of 60 to 300 per minute, depending on the thickness of the muscle.

2. Increases in peak tension were paralleled by increases in maximum rate of contraction $(dT/dt)_{max}$ proportionally greater than the reduction in time to peak tension which was also observed.

3. Time to 90% decline in peak tension from the peak tension point shortened more with increases in frequency than did time to peak tension. The muscle, therefore, exerted tension for a greater part of each contraction cycle at higher frequencies.

This implies that maximum rate of relaxation, $(-dT/dt)_{max}$ increases with peak tension as well. We therefore first examined the relationship of peak tension to maximum rate of relaxation. For reasons to be given later, we used frequencies of 3, 6, 12, and 30 per minute

in most experiments.

The upper portion of Figure 3 indicates the linear relationship of peak tension to maximum rate of relaxation (MRR) in beats stabilized (see Methods) at each of these frequencies. This relationship was observed in some 50 different papillary muscles.

This relationship was found to be independent of the resting length of the muscle at resting tensions from 0.5 to 4.5 grams, i.e. even on the descending portion of the length/tension curve (Dresel & Schluter, unpublished).

The lower portion of Figure 3 indicates the relationship of peak tension to average rate of relaxation from the point of peak tension to 10% of peak tension (ARR) in the same muscle. This has been found to be essentially similar to the relationship of peak tension to maximum rate of relaxation in all muscles studied. Both maximum rate and average rate of relaxation are affected by interventions in the same way. We have therefore chosen to report rate of tension decline as maximum rate of relaxation in subsequent results.

Two measures of duration of decline in tension are commonly used by other workers (15, 30, 46). These are time for tension to decline by 50% from the point of peak tension, and time for tension to decline by 90% from this point.

Sonnenblick (15) has reported that time for 50% decline tends to decrease with increasing frequency of contraction. Koch-Weser (30) has indicated slight decreases in time to 90% decline with increasing frequency in the range used in the present experiments. Both of these workers used

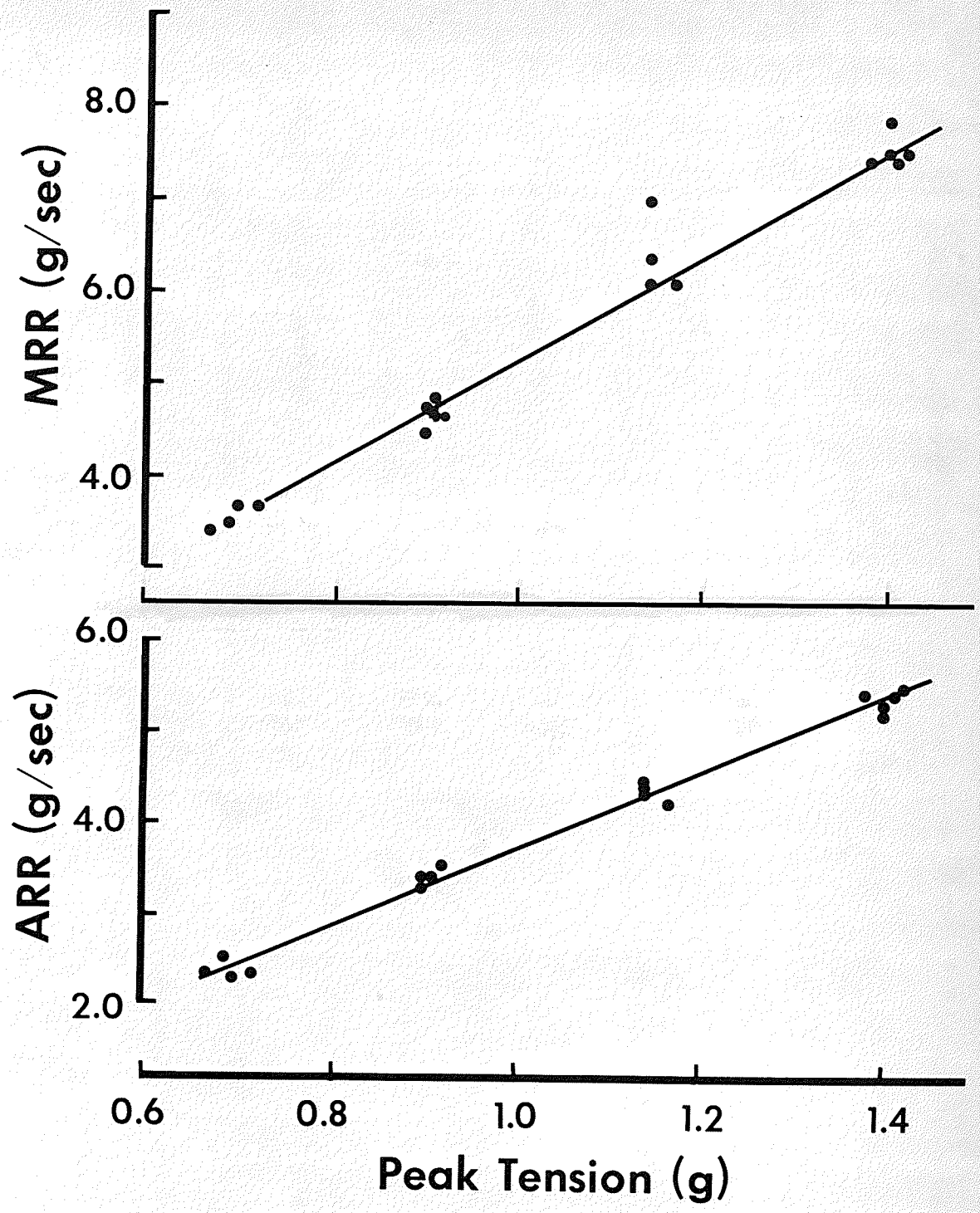


Figure 3

The linear relationship in a single muscle of peak tension to maximal (MRR) and average (ARR) rates of relaxation. Each point represents one beat. Changes in strength were caused by stimulating at increasing frequencies of 3, 6, 12, and 30 per minute, i.e. the interval/strength effect.

data pooled from several muscles, and reported them as means ± standard errors.

We have found considerable variation between individual muscles in the relationships of both indices to frequency and to peak tension.

Figure 4 illustrates the relation of peak tension to time to 50% tension decline (TR/2) for three different muscles. The top portion illustrates this relationship for the same muscle used in Figure 3.

The results in the two top portions were observed most commonly. The inconsistency of this relationship from muscle to muscle is clearly illustrated.

Figure 5 illustrates the relationship of peak tension to time to 90% decline in tension (RT) for the same muscles used in Figure 4. The relationship is seen to parallel that of peak tension to time to 50% decline in tension closely in corresponding muscles.

The authors cited did not relate the indices to contraction strength, but to frequency. We therefore examined this relationship next.

Figure 6 illustrates the relationship of frequency to time to 50% decline in tension in 6 different muscles. Each data point represents an average of some 4 or 5 events recorded at that frequency in a single muscle. The same individual variability occurs in this relationship as in that of peak tension shown in Figure 4.

However, if the data points at each frequency for all six muscles are pooled, as indicated by the means with standard error bars, the slight tendency to decline reported by Sonnenblick is clearly shown. Data for the relationship of peak tension to time to 90% decline in tension from

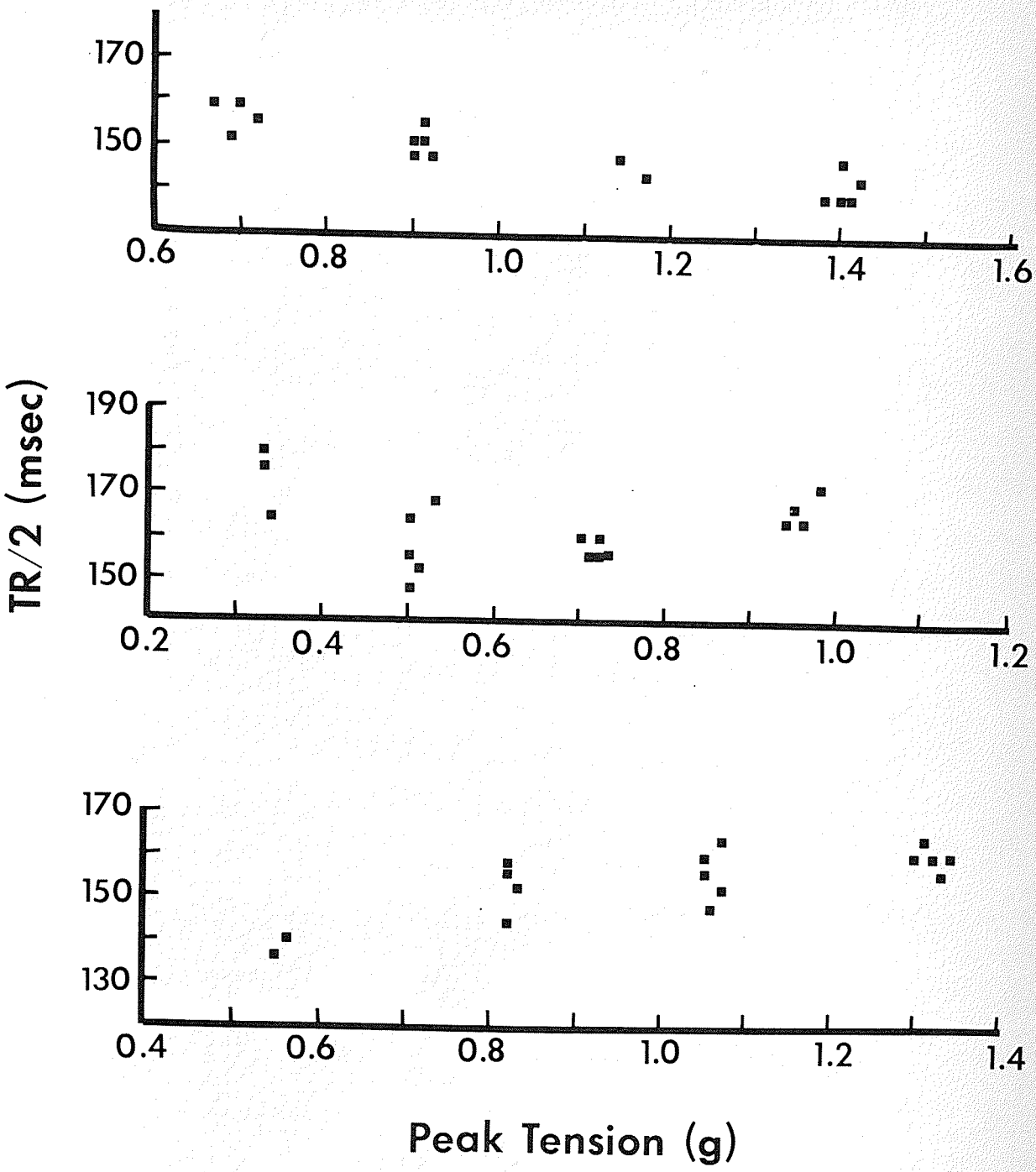


Figure 4

The relationship of peak tension to time to 50% decline from peak tension (TR/2) in 3 different muscles. Each point represents one beat. Note that the relationship differs in each muscle. Changes in strength were caused by the interval/strength effect as in Figure 3.

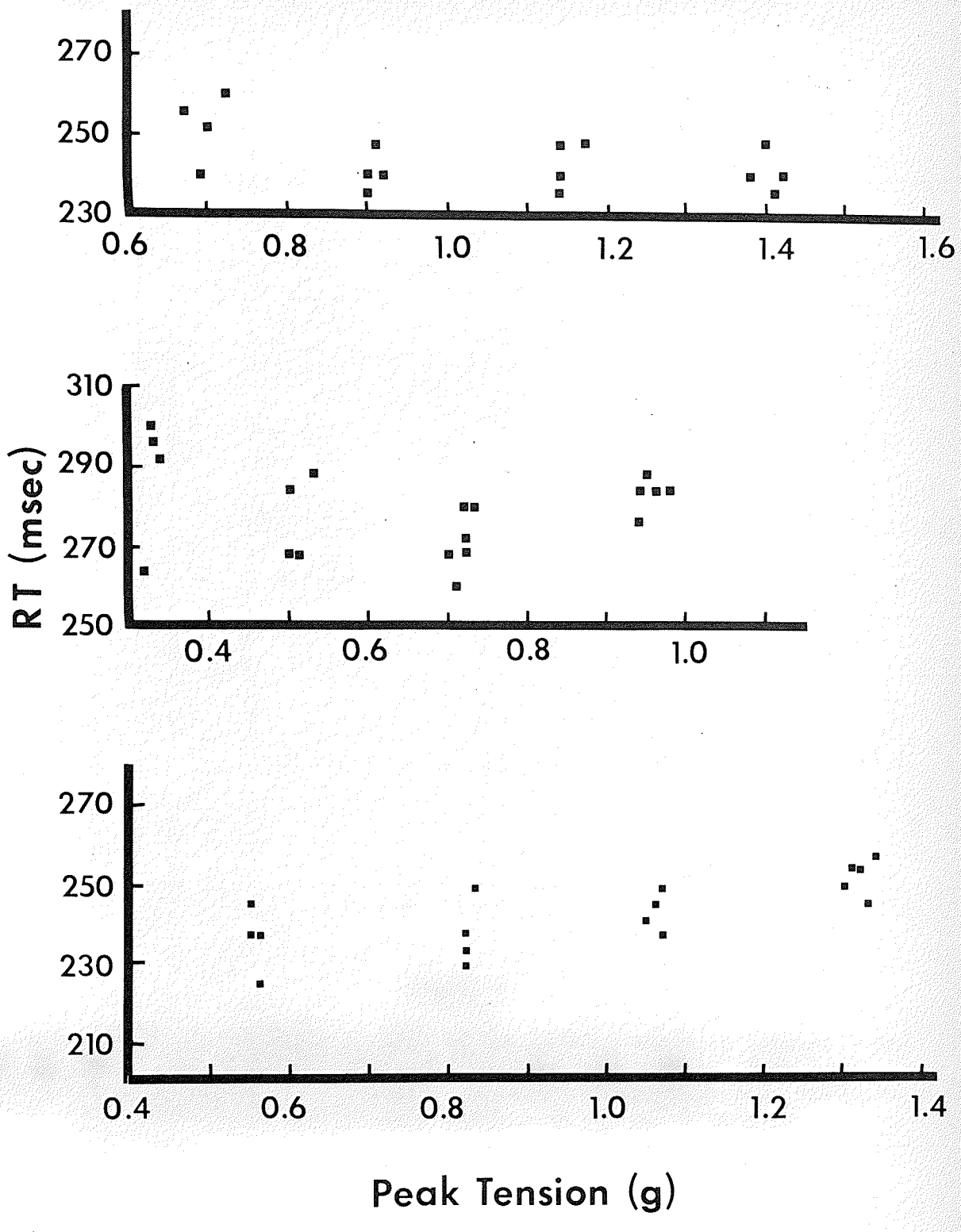


Figure 5

The relationship of peak tension to time to 90% decline from peak tension, (RT) in the same events shown in Figure 4. Note the variability from muscle to muscle, and the wider spread of values at some frequencies in some muscles compared to TR/2.

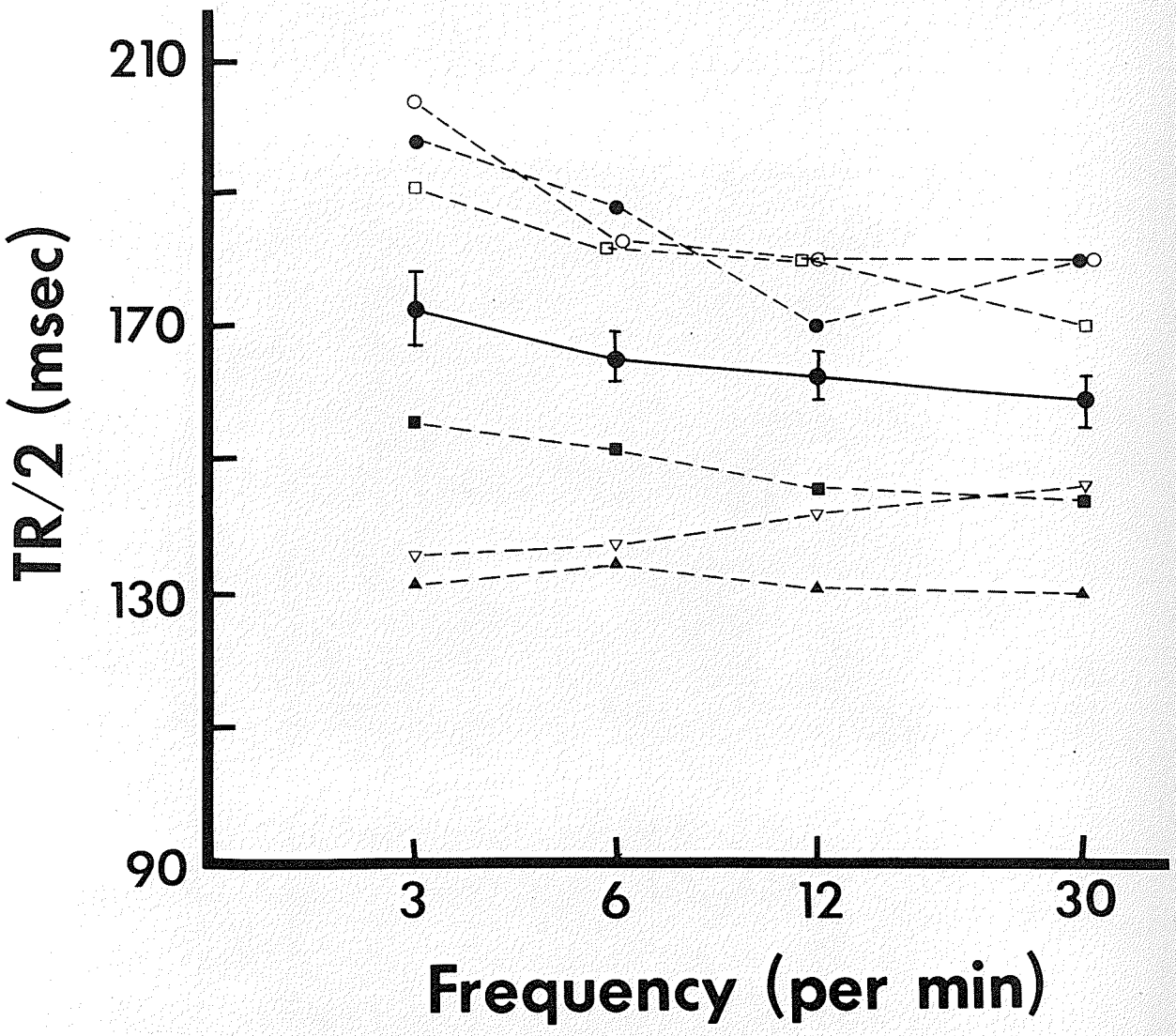


Figure 6

The relationship of frequency to TR/2 in the muscles shown in Figures 4 and 5 (■ , ○ , ▽), and in three other muscles (▲ , □ , ●). Each point represents an average of 4 to 5 beats. Note the variability from muscle to muscle. The large solid circles show means ± SE for all 6 muscles.

the same muscles behaves in the same way. Mean values with standard error bars are virtually superimposeable upon the figure drawn by Koch-Weser (30).

This indicates that pooling data from several muscles and reporting mean values may obscure individual variability between muscles that may itself be an important indication of muscle behaviour.

Changes in time to 90% tension decline have been shown to parallel those in time to 50% tension decline on the interval/strength relationship. Changes are also parallel in the presence of inotropic interventions used. It is possible, however, that time to 90% decline in tension falls on that final portion of the relaxation curve influenced by viscous elements, particularly in events affected by interventions. Therefore we have used time to 50% as the index of duration of decline in tension in reporting our results.

Many workers consider it necessary to correct measures of contractility for variations in cross-sectional area when comparing different muscles. To determine how such variation affects the relationship of peak tension to maximum rate of relaxation, we plotted in Figure 7 data for 9 different muscles varying in thickness from 0.5 to 1.0 mm, and in length from 3 to 5 mm. Data from a single muscle is indicated as open circles. The overall correlation coefficient is 0.98. Therefore this index would appear to be independent of cross-sectional area.

Figure 8 shows the data for the relationship of peak tension to time to 50% decline in tension for the same muscles. The coefficient of correlation was less than 0.1, and the variability indicated in Figure 4

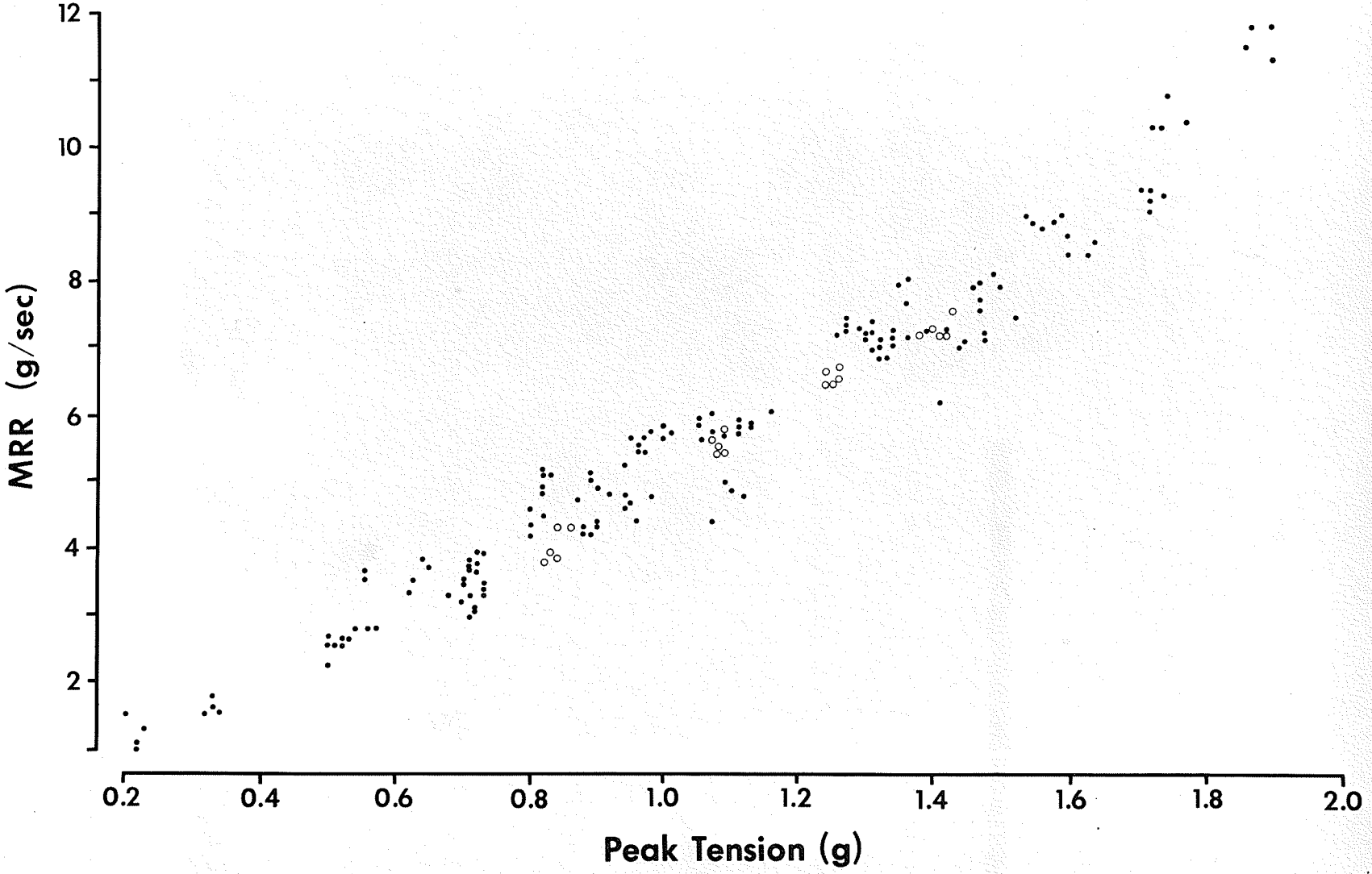


Figure 7

The linear relationship in 9 different muscles examined over a period of 1 month. Changes in strength were caused by the interval/strength effect. Each point represents a single beat. Data from a single muscle is indicated separately (o). Coefficient of correlation = 0.98.

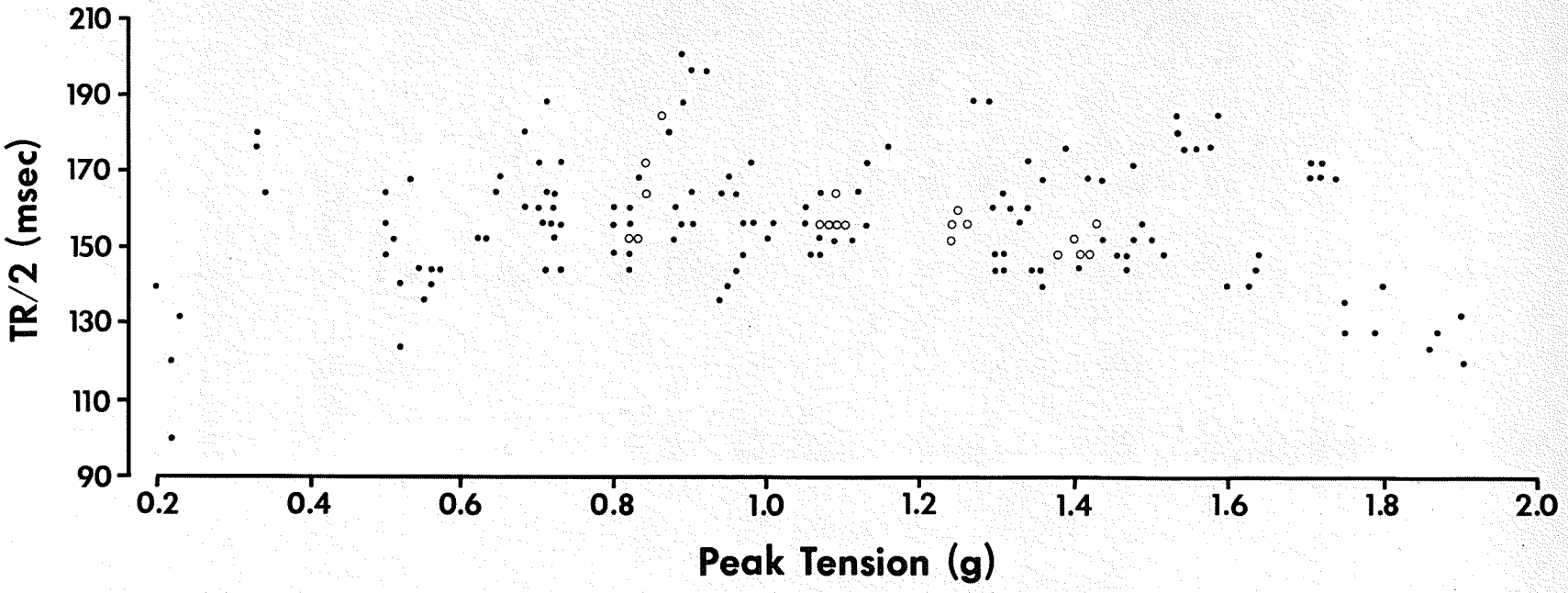


Figure 8

The inconsistent relationship of peak tension to TR/2 in the same events represented in Figure 7. Data from the same single muscle is indicated separately (o). Coefficient of correlation < 0.1.

is confirmed. Data from the same muscle as in Figure 7 is indicated as open circles.

A number of parameters of muscle size, including width, weight, length, weight/length, length times width, and cross-sectional area, were tested to determine if variations in time to 50% decline in tension were related to any of them. There was no correlation with any of them.

From the irregularity of its relationships to peak tension and to frequency, time to 50% decline in tension would seem to be an indefinite index of relaxation at best. However, the fact that the relationship to peak tension does differ, in being irregular, from that of maximum rate of relaxation, may indicate that time to 50% decline in tension is measuring something at least partly different. In keeping with the use of both a rate index and a duration index in characterising the contractile phase, we shall report our results for the relaxation phase in terms of both maximum rate of relaxation and time to 50% decline in tension, related to peak tension.

B. EFFECTS OF INOTROPIC INTERVENTIONS ON THE RELATIONSHIPS

Previous work has not consistently related changes in the relaxation phase to inotropic changes, particularly in peak developed tension. We feel that this is important, and have shown that one index, maximum rate of relaxation, bears a consistent relationship to peak tension. We have therefore examined the effects on this relationship and on that of peak tension to time to 50% decline in tension, of various inotropic inter-

ventions stated to affect relaxation (see Introduction).

1. CHANGES IN CALCIUM CONCENTRATION

If changes in calcium sequestration are involved in changes in relaxation, changes in calcium concentration in the external medium might be expected to have an effect, because they change the influx of calcium during the action potential (27). Increases in external concentration of calcium are well known to have a positive inotropic effect exclusively by means of increases in maximum rate of contraction. Time to peak tension is in fact reduced (21, 50, 51). We have therefore examined the effects of increased calcium concentration on the relationships of peak tension to maximum rate of relaxation (MRR) and time to 50% decline in tension from the peak tension point (TR/2).

The following protocol was used for these experiments : the muscle was equilibrated for one hour at a frequency of 30 per minute in the lower concentration of calcium. The interval/strength relationship was then determined (see Methods). Calcium was added up to the higher concentration, and the muscle was equilibrated 30 minutes at a frequency of 30 per minute before recording the interval/strength relationship at this concentration.

Figure 9 illustrates the effects of changing from 1.25 to 2.5 mM calcium, Figure 10 of a change from 2.5 to 5.0 mM calcium.

The upper portion of both figures shows the relationship of peak tension to maximum rate of relaxation (MRR).

It is important to note that increases in concentration of calcium

Figure 9

The effect of increase in external calcium concentration on the relationship of peak tension to MRR and on TR/2 in a single muscle. Note the shift of the interval/strength line for MRR in 2.5 mM calcium from that obtained in 1.25 mM calcium and the lack of increase in TR/2.

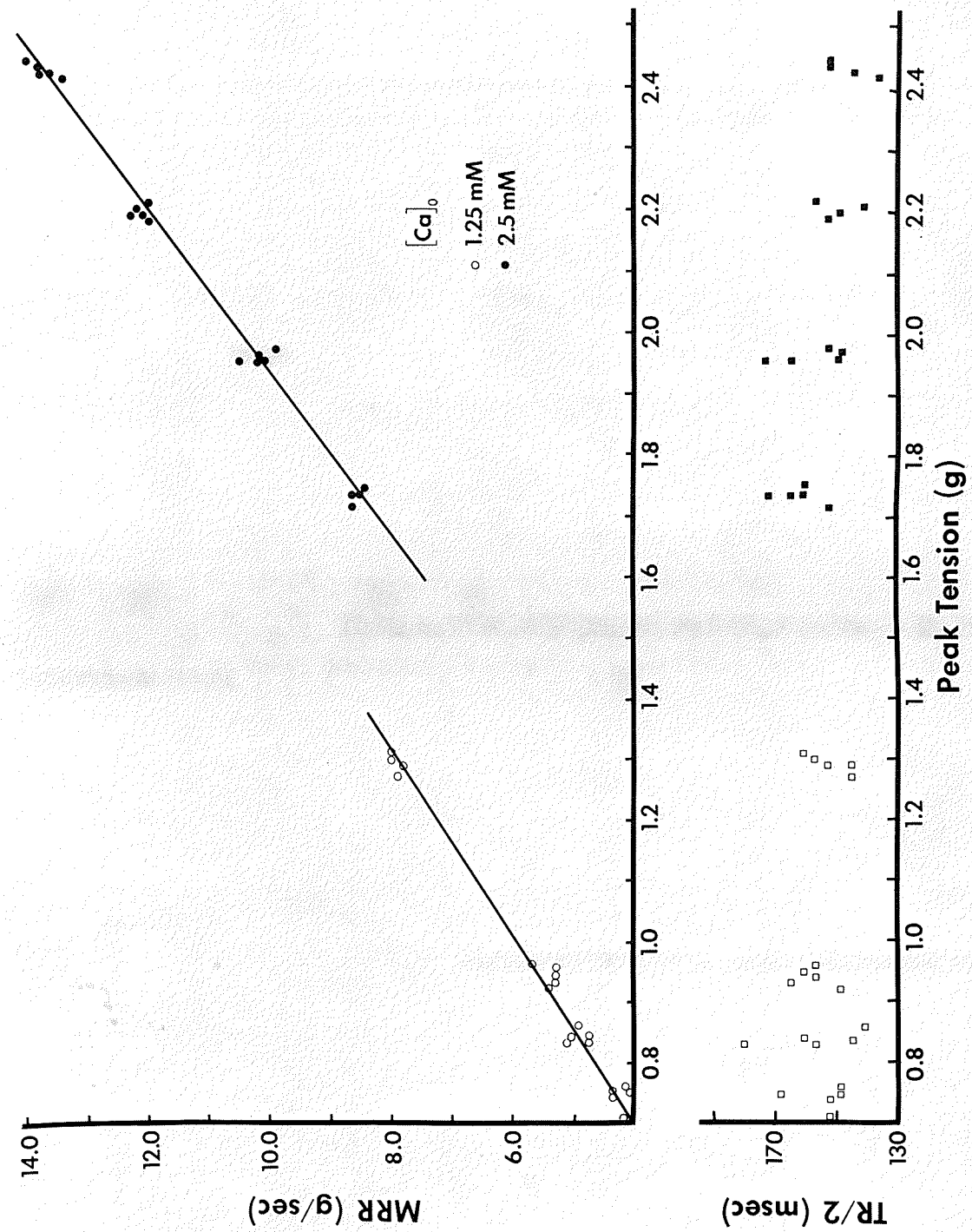
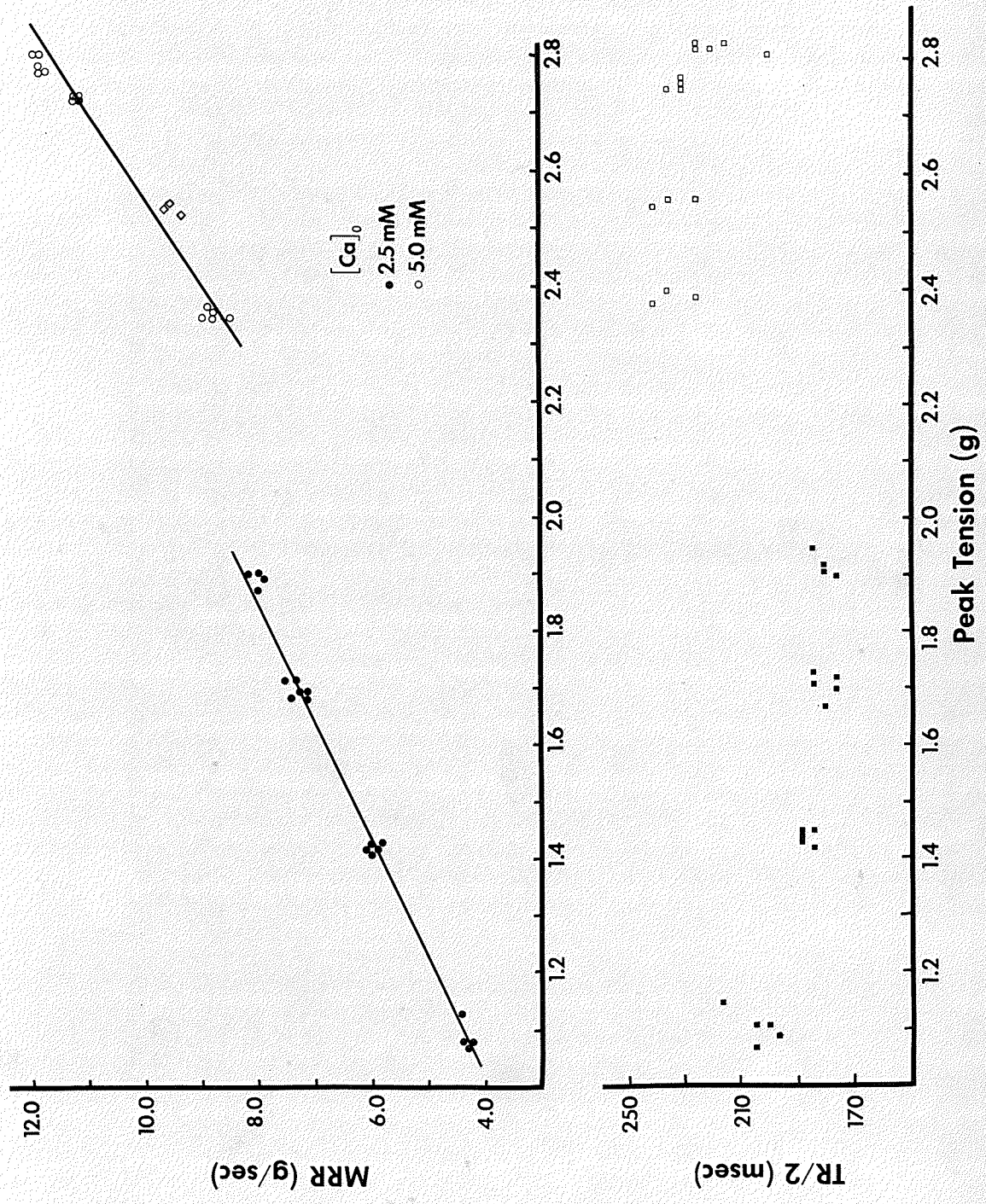

MRR (g/sec)
14.0
12.0
10.0
8.0
6.0
$[Ca]_o$
1.25 mM
2.5 mM
TR/2 (msec)
170
130
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Peak Tension (g)

Figure 10

The effect of increase in external calcium concentration from 2.5 mM (● and ■) to 5.0 mM (○ and □) in a single muscle. MRR is slowed as in Figure 9. Note in addition a change in TR/2 which was not seen in Figure 9. The negative inotropic effect at 30 per minute in 5.0 mM calcium is indicated by a different symbol (◇) for MRR.


MRR (g/sec)
12.0
10.0
8.0
6.0
4.0
$[Ca]_0$
• 2.5 mM
○ 5.0 mM
TR/2 (msec)
250
210
170
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Peak Tension (g)

cause an INCREASE in maximum rate of relaxation at any given frequency. However, when MRR is related to peak tension, it is shown to be DEPRESSED at all frequencies in the higher concentrations of calcium. There was a shift to the right in each of 3 muscles changed from 1.25 to 2.5 mM calcium, and each of 5 muscles changed from 2.5 to 5.0 mM calcium.

Change from 1.25 to 2.5 mM calcium caused no change in the linearity of the relationship of peak tension to maximum rate of relaxation. However, the slope of the interval/strength line was slightly increased in one muscle, shown in Figure 9, and slightly reduced in two other muscles.

Change from 2.5 to 5.0 mM calcium caused no change in the slope of the interval/strength line in one muscle, slightly reduced the slope in a second, and slightly increased the slope, as shown in Figure 10, in three others. In three of five muscles tested at 5.0 mM calcium, the maximum rate of relaxation failed to increase proportional to the increase in peak tension at the highest frequency, producing a plateau. In two other muscles there was a negative inotropic effect at the highest frequency, peak tension and maximum rate of relaxation both being depressed. We have no explanation for the negative inotropic effect, an example of which is shown in Figure 10.

In order to determine whether this interruption of linearity in 5.0 mM calcium depended on peak tension or on frequency, the relationship of MRR to frequency for the muscle shown in Figure 10, in which a negative inotropic effect occurred at the highest frequency, was plotted together with data from another muscle in which a positive inotropic effect had been observed. Figure 11 shows the results. In these two muscles, and in three others, MRR was consistently depressed at a frequency of 30 per minute,

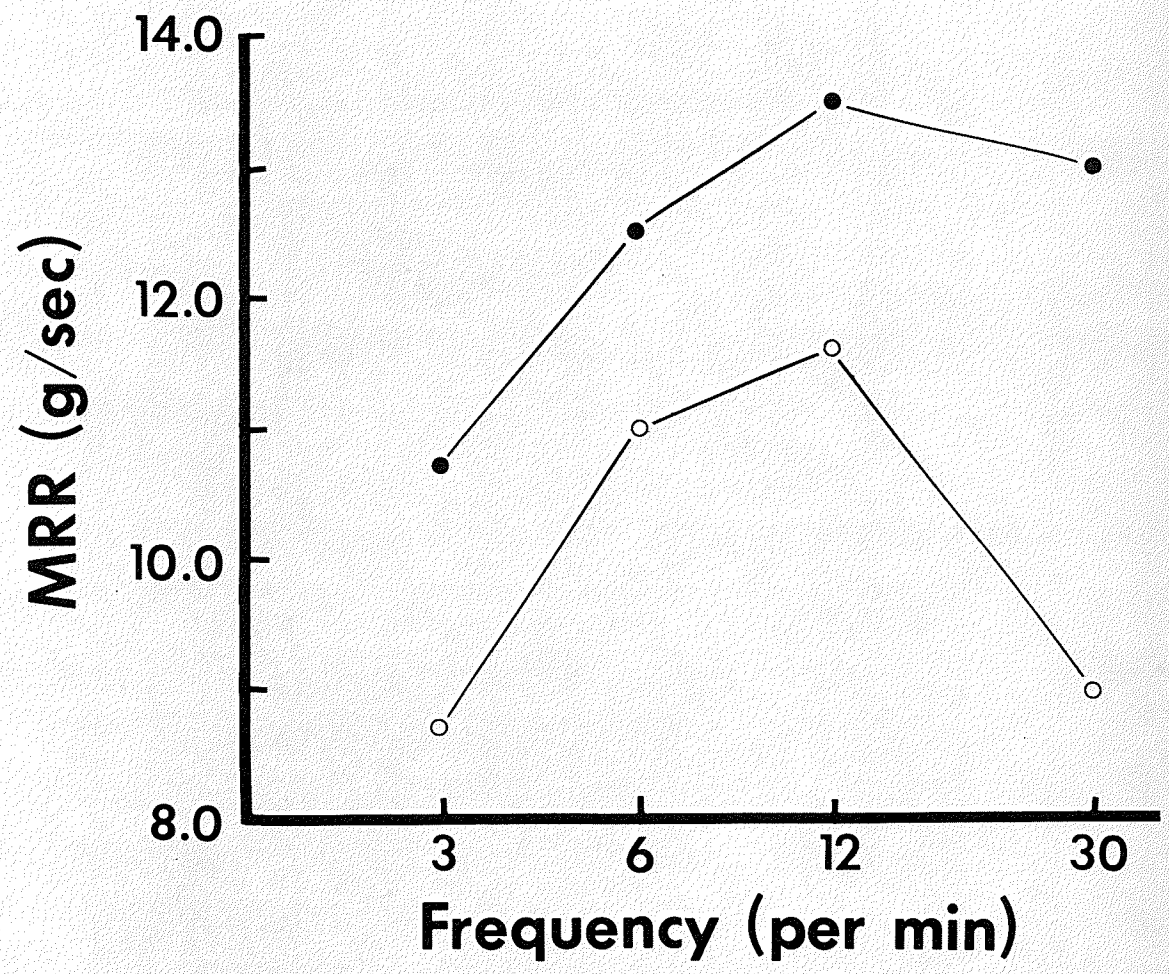


Figure 11

The frequency-dependent additional depression of MRR in 5.0 mM calcium. Each point represents the average of 4 to 5 beats. The figure includes one of two muscles in which a frequency of 30 per minute produced a negative inotropic effect (o), and one of three muscles in which no negative inotropic effect occurred (●).

whether peak tension declined as it did in two muscles, or increased as it did in three.

In contrast to the relationship of peak tension to MRR, the effect of changes in concentration of calcium on time to 50% decline in tension (TR/2) appears to depend on the concentration of calcium from or to which the change is made.

TR/2 was not changed in the 3 muscles tested at calcium concentrations of 1.25 and 2.5 mM. Data for one muscle are shown in the lower portion of Figure 9.

The lower portion of Figure 10 illustrates the increase in duration of TR/2 in 5.0 mM calcium. This effect occurred in each of 5 muscles. Note that there was no change in TR/2 at the highest frequency, independent of the negative inotropic effect.

The effects of different concentrations of calcium on TR/2 further illustrate the inconsistency of the relationship of this index to peak tension developed.

Reductions in concentration of calcium have a negative inotropic effect, reducing peak tension at all frequencies. Since increasing the concentration of calcium slows maximum rate of relaxation relative to peak tension, it might be expected that reducing the concentration would speed it. However, the effects of reducing the concentration of calcium from 2.5 to 1.25 mM were extremely variable among 5 muscles studied. In one muscle the interval/strength relationship in 1.25 mM calcium shifted with slight reduction in slope in the direction of speeded MRR at all peak tensions. In two muscles the interval/strength line in 1.25 mM calcium displayed a

shallower slope and crossed the 2.5 mM interval/strength line, indicating more rapid MRR at low frequencies, and a slower MRR at high frequencies in 1.25 mM calcium. In the two remaining muscles there was a shift with reduced slope in the direction of slowed MRR at all frequencies.

Reduction in concentration of calcium from 2.5 to 1.25 mM produced an INCREASE in time to 50% tension decline (TR/2) at all levels of peak tension. Figure 12 indicates this effect in a single muscle in which the increase was greatest. These results must be contrasted with the lack of any change shown in Figure 9 when the calcium concentration is changed in the reverse direction.

The effects of changes in concentration of calcium may be summarized as follows :

1. Increases in concentration of calcium affect the relationship consistently in that maximum rate of relaxation is slowed at all levels of peak tension in the higher concentration.

The effect cannot be dissociated from the positive inotropic effect in 2.5 mM calcium or at lower frequencies in 5.0 mM calcium, but has been dissociated at a frequency of 30 per minute in 5.0 mM calcium. This effect of increased extracellular calcium may thus be dependent both on changes in strength and changes in frequency.

2. Time to 50% decline in tension is not changed by increasing concentration of calcium from 1.25 mM to 2.5 mM, but is prolonged by increase from 2.5 to 5.0 mM.

3. Decreases in concentration of calcium from 2.5 mM to 1.25 mM affect the relationship of peak tension to maximum rate of relaxation

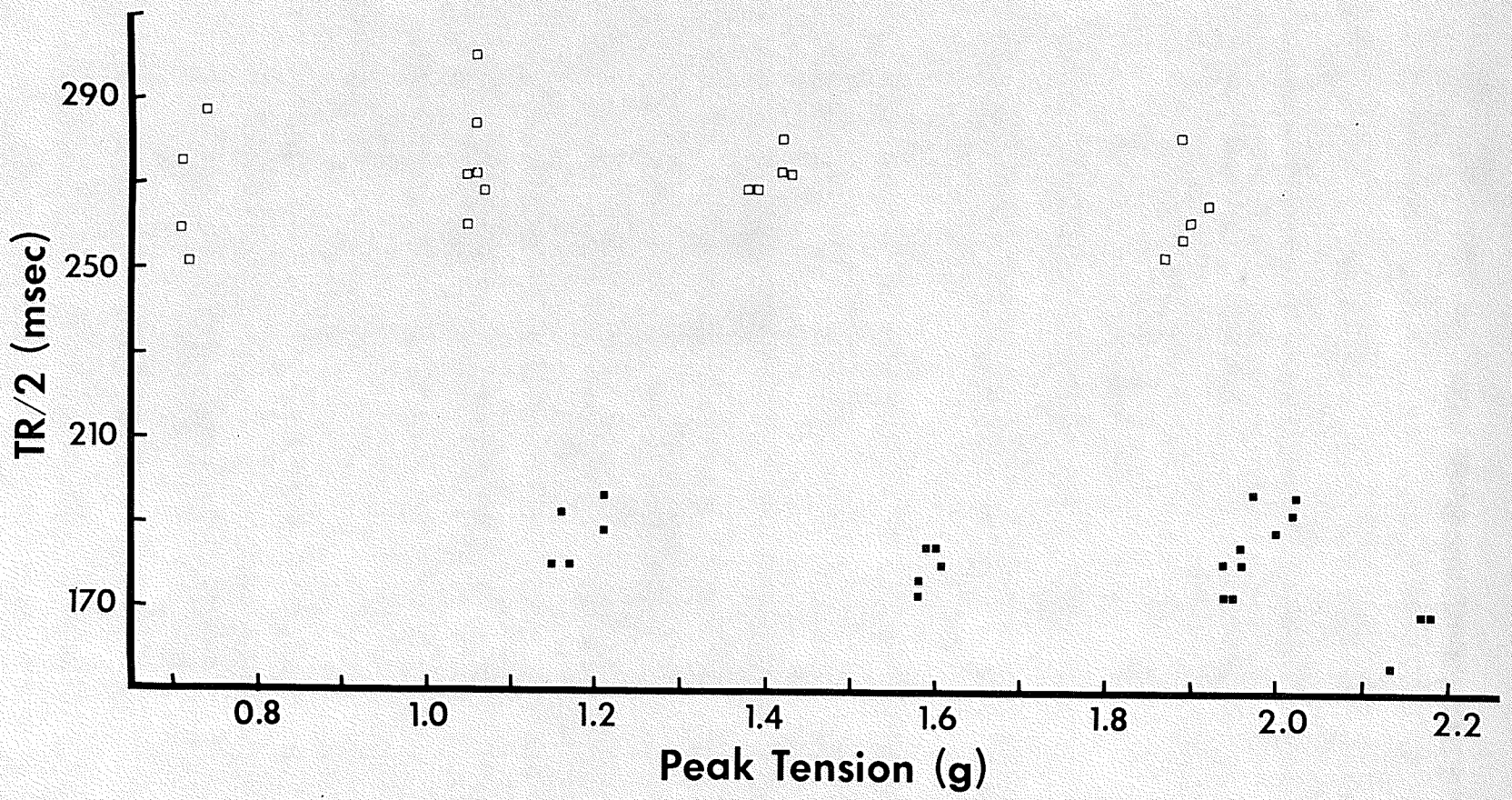


Figure 12

The increase of TR/2 produced by reduction of calcium concentration from 2.5 mM (■) to 1.25 mM (□).

inconsistently, except that the slope of the interval/strength line is always reduced in 1.25 mM calcium.

4. Reducing concentration of calcium produces a consistent prolongation of time to 50% decline in tension at all levels of peak tension.

It therefore appears that the concentration of calcium in which the muscle is equilibrated influences the effect of subsequent changes in concentration, and possibly of other interventions as well.

2. EFFECTS OF DRIVE

Prolongation of relaxation during recovery from drive has been previously observed in this laboratory (57, 58) and has been reported by others (51).

Our early experiments showed that maximum rate of relaxation was depressed at a frequency of 12 per minute following stabilization at 60 per minute at the end of the interval/strength determination. Beats fell below the interval/strength line compared to those recorded at 12 per minute during the determination. Recovery appeared to require 15 to 20 minutes. When frequency of 60 per minute was excluded from the protocol, depression at 12 per minute was still observed following stabilization at 30 per minute. Recovery appeared to require 5 to 10 minutes. For this reason 30 per minute was the highest frequency used in determining the interval/strength relationship in the present work. We then tested the effects of various periods of drive, at 120 per minute. 30 seconds of drive at this frequency was found to produce a considerable slowing of relaxation while

allowing complete recovery within 15 to 20 minutes when frequency was reduced to 12 per minute.

The following protocol was used : After recording the interval/strength relationship, the muscle was allowed to restabilize at a frequency of 12 per minute. Several events were then recorded. The muscle was driven at 120 per minute for 30 seconds, and frequency reduced to 12 per minute. One event was recorded every 30 seconds for 10 minutes to monitor recovery. The factor of time is therefore involved as a variable in a number of subsequent figures (13 to 18) which illustrate the effects of interventions. However, it was not felt necessary to make 3-dimensional representations of peak tension, maximum rate of relaxation, and time. The results can be understood more easily if it is realized, as is shown by arrows in some of the figures, that points corresponding to the effects of interventions follow one another at 30 second intervals on the 2-dimensional graph. Using the interval/strength line as a baseline, overall shift from the interval/strength line and changes in position of successive recorded beats with time are therefore discussed. The irregularity of the relationship of peak tension to time to 50% decline in tension (TR/2) has been discussed above. Nonetheless subsequent figures relate TR/2 to peak tension to enable changes in MRR and TR/2 to be compared against the same variable. The introduction of time as a third variable should also be considered in examining these figures.

Both Figures 13 and 14 show recovery from drive in 2.5 mM calcium. The upper portion of each illustrates the relationship of peak tension to maximum rate of relaxation (MRR).

Figure 13

Recovery from drive in a single muscle at calcium concentrations of 2.5 mM and 5.0 mM. Note the shift of the interval/strength line produced by increase from 2.5 mM (● and ■) to 5.0 mM calcium (△), as in Figure 10. Recovery from a 30-second period of drive at 120 per minute is then shown for both concentrations of calcium. Beats at a frequency of 12 per minute were recorded at 30-second intervals, (▲——▲) and beats joined by this line must be considered to represent progressive changes in the relationship, the direction of which are indicated by arrows. Note the greater deflection of points in 5.0 mM calcium, and the longer duration of deflection from the interval/strength baseline. Note the parallel changes in TR/2. For further information, see text.

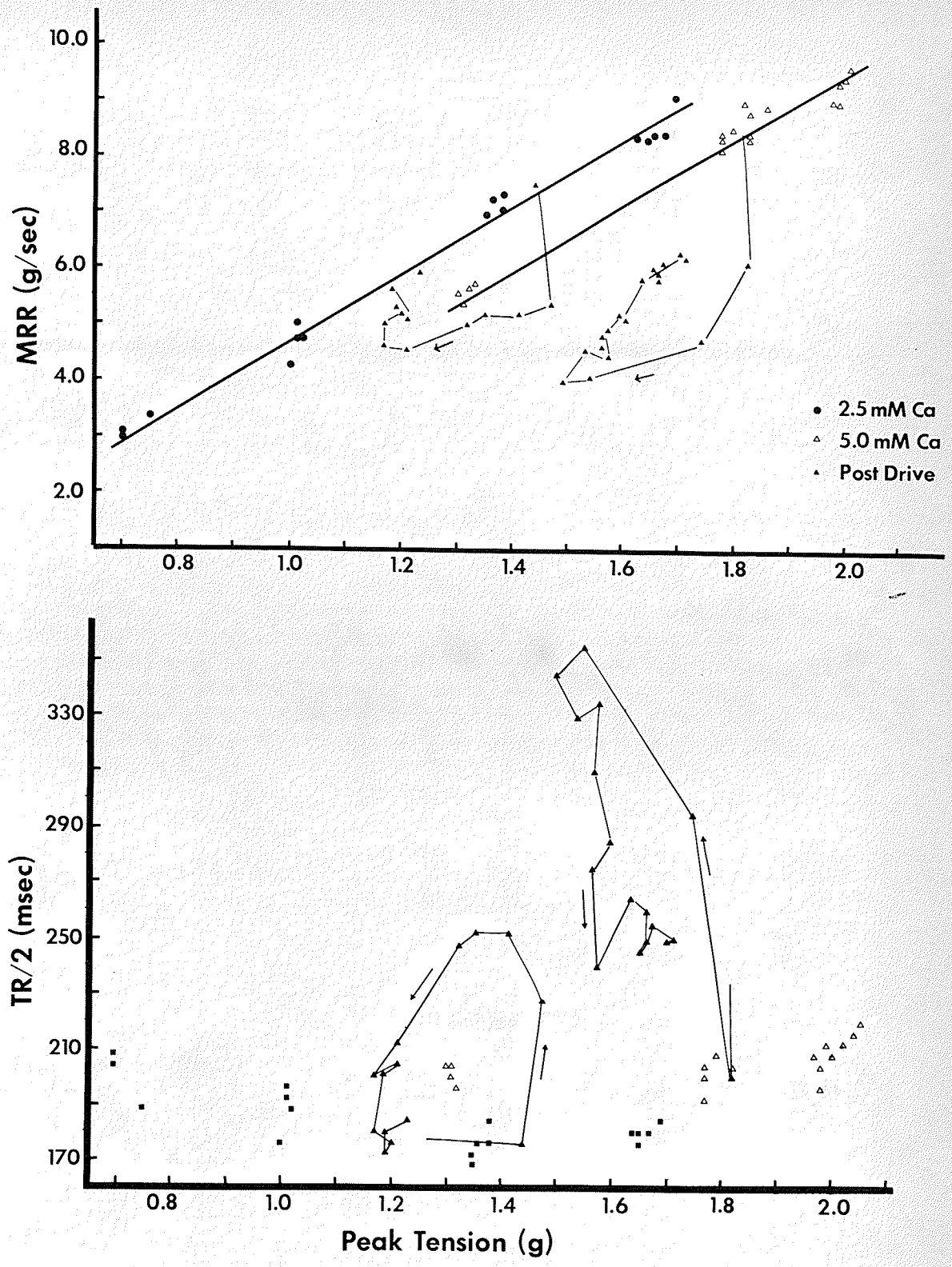
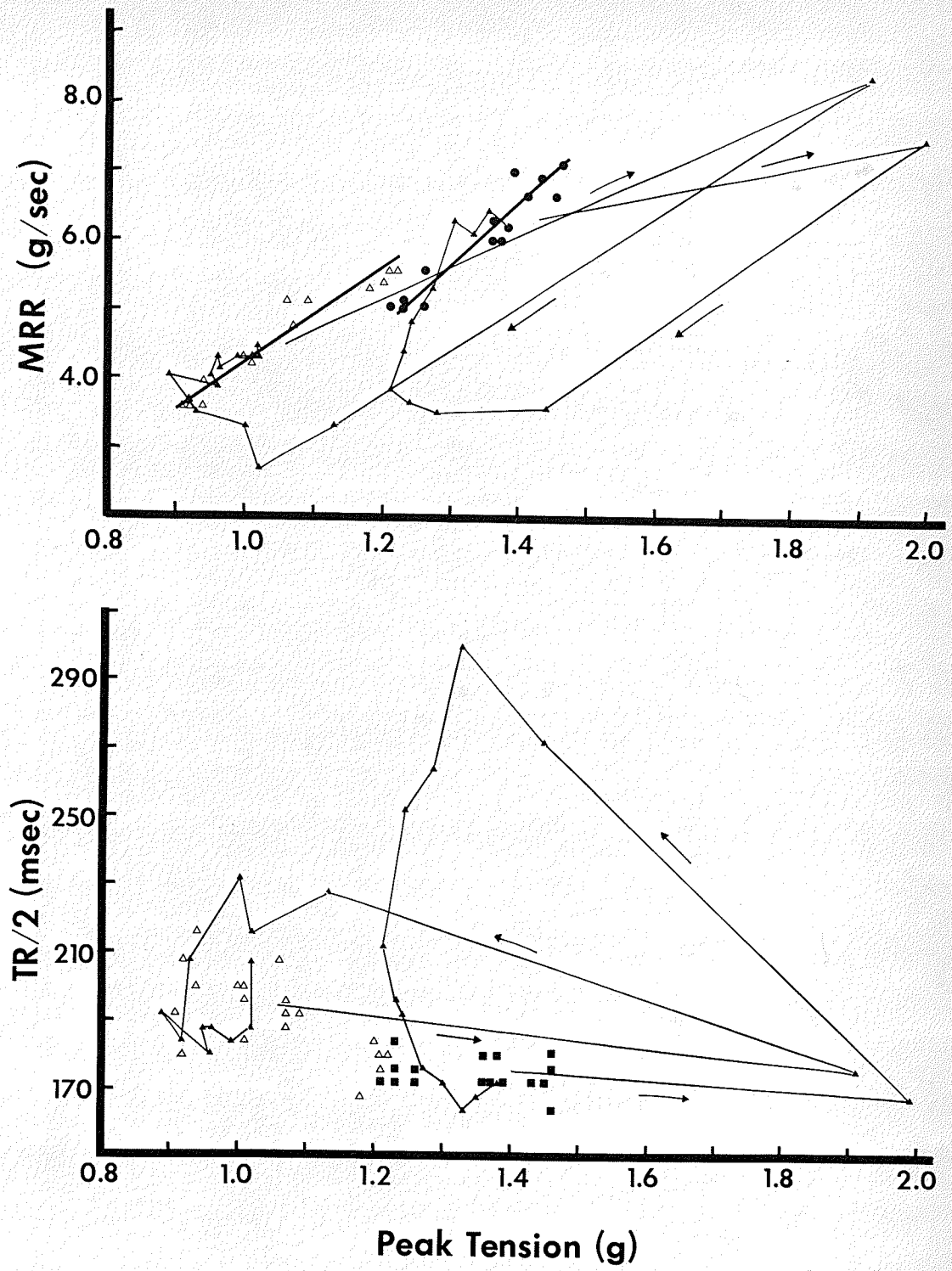

10.0
8.0
6.0
4.0
2.0
MRR (g/sec)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.5 mM Ca
5.0 mM Ca
Post Drive
330
290
250
210
170
TR/2 (msec)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Peak Tension (g)

Figure 14

Recovery from drive in a single muscle in calcium concentrations of 2.5 mM and 1.25 mM. Note the shift of the interval/strength line produced by decrease from 2.5 mM (● and ■) to 1.25 mM calcium (△). Protocol was as in Figure 13. Recovery at 12 per minute was monitored at 30-second intervals (▲——▲). Note the smaller deflection of points in 1.25 mM calcium, and the shorter duration of deflection from the interval/strength baseline. Note the parallel changes in TR/2.


MRR (g/sec)
8.0
6.0
4.0
0.8
1.0
1.2
1.4
1.6
1.8
2.0
TR/2 (msec)
290
250
210
170
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Peak Tension (g)

Due to accumulated positive inotropic effect of activation (PIEA) (51), the first beats often show higher peak tension and MRR than those prior to drive. The increase either follows the line or falls below it, i.e. MRR increases in proportion to or is slowed, but never speeded, relative to the interval/strength relationship.

Within 1 to 2 minutes, while peak tension drops only slightly below predrive values, MRR has fallen considerably. Both values then decline together, and begin to recover as they approach the interval/strength line. They return to the line at values much lower than the predrive beats within 6 to 7 minutes. From this point successive beats recover along the relationship, returning to predrive values after 15 to 20 minutes. This effect occurred in each of 4 muscles used.

The lower portions of Figures 13 and 14 illustrate the effect of drive on time to 50% tension decline (TR/2). The parallelism to MRR appears to be quite good. Abrupt depression of MRR is paralleled by increase in TR/2. The time course of recovery is paralleled by return of TR/2 to values equal to those seen before drive.

In view of the effects of increased concentration of calcium alone, we next examined the effects of elevated concentrations of calcium on recovery from drive. As recovery was found to be complete within 15 to 20 minutes, two 30 second drive procedures in different concentrations of calcium could be carried out in the same muscle. The muscle was equilibrated for one hour in 2.5 mM of calcium before recording the interval/strength relationship and instituting drive. It was then equilibrated for 30 minutes in the new calcium concentration before proceeding as before.

Figure 13 indicates the effect of change from 2.5 to 5.0 mM calcium on recovery from drive. The upper portion indicates the relationship of peak tension to maximum rate of relaxation (MRR). On comparing patterns of recovery : a. The positive inotropic effect of activation in the first beat appears to be less in 5.0 mM calcium.

b. Depression of MRR is greater at any level of peak tension in 5.0 mM calcium. This is especially apparent in the first 2 to 3 beats in which peak tension is greater than or only slightly below predrive values.

c. MRR takes longer to recover to the interval/strength line in 5.0 mM calcium. In 2.5 mM calcium it has returned to the line within 6 minutes. In 5.0 mM calcium it has not yet returned after 10 minutes.

The lower portion of Figure 13 shows the effects on time to 50% tension decline (TR/2) in the same muscle. Greater depression of MRR is paralleled by greater increase in TR/2. The time course of approach toward predrive values is also similar in the two indices.

The effects of reduction in concentration of calcium from 2.5 mM to 1.25 mM on recovery from drive were examined next. Figure 14, upper portion, illustrates the relationship of peak tension to maximum rate of relaxation. In each of two muscles examined :

a. Positive inotropic effect of activation in the initial beats is greater in 1.25 mM calcium.

b. The extent of depression in MRR is less in 1.25 mM calcium.

c. The duration of depression in MRR is less in 1.25 mM

calcium, as beats have recovered to the interval/strength line within 2½ minutes, compared to 4 minutes in 2.5 mM calcium.

The lower portion of Figure 14 indicates the effect on time to 50% decline in tension (TR/2). Changes in TR/2 parallel changes in MRR quite well.

To summarize the effects of changes in concentration of calcium on recovery from drive :

1. Changes in the relationship of peak tension to maximum rate of relaxation are compatible with an effect on the ability of the cell to handle calcium. This appears to be facilitated by decreases in concentration of calcium, and retarded by increases.

2. The time course and degree of change in maximum rate of relaxation is paralleled quite well by changes in time to 50% decline in tension in both increased and decreased concentrations of calcium after drive.

3. EFFECTS OF HYPOXIA

The relaxation-prolonging effects of rapid drive have been suggested to be due to hypoxia of the muscle core (30, 51). Several workers (11, 46, 47) have recently reported the relaxation-prolonging effects of recovery from hypoxia. We therefore proceeded to examine effects of hypoxia on relaxation.

The muscle was equilibrated for one hour before determining the interval/strength relationship. It was then allowed to stabilize at 12 per minute, and 5 beats recorded. Frequency was kept at 12 per minute throughout

hypoxia and reoxygenation.

Hypoxia was induced by bubbling the muscle bath with 95% nitrogen/ 5% carbon dioxide for 10 minutes. Beats were recorded every 30 seconds.

95% oxygen/5% carbon dioxide was then reintroduced and recovery monitored every 30 seconds for 10 minutes. Recovery was followed for up to one hour in several experiments.

Figure 15 indicates the time course of relative change in oxygen tension. Due to the rapidity of gas delivery, large changes in oxygen tension occurred within 1 - 2½ minutes of changing the gas.

The time course of major changes in the indices studied paralleled phases of rapid decline and rapid increase in oxygen tension during induction of and recovery from hypoxia.

Recovery of developed tension after 20 minutes of hypoxia was stated to be complete within 30 minutes of reoxygenation (46). Sonnenblick (11) reported incomplete recovery of time to peak tension and time to 50% decline in tension up to 1 hour after terminating 60 minutes of hypoxia. In several muscles we have observed incomplete recovery of peak tension, or recovery followed by depression. In one muscle peak tension recovered completely after 30 minutes of reoxygenation, and was substantially depressed after 1 hour. Thus, even when recovery appears to be complete, the muscle may not be normal. Adrenaline 5×10^{-8} g/ml induced independent beating in a muscle which had appeared to have recovered completely 30 minutes after reoxygenation. Adrenaline had not caused this effect before hypoxia. We concluded that recovery from a single period of hypoxia might not be complete, and that the effects of a second period of hypoxia in the

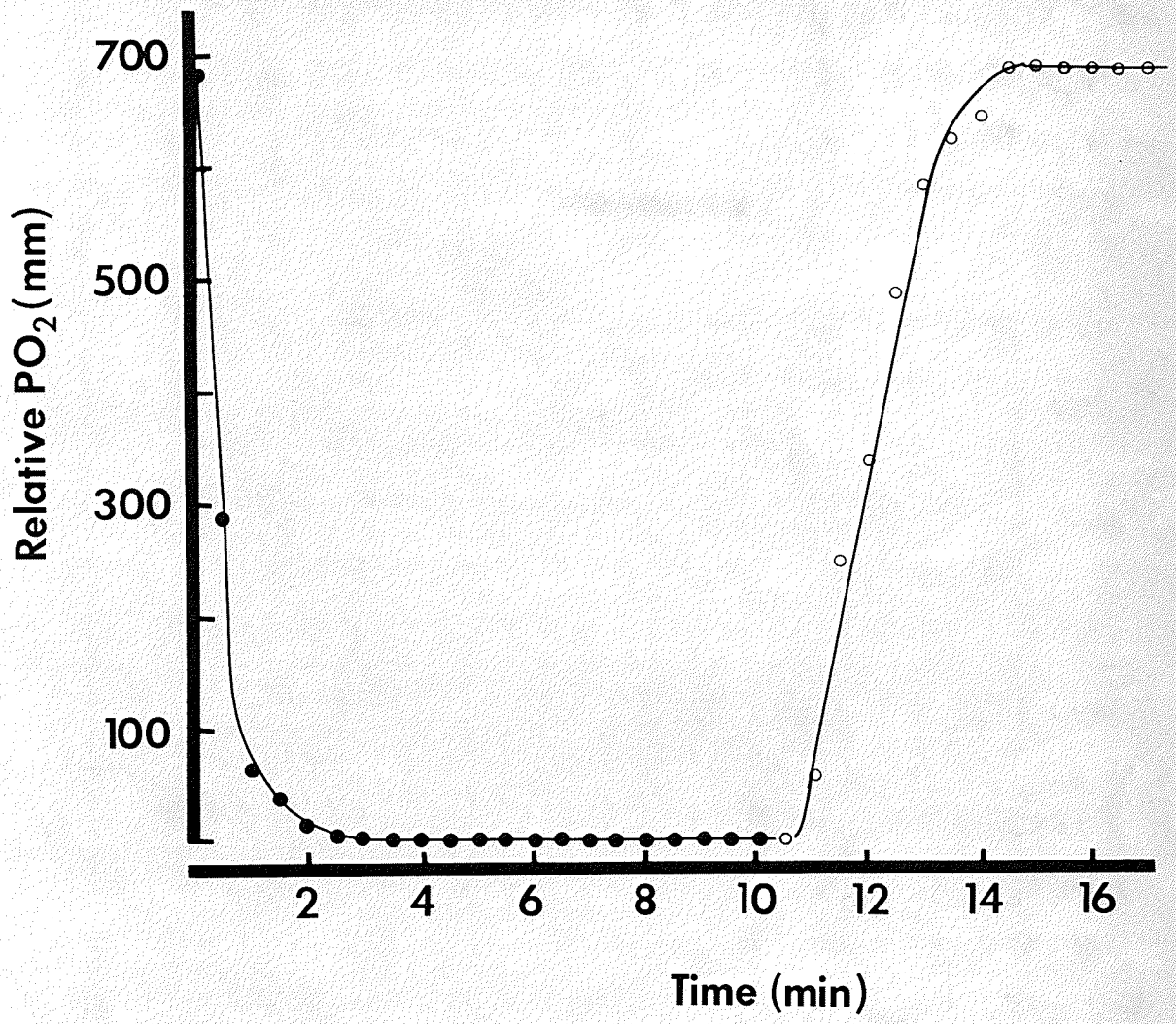


Figure 15

The time course of change in oxygen tension during induction of hypoxia (●——●) and reoxygenation (○——○). The point at 0-time is calculated. Dial readings from the oxygen electrode device were then referred to this PO_2. Measurements were made at 30-second intervals.

same muscle might not be compared reliably to the first period in a paired experimental design. Effects of changes in calcium concentration were therefore tested in different muscles.

Figure 16 indicates the effects of hypoxia and recovery therefrom on a single muscle in 2.5 mM calcium. Each of 4 muscles gave the same results.

Figure 17 indicates the same relationships for another muscle in 5.0 mM calcium. This is typical of effects observed in 3 muscles.

Hypoxia in both calcium concentrations caused a speeding of maximum rate of relaxation (MRR) relative to peak tension as contractility declines. In both calcium concentrations this speeding tended to occur abruptly within 2 to 3 minutes of oxygen deprivation, coinciding with the point at which oxygen tension approaches 0 in the muscle bath.

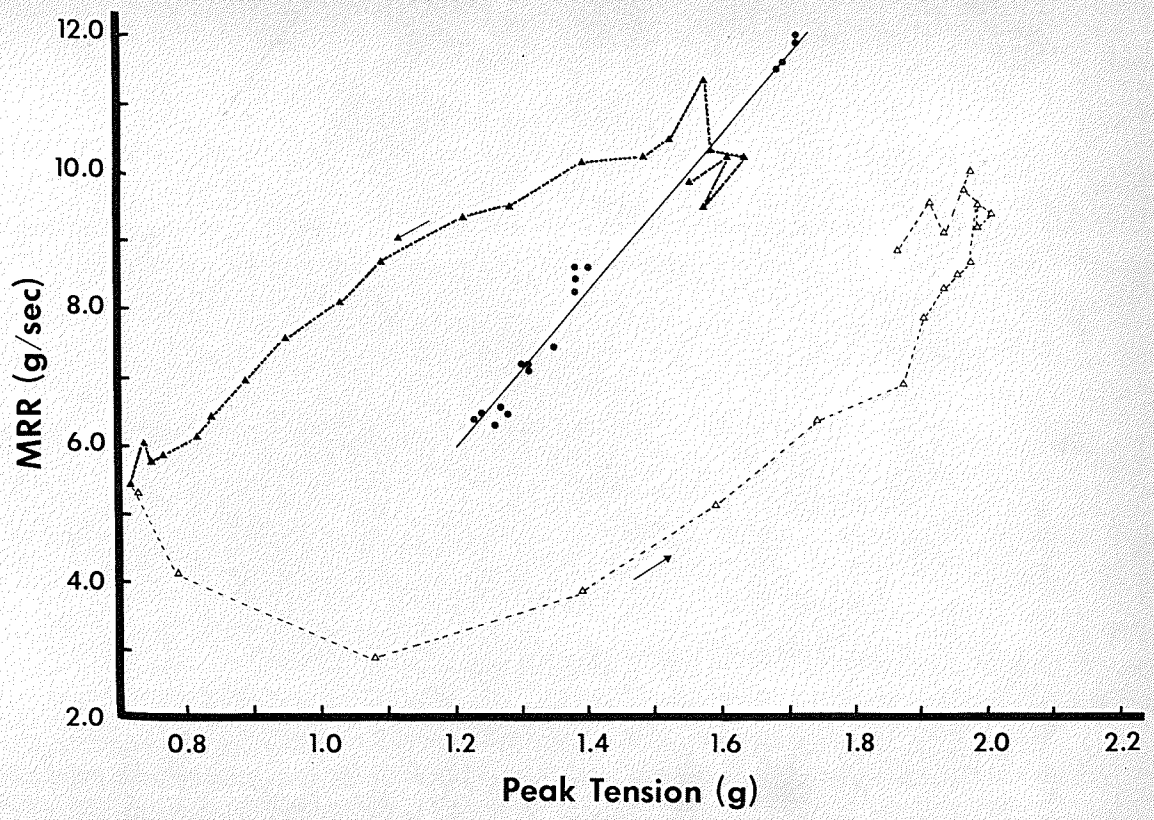
Figure 16 shows that beats recorded at 30 second intervals during hypoxia shifted further and further away from the interval/strength line as time progressed. This was observed in 2 of the 4 muscles tested at 2.5 mM calcium. In the other 2 preparations there was no further increase in the difference from the normal relationship.

In 5.0 mM calcium each of 3 muscles displayed the parallel shift shown in Figure 17.

Within 1 to 2 minutes of reintroducing oxygen MRR is substantially depressed, and is further depressed for some 2 to 3 minutes more relative to a rapidly recovering peak tension. As in the presence of a change in calcium concentration *per se* (Figures 9 and 10), in absolute terms MRR is increasing. Only when it is related to peak tension does progressive

Figure 16

The effects of hypoxia and of reoxygenation in 2.5 mM calcium. A frequency of 12 per minute was maintained during both phases. The interval/strength effect is the baseline (● and ■). As in Figures 13 and 14, directional arrows indicate changes with time in beats recorded every 30 seconds. Changes were followed for 10 minutes of hypoxia (▲) and 10 minutes of reoxygenation (△). Note the speeding of MRR during induction of hypoxia, with a slight decrease in TR/2, and the significant depression of both MRR and TR/2 within 2 minutes of reoxygenation.


12.0
10.0
8.0
6.0
4.0
2.0
MRR (g/sec)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Peak Tension (g)

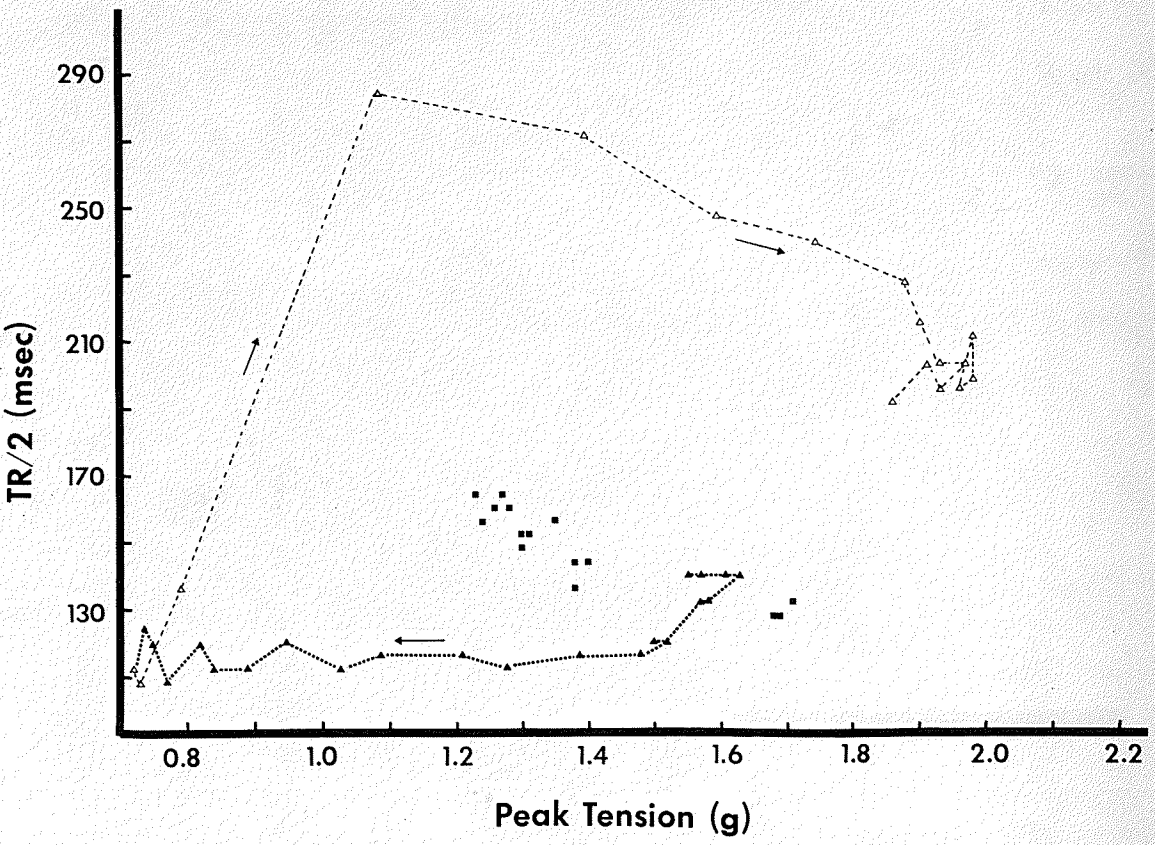
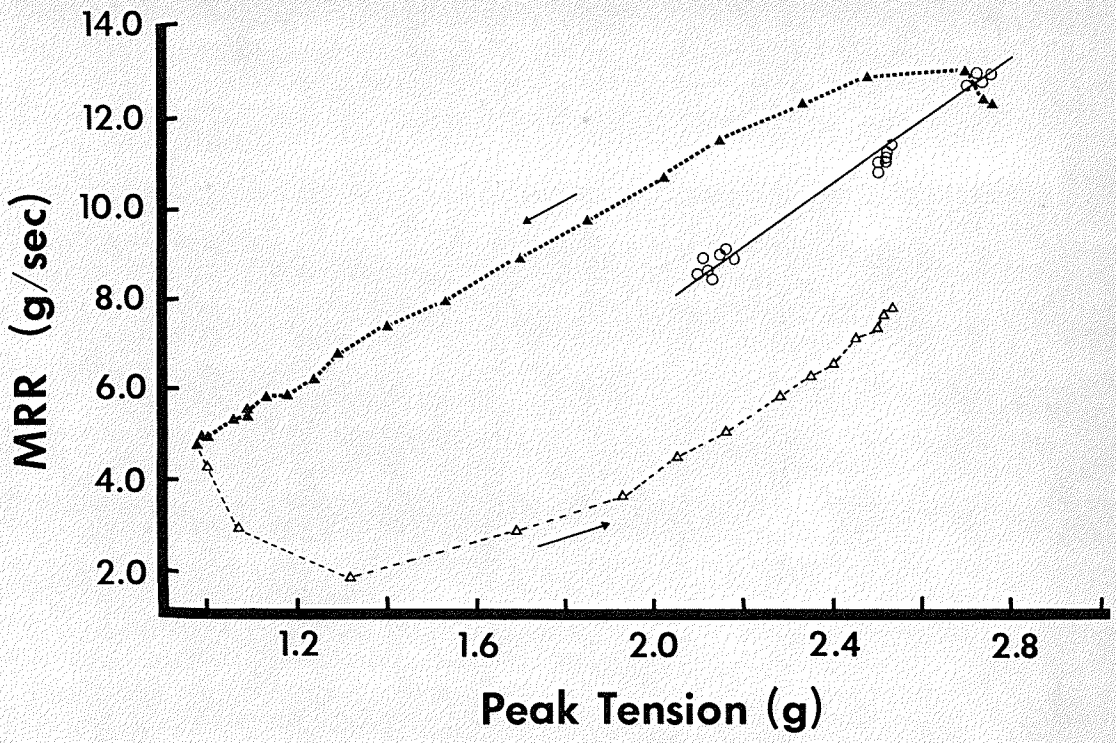
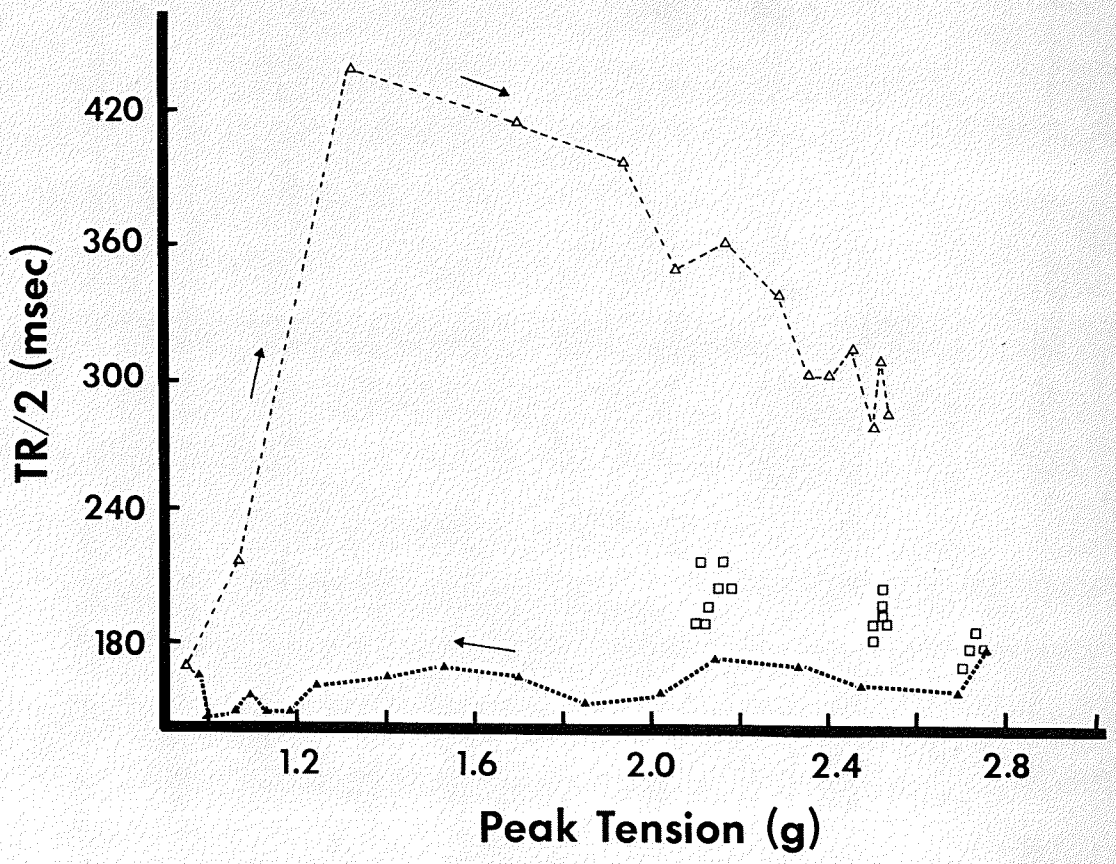

290
250
210
170
130
TR/2 (msec)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
Peak Tension (g)

Figure 17

The effects of hypoxia and of reoxygenation in 5.0 mM calcium. The interval/strength effect is the baseline (○ and □). Protocol as in Figure 16, but note change in scales.


14.0
12.0
10.0
8.0
6.0
4.0
2.0
MRR (g/sec)
1.2
1.6
2.0
2.4
2.8
Peak Tension (g)


420
360
300
240
180
TR/2 (msec)
1.2
1.6
2.0
2.4
2.8
Peak Tension (g)

depression become apparent. Peak tension has recovered to values close to or greater than control after 10 minutes, but MRR remains depressed.

The pattern of recovery toward the interval/strength line differs from muscle to muscle. In most muscles recovery was not followed for a sufficient period of time to determine when the beats rejoined the line. Recovery had occurred after 25 to 30 minutes of reoxygenation in 2 muscles observed for longer periods in 2.5 mM calcium. In 2.5 mM calcium peak tension may decline in successive recorded beats at a fairly constant MRR, as in Figure 16, and approach the line. This occurred in 3 of 4 muscles. In the fourth muscle, peak tension first declined to the level of control beats at a constant MRR, and then stayed at this value while MRR increased and beats rejoined the line.

In 5.0 mM calcium peak tension may decline while MRR increases, as it did in 1 of 3 muscles. In a second, peak tension declined at constant MRR. In the third, shown in Figure 17, the pattern is not yet apparent after 10 minutes.

Attempts were made to quantitate the degree and duration of depression of MRR to compare effects of different calcium concentrations. Duration could not be adequately compared as full recovery had not been monitored.

As we felt that effects of change in calcium concentration could not be examined in the same muscle by 2 successive periods of hypoxia, differences in strength and in the time course of recovery between individual muscles made the meaning of comparisons as total area of deviation from the interval/strength relationship, or maximum deviation from the

relationship, indefinite.

The subjective impression overall was that there was no difference between the effects of hypoxia in 2.5 mM and 5.0 mM calcium.

The lower portions of Figures 16 and 17 indicate the effect on time to 50% decline in tension (TR/2). Reductions in TR/2 parallel speeding of MRR during induction of hypoxia. Extensive increase in TR/2 parallels the greatest depression of MRR during recovery, and the time course of recovery is the same for TR/2 and MRR.

In contrast to the lack of apparent difference between effects of 2.5 mM and 5.0 mM calcium, hypoxia does have a different effect on the muscle in 1.25 mM calcium.

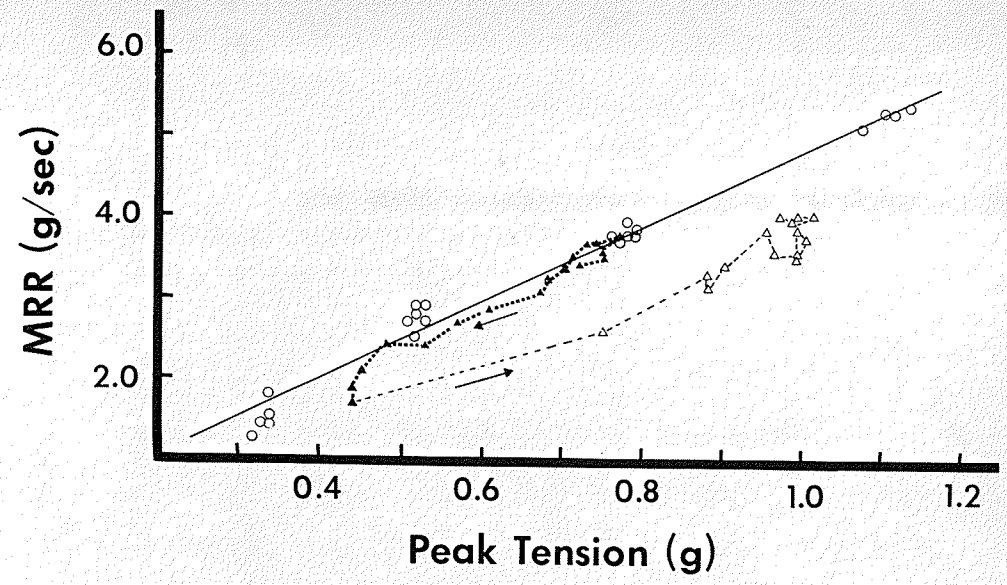
Figure 18 illustrates the effects on maximum rate of relaxation and time to 50% decline in tension observed in each of 2 muscles treated. The most important difference is the lack of speeding of MRR during induction of hypoxia in 1.25 mM calcium. MRR declines down the interval/strength line in proportion to peak tension decline.

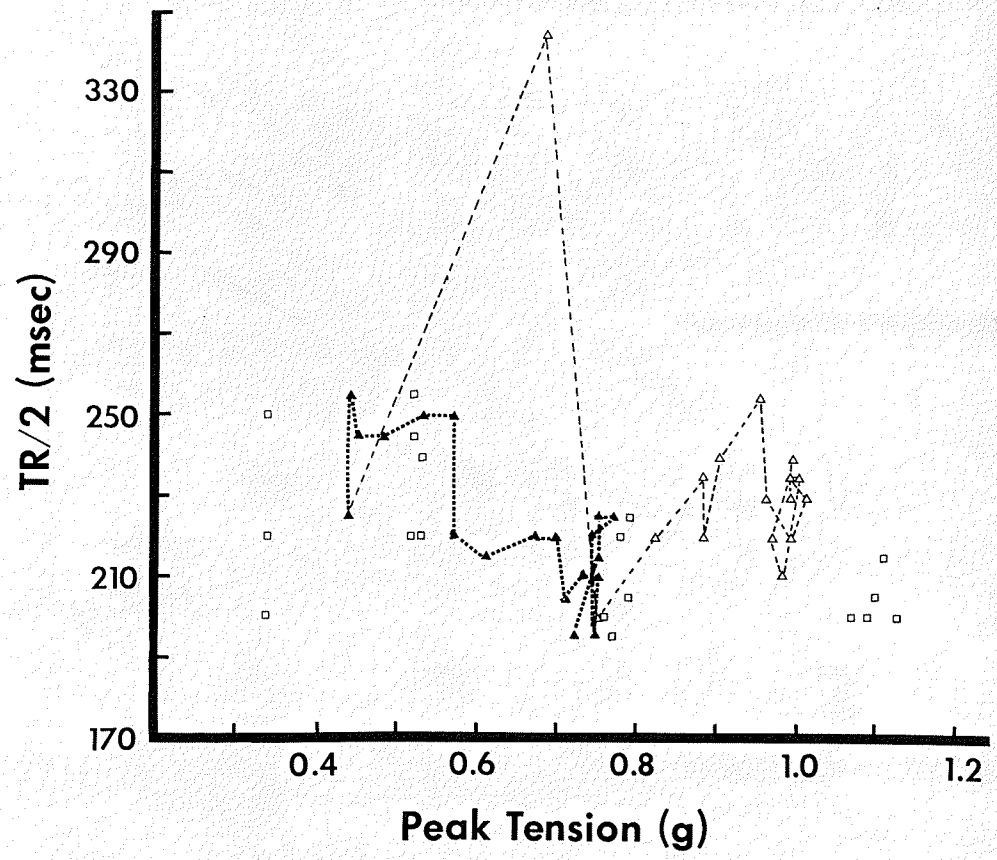
During recovery from hypoxia MRR is depressed to a much smaller extent than in 2.5 or 5.0 mM calcium. Reoxygenation causes the initial depression in MRR and the surge of recovery in peak tension seen in 2.5 and 5.0 mM calcium. From this point, however, MRR appears to recover in tandem with peak tension, so that recovery is parallel to the interval/strength line. In both muscles peak tensions higher than control values at maximum rates of relaxation that were relatively depressed, were achieved after 10 minutes of reoxygenation.

The lower portion of Figure 18 indicates the effect on TR/2.

Figure 18

The effects of hypoxia and of reoxygenation in 1.25 mM calcium. The interval/strength effect is the baseline (○ and □). Protocol as in Figure 16. Note the lack of speeding of MRR during hypoxia, and the smaller depression during reoxygenation, compared to Figures 16 and 17.


6.0
4.0
2.0
MRR (g/sec)
0.4
0.6
0.8
1.0
1.2
Peak Tension (g)


330
290
250
210
170
TR/2 (msec)
0.4
0.6
0.8
1.0
1.2
Peak Tension (g)

Lack of deflection during induction of hypoxia is consistent with the lack of deflection of MRR from the interval/strength line. The pattern during recovery is scattered, however. The other muscle examined did not show these inconsistencies during recovery.

In summary : 1. Induction of hypoxia in 2.5 or 5.0 mM calcium causes a speeding of maximum rate of relaxation relative to peak tension. This speeding does not occur in 1.25 mM calcium. In terms of calcium movement, it is unexpected that the smaller intracellular calcium load that would be expected in 1.25 mM calcium should not be handled even more readily, i.e. produce an even greater speeding of MRR, than 2.5 or 5.0 mM calcium.

2. In contrast to the greater depression of MRR by 5.0 mM calcium compared to 2.5 mM calcium during recovery from drive (Figure 13), no difference could be shown between these concentrations on recovery from hypoxia. This may be due to the lack of paired comparisons, and the differences in strength between muscles.

In 1.25 mM calcium the degree of depression was definitely less than in 2.5 or 5.0 mM. The duration could not be compared due to failure to monitor recovery until beats returned to the line. This was an inadequacy in the protocol.

3. Changes in TR/2 parallel those in MRR at all calcium concentrations during induction of hypoxia. This consistency held during recovery in 2.5 mM and 5.0 mM calcium, but 1 of 2 muscles in 1.25 mM calcium showed discrepancies. The consistency of change in TR/2 following reduction in calcium concentration from 2.5 to 1.25 mM (Figure 12),

and on recovery from drive in 1.25 mM calcium (Figure 15) may therefore not hold during recovery from hypoxia in 1.25 mM calcium.

4. EFFECTS OF ADRENALINE

The property of adrenaline to speed relaxation has been reported by many workers (4, 38, 39, 40, 42). We examined the effects of 5×10^{-8} g/ml of adrenaline on the relationship of peak tension to maximum rate of relaxation, and on time to 50% decline in tension.

The procedure was as follows : after recording the interval/strength relationship, the muscle was stabilized at the lowest frequency, 3 per minute. 5 events were recorded. Adrenaline was added, and 5 beats were recorded when the beats had stabilized, after 30 seconds to 1 minute. The bath was rinsed twice, and frequency raised to the next highest. After 5 minutes the bath was rinsed twice again, and left 10 minutes more. Stabilized beats were recorded, and adrenaline 5×10^{-8} g/ml was added as before.

The upper portion of Figure 19 shows effects on maximum rate of relaxation (MRR). As occurred previously, the speeding of MRR in absolute terms is in fact shown to represent depression when related to the increase in peak tension produced. The shift in the interval/strength relationship resembles that produced by increased calcium concentrations (Figures 9 and 10). In the muscle shown in Figure 19, the slope of the shifted line is reduced. In a second muscle the shift was approximately parallel, and in the third, the increase in MRR failed to parallel increase in peak tension

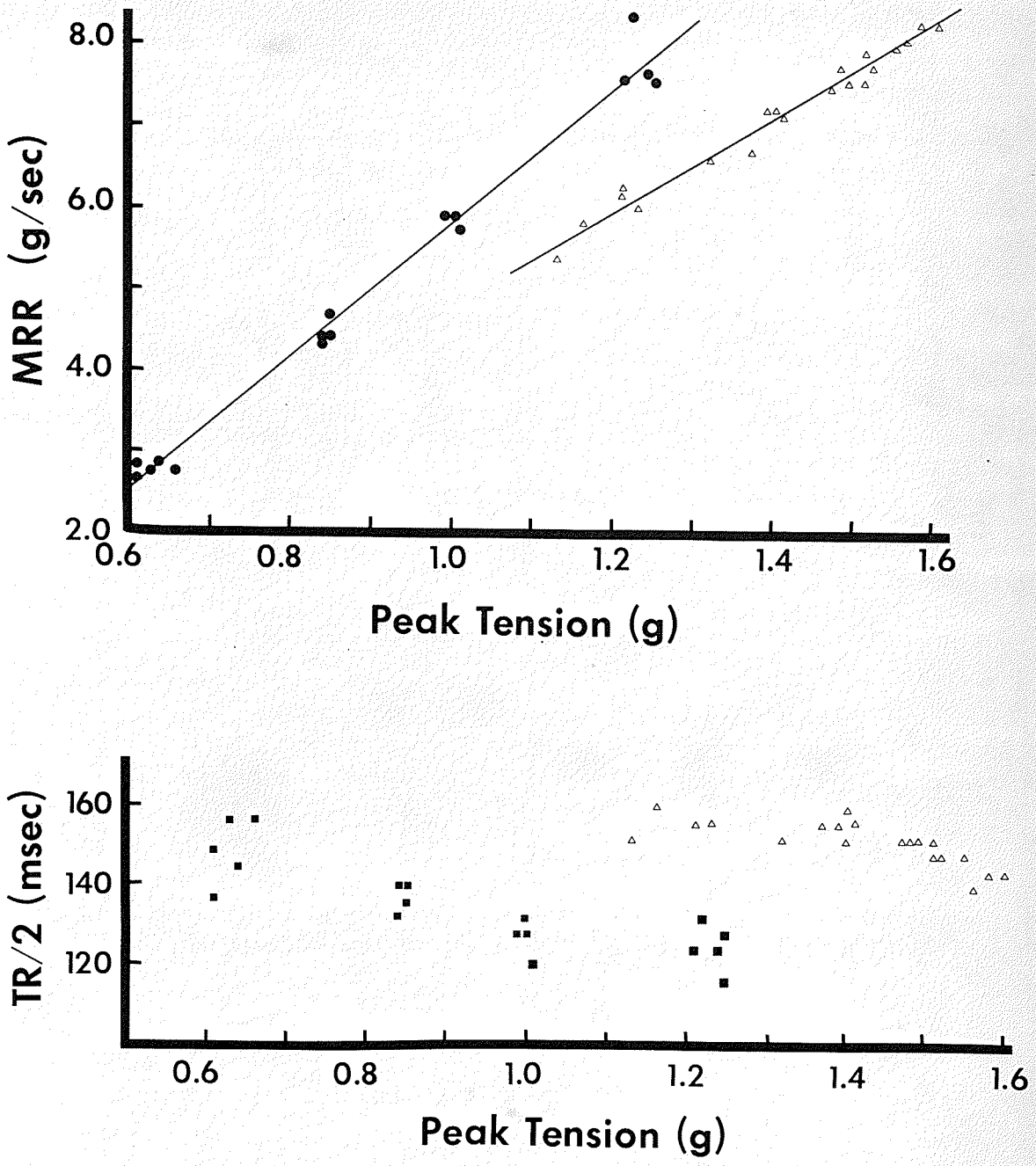


Figure 19

The effect of the addition of adrenaline 5 x 10^{-8} g/ml. The interval/strength effect was first determined in 2.5 mM calcium (● and ■), and was then determined again in the same muscle in the presence of adrenaline (△). Note the similarity of these results to those of calcium (Figure 10).

at the highest frequency, and a plateau occurred as it had in some muscles at 5.0 mM calcium (see above).

The lower portion of Figure 19 shows the effect on time to 50% decline in tension in the same muscle. In each of three muscles examined, increase in TR/2 occurred at all frequencies, in parallel with the slowed MRR.

Workers reporting speeding of relaxation by catecholamines all used concentrations higher than 5×10^{-8} g/ml. We therefore decided to do a dose/response determination at concentrations of adrenaline of 1, 4, 16, 64, and 256×10^{-8} g/ml.

High calcium concentrations have been used in adrenaline studies (38, 39) to achieve a maximum inotropic effect so that effects on relaxation can be seen in the absence of increases in peak tension.

Reductions in sodium concentration have also been shown to have a positive inotropic effect, effective calcium concentration being proportional to $(Ca^{++})/(Na^{+})^2$ (21). We therefore replaced 43 mM (30%) of sodium by an osmotic equivalent of sucrose and used 6.25 mM calcium. Higher concentrations of calcium precipitated out of our solution. At the frequency used (12 per minute) and the highest concentration of adrenaline (2.56×10^{-6} g/ml) a maximum inotropic effect was observed only in one of three preparations (see below).

The procedure was as follows : after recording the interval/strength relationship, the muscle was allowed to stabilize at 12 per minute and 5 beats recorded.

Increments of adrenaline were added up to the next highest dose

2 minutes after adding the previous dose. Beats were allowed to stabilize, and 5 consecutive signals were then recorded.

Evidence of breakdown of adrenaline in the form of a change in color was not seen up to 10 minutes after the first addition, i.e. after completion of the expériment. Monitoring the effects of the highest doses 12 minutes after addition of the lowest dose showed no decline from the maximum peak tension achieved. We therefore concluded that we were observing the effects of the full concentration 8 minutes after the first addition when the final events were recorded.

The upper portion of Figure 20 illustrates effects on the relationship of peak tension to maximum rate of relaxation (MRR). The flattening of the interval/strength line due to elevated calcium concentrations, as mentioned previously, is shown clearly here.

At concentrations of up to 16×10^{-8} g/ml of adrenaline, peak tension is increased more than MRR, causing shift to the right. This concentration exerted very close to the maximum inotropic effect, and caused some speeding of MRR. At 64 and 256×10^{-8} g/ml, speeding of MRR relative to peak tension is quite apparent. These results occurred in each of 3 muscles examined.

The lower portion of Figure 20 shows the effects on time to 50% decline in tension in the same muscle. In all three muscles the course of reduction in TR/2 parallels speeding of MRR at the higher concentrations of adrenaline.

Figure 21 indicates the relationship of concentration of adrenaline to maximum rate of relaxation under the same conditions as above. In this

Figure 20

The effect of increasing concentrations of adrenaline at a single frequency, 12 per minute, in 6.25 mM calcium with 43 mM sodium replaced by sucrose. The interval/strength effect at these concentrations is the baseline (○ and □). Note the depression of MRR produced at 30 per minute in the high calcium concentration. Concentrations of adrenaline added were 1×10^{-8} g/ml (▲), 4×10^{-8} g/ml (△), 16×10^{-8} g/ml (■), 64×10^{-8} g/ml (+), and 256×10^{-8} g/ml (●). Note the lack of prolongation of TR/2 at 4×10^{-8} g/ml, compared to the prolongation produced by 5×10^{-8} g/ml in 2.5 mM calcium in Figure 19.

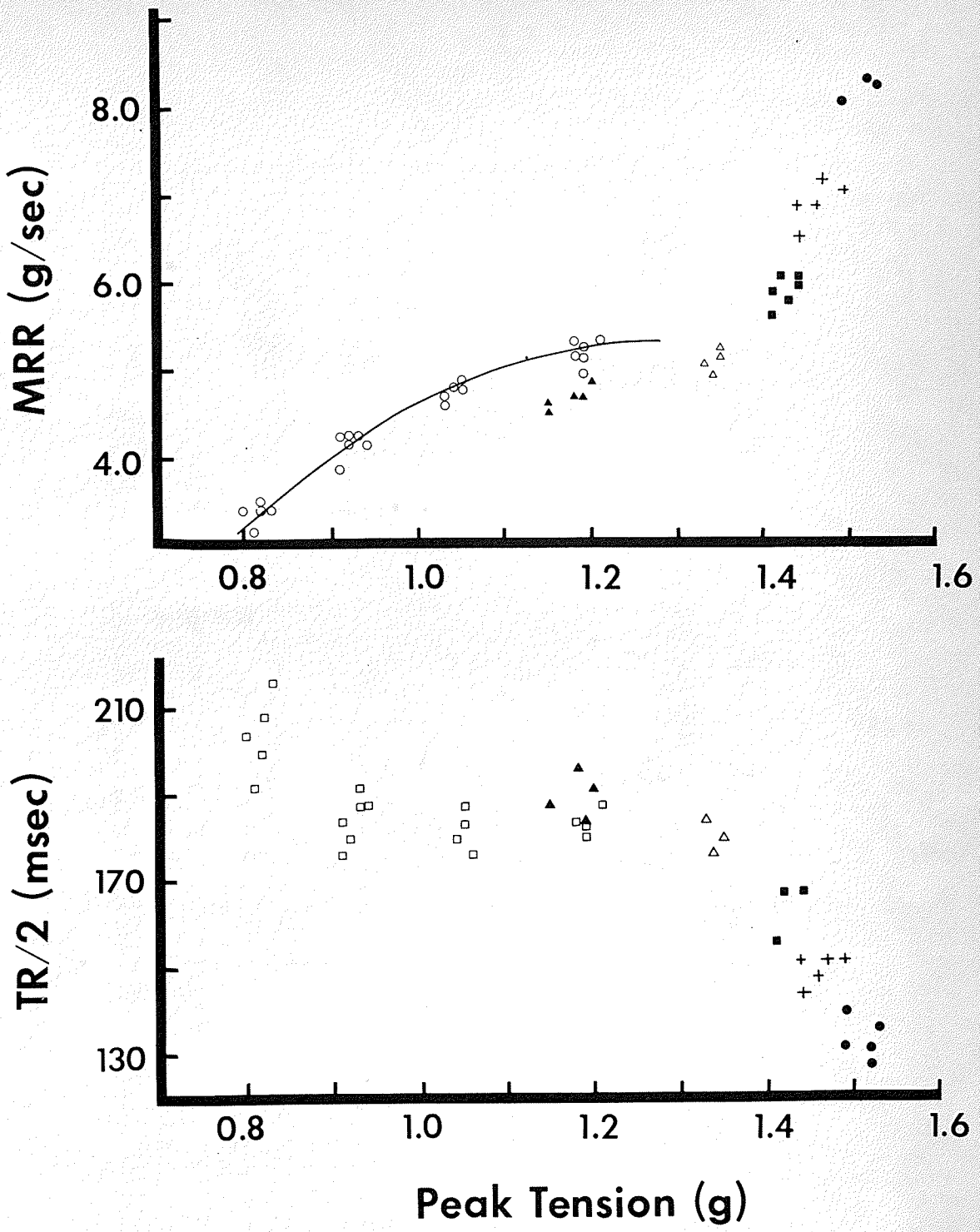
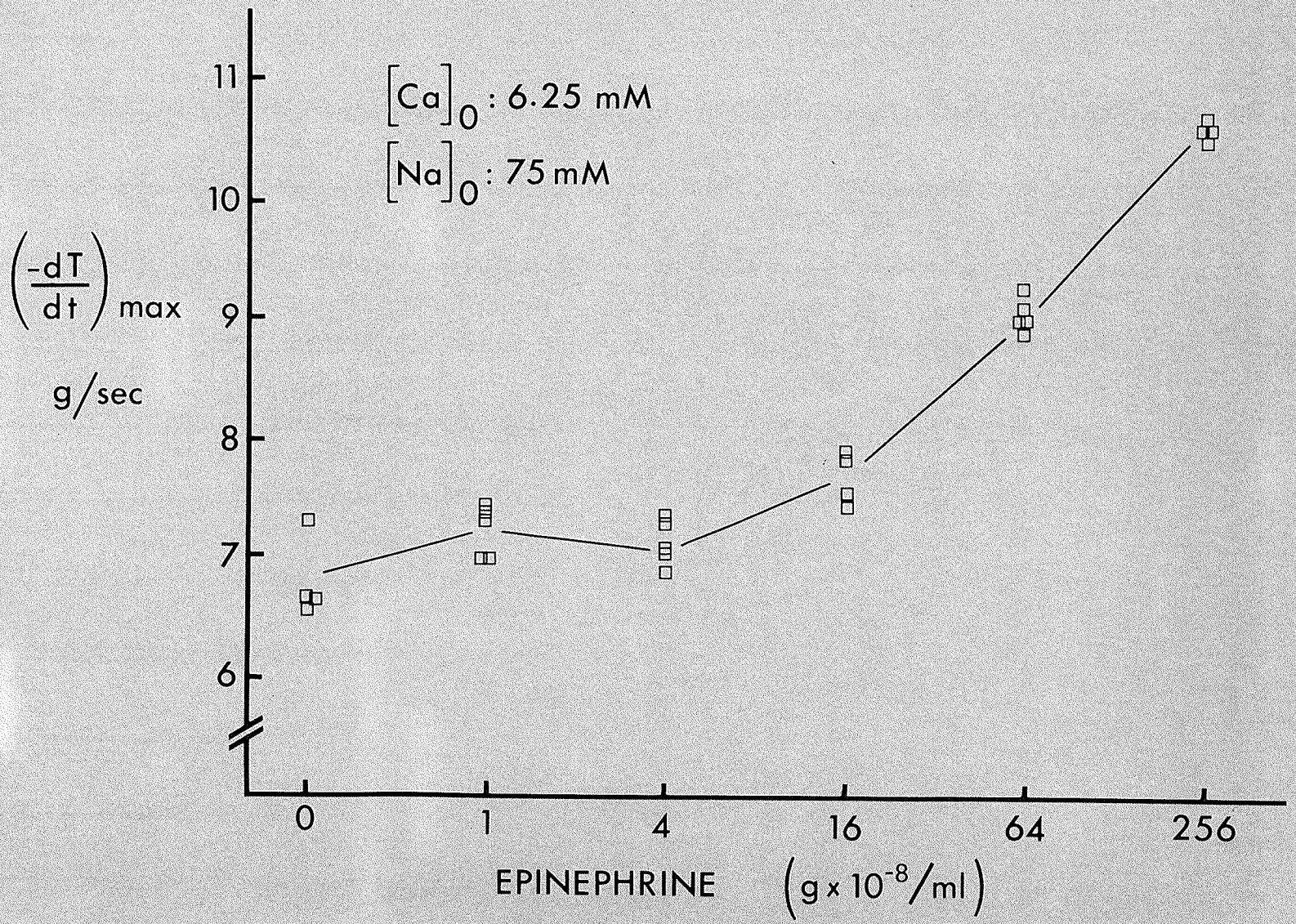

MRR (g/sec)
8.0
6.0
4.0
0.8
1.0
1.2
1.4
1.6
TR/2 (msec)
210
170
130
0.8
1.0
1.2
1.4
1.6
Peak Tension (g)

Figure 21

The influence of increasing concentrations of adrenaline on beats at 12 per minute. Protocol was as in Figure 20. In this muscle, no significant positive inotropic effect was seen at any concentration of adrenaline.


$\left(\frac{-dT}{dt}\right)_{max}$
g/sec
11
10
9
8
7
6
$[Ca]_0$: 6.25 mM
$[Na]_0$: 75 mM
0
1
4
16
64
256
EPINEPHRINE $(g \times 10^{-8}/ml)$

muscle peak tension did not increase significantly at any concentration of the drug. The lack of increase of MRR at lower doses, followed by progressively greater increases at higher doses is clearly displayed.

To summarize the effects of adrenaline :

1. Slowing of maximum rate of relaxation and increase in time to 50% decline in tension at all frequencies by 5×10^{-8} g/ml of adrenaline was the same pattern observed on changing calcium concentration from 2.5 mM to 5.0 mM (Figure 10).

2. The adrenaline dose/response effect is consistent with a positive inotropic effect by adrenaline at all concentrations used, while the effect on relaxation only appears at higher concentrations.

3. Changes in time to 50% decline in tension consistently parallel changes in MRR at different concentrations of adrenaline.

SECTION IV

DISCUSSION

DISCUSSION

It has long been accepted that measurements of the contraction phase of the isometric event reflect actual processes of activation. Maximum rate of contraction (MRC) is thought to parallel the rate of activation of force-generating sites per unit of cross-sectional area, or the rate of delivery of calcium to contractile proteins (59). Peak tension is considered to be the resultant of interaction between intensity of active state (MRC) and the duration of maximum intensity of active state measured as time to peak tension (TTP). These two indices have been shown to be affected to different degrees by such interventions as changes of frequency (51), and adrenaline (42).

When cardiac muscle beats more rapidly, TTP declines but MRC increases. Up to a frequency of 60 to 180 per minute in cat papillary muscle, the increase of MRC outweighs the decline of TTP and peak tension increases. The total duration of the event also becomes shorter (30). The ability of cardiac muscle to beat more rapidly without going into contracture may be due to more rapid attainment of peak activation, earlier decline, more rapid decline, or some combination of these. Study of the relaxation phase, then, must touch on some aspect of this fundamental property of cardiac muscle.

Most workers avoid a measure of relaxation analagous to MRC, i.e. maximum rate of relaxation (MRR). Also, they generally use only one measure, and do not relate it to peak tension. The most common measures of relaxation are durations, such as time to 50% decline from peak tension (TR/2), or

time to 90% decline (RT). When MRR is used, there is no indication of what it might be a measure of.

Average rates are composites of the duration and intensity of onset or decline of active state. Any change in an average rate would require separate determinations of duration and of intensity. Therefore it seems most sensible to determine these indices separately from the beginning.

Series elastic element stiffness has been shown to be independent of contraction frequency (9), of muscle length (10), unchanged by up to 60 minutes of hypoxia (11) and to increase in proportion to tension exerted, independent of the phase of the contraction/relaxation cycle (7, 8).

Relaxation, then, can be considered a function of the interaction between declining activation (9, 15), and the elasticity of the stretched series elastic element (SEE). This interaction would be analagous to an independently driven ratchet (level of activation) intersperced between the tiers of and interfering with the shortening of a stretched spring (SEE). Series elasticity is directly proportional to tension, and is independent of other factors except large reductions in temperature (12), and changes in osmolarity of 100 m osmoles or more (13). Both of these conditions were held constant in our experiments.

SEE will contribute to muscle relaxation to a degree directly proportional to the level of tension at any instant. The nature of its contribution will be always the same.

The location and behaviour of viscous elements is still an area of controversy. Changes in viscous compliance may affect indices of duration

of relaxation, especially relaxation time (RT). Changes in viscous compliance have been shown to be proportional to peak developed tension (3, 5). Therefore if indices of relaxation are related to peak tension, the contribution of viscous elements can be taken as a constant, provided further studies show that given interventions do not change the properties of the viscous elements. Such studies have not been done to the extent that they have been done for SEE.

Changes in characteristics of relaxation could therefore be considered to be due to changes in the decline of activation. The declining level of active state shown to persist during the relaxation phase (9, 15) would be due to actinmyosin bond formation in proportion to the declining concentration of calcium in the myofibrils. Relaxation would be prolonged by any factor causing systems of calcium sequestration to compete less effectively with troponin binding sites for free calcium. The rate of decline of active state would be increased by any factor increasing the rate of calcium sequestration.

Unless calcium sequestration begins abruptly at the peak of active state, it is not unlikely that it begins at some point, perhaps at some critical intracellular calcium concentration, before peak activation (31, 60). As peak active state is so short that the contractile element is thought not to develop the tension it is capable of, removal of calcium from the cycle of binding to and unbinding from troponin sites would therefore not only influence the duration of maximum intensity of active state, measured as time to peak tension (TTP), but might also limit the maximum intensity itself, i.e. peak tension.

Skeletal muscle can utilize internal stores of calcium and beat for hours in a calcium-free medium (17). Nonetheless, internal redistribution of calcium from sequestration to storage sites has been shown to be necessary for complete recovery (i.e. complete relaxation) following a tetanus (71).

Calcium efflux from cardiac cells occurs during the contractile event (21). Lanthanum, a metal shown to bind on the external surface of the sarcolemma and not to penetrate (21), has been shown to retard calcium movement in a compartment tentatively associated with relaxation in the gas-perfused kitten heart (72). This was associated with some degree of contracture. Removal of Lanthanum restored this compartment and reduced contracture.

Efflux of calcium may be necessary for complete relaxation in the cardiac cell. Calcium movement from sequestration sites to the sarcolemma and out may be a part of normal relaxation. Lanthanum, then, might retard relaxation by causing a backup of calcium from blocked efflux sites, eventually causing saturation of sequestration sites and contracture.

To the extent that the course of the isometric event parallels the course of active state (4, 14), the following indices of relaxation may be considered :

Time to 50% decline in peak tension (TR/2) may be considered to indicate the rate of decline of active state, or the rate of calcium sequestration. Increase in TR/2 at a given level of peak tension and at a given frequency may indicate a reduced rate of sequestration, or an increased load delivered. Decrease in TR/2 under these conditions might

indicate increased rate of sequestration. TR/2 would be an extremely useful index if it were independent of peak tension, but our results show this relationship to be inconsistent. However, in the presence of inotropic interventions other than changes in frequency, as drive or hypoxia, it appears to register change as consistently as maximum rate of relaxation.

Maximum rate of relaxation (MRR) reflects that point at which rate of return to resting length is maximal. It may be considered to represent the point of maximum rate of calcium removal and decline of activation. In the presence of change in frequency of contraction it appears to be a better index of relaxation than TR/2 as it has been shown in our results to display a consistent dependence on peak tension that is independent of muscle length or thickness. It is also consistently affected by other interventions.

Reductions in calcium concentration verify neither index, for reduction per se causes inconsistencies in MRR, while reduction in the presence of hypoxia appears to cause inconsistencies in TR/2.

Relaxation may be prolonged by retardation of sequestration of calcium, or by prolonged release of calcium to a sequestering system of unimpaired activity. Neither MRR nor TR/2 should be able to distinguish between these two possibilities, because the presence of a higher concentration of calcium for a longer period of time should affect both indices so as to indicate slowed or prolonged relaxation.

Time to peak tension (TTP) is a measure not only of the time during which active state is increasing, but also of the point at which relaxation begins. Interventions that increase the intensity of active state may shorten it, such as catecholamines (42), or prolong it, such as methylxanthines

(22). These drugs are known to have opposite and opposing effects on relaxation (22).

TTP progressively shortens as frequency of contraction is increased (30). TTP is reduced when extracellular calcium concentration is raised at a given frequency (37, 38, 50). If TTP may be considered to mark the point at which processes responsible for decline of active state, which we would equate with calcium sequestration, become dominant over those responsible for increase (31), the above data might suggest that some mechanism limits the degree to which activation can occur under a given set of conditions. Some factor, possibly a critical intracellular free calcium concentration, may trigger sequestration at this point.

Catecholamines (42), increases in frequency (51), increases in external calcium concentration (21), and methylxanthines (22) have all been suggested to increase activation by increasing the quantity of calcium delivered to the myofibril. The first three have been shown to shorten TTP, consistent with an earlier attainment of the critical intracellular calcium concentration, or, in the case of higher concentrations of adrenaline (40), possible stimulation of sequestration. Methylxanthines, which prolong TTP, have been shown to impair uptake of calcium by isolated sarcoplasmic reticulum (70), suggestive of a direct inhibition of sequestration independent of the positive inotropic effect (22).

On increasing frequency of contraction in 2.5 mM calcium, maximum rate of contraction and peak tension increase. Calcium influx with each action potential has also been shown to increase (18). Increases in maximum rate of relaxation (MRR) proportional to peak tension shown in Figure 3

indicate that calcium-sequestering sites can handle the increasing calcium load due to this factor, and that the rate of sequestration adapts to the load (21).

Increase in external calcium concentration is correlated with increased peak tension and maximum rate of contraction at a given frequency. On increasing to concentrations of 4.0 mM or greater, calcium entry does not increase in proportion to the concentration increase (21). The interval/strength line for the relationship of peak tension to MRR shifts in the direction of slowed MRR at a given peak tension (Figures 9 and 10). Some prolongation of calcium sequestration might also be indicated by prolonged time to 50% decline in tension (TR/2) on increasing calcium from 2.5 to 5.0 mM (Figure 10). This might indicate that calcium sequestration sites cannot handle increased load due to this factor. This seems unlikely if the capacity and adaptability to load is as high as reported (21).

Release from release sites for a longer period of time may be involved. If efflux of calcium from the cell is a necessary final step in a chain of calcium movements involved in relaxation, some backup effect from efflux sites may occur due to the higher gradient. The data of Reuter and Seitz (61) showing increased efflux in the presence of a higher external calcium concentration may be quoted as evidence against this latter possibility. The failure of TR/2 to increase on changing from 1.25 to 2.5 mM calcium, despite depression of MRR, is difficult to reconcile with any mechanism proposed.

Reduction in external calcium from 2.5 to 1.25 mM produced varied effects. Prolongation of TR/2 (Figure 12) in all muscles suggests a

prolongation of sequestration that was not verified by the direction of change of MRR in all muscles. A reduction of slope of the relationship of peak tension to MRR in all muscles at the frequencies used does indicate that the increase in MRR in proportion to increases in peak tension was less in 1.25 mM than in 2.5 mM calcium. These would seem to be indications of a greater load on the sequestration system, for, as previously stated, there is no reason to suspect that the sequestering system itself should be impaired by simple change of calcium concentration. This greater load is unexpected in view of the reduced external concentration. Backup due to retarded efflux because of a reduced gradient across the sarcolemma (61), and consequent overload of sequestration sites would fit the data, but would mean that the rules of calcium handling change depending on the initial calcium concentration, and the direction in which concentration is changed. The effects of reduction in calcium concentration on the relationship of peak tension to maximum rate of relaxation are ambiguous, and deserve further study.

The effects on relaxation of drive and hypoxia may also be explained in terms of calcium sequestration.

Drive (Figures 13 and 14) would greatly increase the quantity of calcium delivered to this system. As long as drive were maintained, whatever mechanisms that adjust relaxation rate and duration in proportion to frequency of contraction would remain operative, and the load would be handled at a rate appropriate to the frequency.

Koch-Weser (30) has shown that time to peak tension (TTP) adjusts rapidly to changes in frequency, while intensity of active state, equated

with quantities of calcium released (59), adjusts more slowly. He used this rapid adaptation of duration of active state measured by TTP, and slower adaptation of intensity $(dT/dt)_{max}$, to explain the inotropic effects on initial beats following changes in frequency. If we consider TTP to be also an indication of a frequency-dependent calcium sequestration process, this process is seen to adapt rapidly to change in frequency, while the calcium release system adapts more slowly. Thus large quantities of calcium would for a short time continue to be delivered to sites of sequestration which have suddenly adapted their activity to a much smaller load. Slowing of MRR relative to peak tension following drive would then indicate the inability of the system to handle a load appropriate to the high frequency with an activity appropriate to the reduced frequency. Some impairment of the supply of energy to sequestration sites could also contribute to the depression of relaxation (See Hypoxia below).

Recovery of relaxation parameters would occur as the load were progressively reduced in the face of declining quantities being released. As the capacity of the system need not be impaired, except in the very core of a thick muscle, recovery of maximum rate of relaxation (MRR) to values appropriate to the peak tension, i.e. return to the interval/strength line with time, would occur as soon as intracellular free calcium was reduced to manageable levels. During recovery from drive, the increase in TR/2 paralleling the depression of MRR, and the fact that depression is greater in the presence of a higher calcium concentration (Figure 13), and less in the presence of reduced calcium (Figure 14) further support an explanation in terms of calcium handling.

The effects of hypoxia are somewhat more complex. Periods of prolonged ischemia have been found to cause uptake of large amounts of calcium, largely by mitochondria (62, 63). Increased uptake of calcium at reduced oxygen tensions (2% to 5%) in a Langendorff preparation of whole kitten heart perfused with 20% blood in Krebs has been observed as well (Bailey and Pang, unpublished). Increase in maximum rate of relaxation relative to peak tension as contractility declines (Figures 16 and 17) in 2.5 mM and 5.0 mM calcium is therefore unexpected, since increased influx would increase the load of calcium that systems of sequestration are nonetheless apparently able to handle at an increased rate. Reduction in TR/2 confirms this facilitation of calcium handling. Failure of MRR to be speeded in 1.25 mM calcium (Figure 18), when presumably less calcium will enter the cell, is also paradoxical, albeit consistent with some degree of prolongation of calcium handling suggested by the effects of reduction in calcium concentration from 2.5 mM to 1.25 mM discussed above.

There are several possible explanations for this speeding of maximum relaxation rate in 2.5 mM and 5.0 mM calcium.

1. Constant infusion of carbon dioxide during induction of hypoxia prevents extracellular pH changes. Intracellular pH may well be decreasing, however, due to accelerated production of lactate by anaerobic glycolysis (65, 66).

Increased concentration of hydrogen ions has been shown to increase the affinity for calcium of isolated calcium-sequestering preparations, while reducing the affinity of troponin sites (35). Increased sequestration in parallel with reduced activation would speed MRR relative to peak tension.

Bing et al. (64) have recently reported the influence of reducing external pH on the effects of 60 minutes of hypoxia on rat papillary muscles and trabeculae at 28° C stimulated at 12 per minute. External pHs of 6.8 and 7.1 increased the depression of peak tension by hypoxia as compared to pH 7.4. Levels of contracture during hypoxia were reduced at pH 7.1, and did not occur at all at pH 6.8. Reduced pH therefore had a protective effect on properties of relaxation. However : (1) change in external pH may not necessarily have caused similar changes intracellularily. (2) external changes in pH may have acted via effects on the sarcolemma, rather than on calcium sequestration.

2. The work of MacLeod and various coworkers (73, 74) on guinea pig papillary muscle has suggested that metabolic depression produced by hypoxia leads to a decreased duration of the cardiac action potential as a result of decreased phase 2 slow inward current. Phase 2 has been associated with influx of calcium that contributes to contractility (20, 27) and reduction thereof could be responsible for decline of developed tension. After prolonged periods of anoxia (3 hours) developed tension has stabilized at a low level. At this point, increases in extracellular glucose concentration from 5 mM to 50 mM will partially restore the plateau phase in parallel with some increase in developed tension (74). As the sole source of energy in the anoxic muscle would be glycolytic ATP, this suggests a link between glycolytic ATP and movement of calcium across cell membranes.

The speeding of maximum rate of relaxation on induction of hypoxia parallels the large increase in production of glycolytic ATP due to stimulation of glycolysis (65, 66, 67). This is certainly suggestive

evidence of involvement of glycolytic ATP in calcium sequestration.

Depression of MRR within 1 to 2 minutes of reoxygenation parallels the large reduction in glycolysis indicated by the Pasteur effect (68). If during recovery oxidative phosphorylation is even more than usually favored in competition with glycolytic sites for ADP, then any process dependent on glycolytic ATP will be depressed. The prolonged depression of MRR even after peak tension has recovered to values appropriate to the frequency in 2.5 mM and 5.0 mM calcium, with extensive increase in TR/2 (Figures 16 and 17), fits the pattern of impaired calcium sequestration. The fact that depression of MRR is not greater in 5.0 mM calcium than in 2.5 mM, if it is genuine and not due to the lack of paired comparisons, could also suggest that the primary cause is an impaired mechanism of sequestration rather than an effect of excessive load of calcium, which should be greater in 5.0 mM calcium.

The effects of hypoxia in 1.25 mM calcium (Figure 18) are consistent with a reduced load that the impaired sequestration system can handle almost adequately. It would be of great interest to determine if the duration of depression of MRR relative to peak tension is as great as in 2.5 or 5.0 mM calcium. This would indicate that the main effect of recovery from hypoxia might be impairment of calcium sequestration, possibly due to reduced supply of glycolytic ATP. Impairment would take a definite period of time to overcome, dependent on the duration of hypoxia and, unless the extracellular calcium concentration and consequently the load delivered were extremely small, independent of calcium concentraion.

Any hypothesis for the effects of hypoxia must ultimately account

for the failure of induction of hypoxia in 1.25 mM calcium to speed MRR.

The effects of 5×10^{-8} g/ml of adrenaline on the interval/strength relationship are exactly the same as that of 5.0 mM calcium compared to 2.5 mM calcium (Figure 19 compared to Figure 10). The depression of MRR relative to peak tension, and the prolongation of TR/2 are both consistent with the delivery of an increased calcium load, or prolonged delivery, to a sequestration system that has not been stimulated. It would appear that at this concentration the predominant effect, or the only effect, of adrenaline is its positive inotropic effect. Depression of MRR could then be due to the mechanisms suggested above for increase in extracellular calcium concentration. Failure of previous workers (See Introduction) to note this effect would be due to their use of much higher concentrations of adrenaline, and to failure to relate changes in indices of relaxation to changes in the peak force developed, i.e. to allow for inotropic effects. The dose/response determination might indicate some ceiling on the degree to which activation can be increased, while stimulation of the sequestering system continues to intensify. At higher concentrations of adrenaline a now relatively constant amount of calcium being delivered would be handled with increasing facility until the speed of relaxation might become disproportionately high, and constitute a limiting factor to the degree of activation that could be achieved (40). In the presence of calcium concentrations so high as to prevent positive inotropic effects, adrenaline 2×10^{-6} g/ml will reduce peak developed tension and time to peak tension while speeding relaxation rate (42).

It may be meaningful that Shinebourne et al. (69) found noradrenaline to speed caclium uptake by cardiac microsomes only at concentrations

greater than 1×10^{-6} M, a concentration just above that (0.64×10^{-6} M) which is associated with marked speeding of MRR in our experiments.

Catecholamines (42) and methylxanthines (22) appear to exert similar positive inotropic effects on the contraction phase of the isometric event, via increased influx or potentiation of release of calcium, but to exert opposite effects on the relaxation phase. Adrenaline in higher concentrations appears to enhance calcium sequestration, shortening time to peak tension and speeding relaxation. Caffeine appears to inhibit calcium sequestration prolonging time to peak tension and slowing relaxation, perhaps due to the reported inhibition of calcium transport by sarcoplasmic reticulum (70).

Both classes of compounds stimulate glycolysis by increasing glucose input via glycogenolysis (68). Adrenaline stimulates production of cyclic AMP which activates phosphorylase. Methylxanthines prolong the activity of cyclic AMP by inhibiting the enzyme that degrades it.

Release of endogenous noradrenaline stimulated by procaine can prevent the prolongation of time to peak tension by caffeine. High concentrations of caffeine can prevent the reduction of time to peak tension caused by isoproterenol. In neither case are the positive inotropic effects interfered with (22). Our proposal that glycolytic ATP may be the source of energy for sequestration of calcium is difficult to reconcile with the opposing effects on relaxation of two drugs that affect the proposed pathway in the same way. It would be of interest to examine the effects of methylxanthines in terms of the relationship of peak tension to maximum rate of relaxation. Study of the interactions of methylxanthines and catecholamines

with insulin, which tends to inhibit glycogenolysis (68) might also be of value.

Conclusions may now be drawn in terms of the questions asked at the end of the Introduction under Statement of the Problem.

First, we have found an index of relaxation that displays a consistent relationship to peak developed tension, i.e. maximum rate of relaxation (MRR). This means that changes in patterns of relaxation can now be examined with allowance made for inotropic effects of the intervention used.

Second, interventions previously reported to influence relaxation, such as changes in calcium concentration, drive, hypoxia, and adrenaline, have been found to produce fairly consistent changes in this index.

Third, the majority of our results can be explained in terms of the handling of calcium by the cell. Some results, such as those due to hypoxia and adrenaline, may be explicable by effects on a particular source of energy, glycolysis.

Examination of the relationship of peak tension to maximum rate of relaxation for all interventions, particularily temperature and methyl-xanthines, may prove a very useful tool in determining their effects on decline in activation, perhaps even in terms of changes in patterns of calcium sequestration.

SECTION V

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BIBLIOGRAPHY

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AUTHOR INDEX

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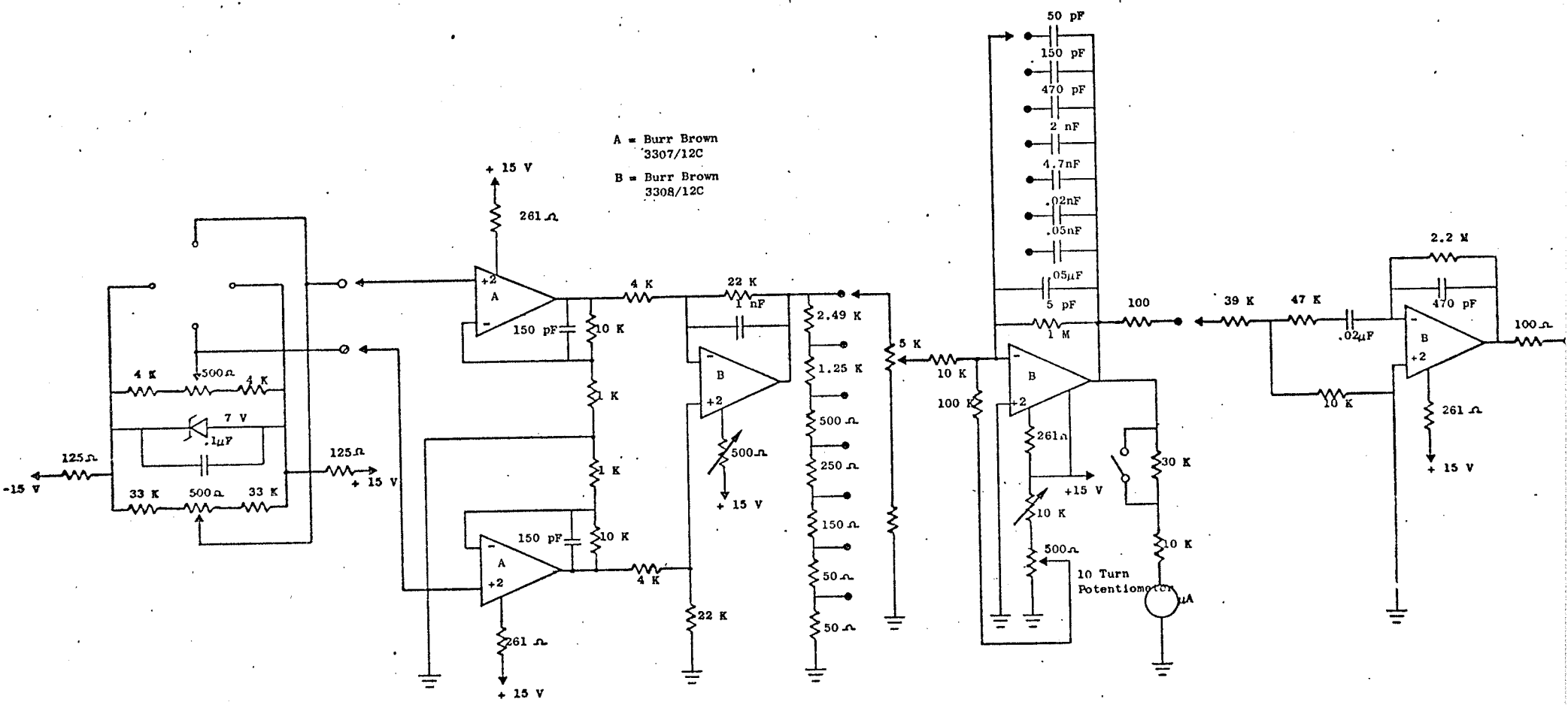
APPENDIX



Appendix 2 : Schematic diagram of amplifier and differentiator. Design by Prof. E.W. Mazerall and Mr. J.C. Delaive of this department.

PROGRAM 1

```
 CS-3-LABFOC-V3-E

01.10 C *** PED1   AUG 21 1972
01.15 E
01.20 S X=FCLK(4,3)
01.23 T !"SET SCOPE - 'X' VERTICAL, 'Y' HORIZONTAL, 'SET' DONE
01.24 A !":"X;I (X-ØX)1.24,1.25;I (X-ØY)1.24,1.27;I (X-ØSET)1.24;G 1.3
01.25 F J=1,30;F I=-500,50,500;S X=FDIS(I,Ø)
01.26 G 1.24
01.27 F J=1,30;F I=-500,50,500;S X=FDIS(Ø,I)
01.28 G 1.24
01.30 T !"CALIBR.   (WHEN DONE 'CTRL/C - NOISE - G 2.1)"
01.35 T !"TENSION RATE
01.40 S X=FSAM(2,256,1,2);D 9;S X(Ø)=Ø;S X(256)=Ø
01.50 F J=Ø,256,256;F I=1,5;S X(J)=X(J)+FB(I+J)
01.60 T !%3," "X(Ø)/5,"  "X(256)/5,"  "
01.70 G 1.4

02.10 C
02.20 F I=1,2;A !"WT:"W(I),"  Y:"YW(I)
02.30 F I=1,2;A !"RT:"R(I),"  Y:"YR(I)
02.40 S BW=(YW(2)-YW(1))/(W(2)-W(1));T !"WEIGHT SLOPE ="%5.1,BW
02.50 S BR=(YR(2)-YR(1))/(R(2)-R(1));T "  RATE SLOPE ="BR
02.60 F I=1,2;S X=FP(W(I)*100,I);S X=FP(YW(I),I+2)
02.62 F I=1,2;S X=FP(R(I)*10,I+4);S X=FP(YR(I),I+6)
02.70 A !"CALIB IN BLOCK:"Y; S X=FDTA(Y,Ø)

03.10 T !!"READY TO SAMPLE
03.20 S B=Y; T !!"EVENTS ACQUIRED
03.30 S X=FSAM(1,256,1,2);D 9;D 8
03.35 I (S)3.3;I (FITR(B/10)-B/10)3.4;T !!
03.40 S B=B+1;T %5,(B-Y);S X=FDTA(B,Ø);I (B-360)3.3
03.50 T !!!"TAPE FULL - MOUNT NEW TAPE - TYPE 'G 2.6'

08.10 S S=1
08.20 I (FB(FMA(1,250))-FB(10))3.4,8.4;I (FB(FMA(1,250))-FB(250))8.4,3.4
08.30 I (FB(260)-FB(FMI(260,500)))8.4,3.4
08.31 I (FB(FMA(260,500))-FB(510))3.4,8.4
08.32 I (FMA(260,500)-FMI(260,500))8.4,8.4;R
08.40 S S=-1

09.10 F I=1,10;S X=FCRT(1,512)
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PROGRAM 2

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 CS-8-LABFOC-V3-E

Ø1.1Ø C *** ANALJ OCT 25,1972
Ø1.2Ø E
Ø1.25 A !"CALIB IN:"Y;A !"SAMPLING RATE:"SR
Ø1.3Ø S X=FDTA(Y,1)
Ø1.31 F I=1,2;S U(I)=FB(I)/1ØØ;S YU(I)=FB(I+2)
Ø1.32 F I=1,2;S R(I)=FB(I+4)/1Ø;S YR(I)=FB(I+6)
Ø1.33 S BU=(YU(2)-YU(1))/(U(2)-U(1))
Ø1.34 S BR=(YR(2)-YR(1))/(R(2)-R(1))
Ø1.35 T !!"EVENT (TO CHANGE SR OR SEQUENCE TYPE CONTR/C,G 6.19 OR 6.2)
Ø1.36 T !!"  NO     FMAX     RR      TTP       RC       AR9     TR/2
Ø1.4Ø S B=B+1;T !%3,B
Ø1.5Ø S X=FDTA(B+Y,1)

Ø2.1Ø I (5-FILT(1,256,15))2.11;S X=FILT(257,512,15);G 2.2
Ø2.11 T "CANCEL";G 1.4
Ø2.2Ø S X=FRAV(1,256);S X=FRAV(257,512)
Ø2.3Ø S P(1)=Ø;F I=4,8;S P(1)=P(1)+FB(I);
Ø2.31 S P(1)=P(1)/5;C---SETS BASELINE(CF LINE 2.3)
Ø2.4Ø S I=FMA(1,256);S T(2)=I;S AM(1)=FB(I)-P(1);D 4;S I=T(2);G 2.42
Ø2.41 C--2.4 FINDS PEAK EXCEPT LASTCOMMAND WHICH STARTS TO FIND BEGIN
Ø2.42 DO 6.1;S I=8
Ø2.5Ø S I=I+1;I (FB(I)-P(1)-1Ø)2.5;C--RUNS UP THE CONTRACTION TO 1Ø
Ø2.6Ø S I=I-1; I (FB(I)-P(1)-2)2.7,2.7,2.6;C--RUNS BACK DOWN
Ø2.7Ø S T(1)=I;DO 6.1;S I=257; C--LAST COMMAND STARTS FIND END

Ø3.4Ø S RA=.1;D 7;D 6.1;S T(3)=TR;G 5.1
Ø3.41 C--TO FIND DT/DT MAX AND Ø-CROSSING POINT:

Ø4.1Ø S P(2)=FB(26Ø)
Ø4.2Ø S I=FMI(26Ø,512);S RC=FB(I)-P(2);D 6.1
Ø4.21 C--GETS POS DT/DT MAX;LAST COMM BEGINS FINDING Ø-CROSS
Ø4.3Ø S I=I+1;I (FB(I)-P(2))4.3;S T(6)=I-256;S AM(2)=FB(I-256)-P(2)
Ø4.4Ø S I=FMA(26Ø,512);S RR=FB(I)-P(2);DO 6.1;C---NEG DT/DT MAX
Ø4.5Ø S RA=.5;D 7;;S T(4)=TR
Ø4.8Ø C--- SEEKS TR HALF

Ø5.1Ø F I=1,5Ø; S X=FCRT(1,512); C----DISPLAYS
Ø5.2Ø T "  "%3.Ø2,(AM(1)/BU)," "%3.Ø2,(RR/BR)
Ø5.3Ø T "   "%3, (T(6)-T(1))*SR,"    "%3.Ø2(RC/BR),"    "
Ø5.31 T %3.Ø2,((.9*AM(1))/BU)/(T(3)-T(6))*(SR/1ØØØ)
Ø5.32 T "   "%3,(T(4)-T(6))*SR;G 1.4

Ø6.1Ø F J=I-2,I+2;S J=FP(FB(J)-5Ø,J); C---OFFSETS DISPLAY AT POINTS
Ø6.19 A "NEW SR:"SR
Ø6.2Ø A "  B:"B; S B=B-1; G 1.4

Ø7.Ø1 C>>>>>>>>>FINDS SPECIFIED PROPORTIONAL FALL
Ø7.1Ø S AR=(FB(T(2))-P(1))*RA+P(1);S I=T(2)
Ø7.2Ø S I=I+1Ø;I (FB(I)-AR)7.3;I (I-255)7.2
Ø7.3Ø S I=I-1Ø
Ø7.4Ø S I=I+1;I (FB(I)-AR)7.5;I (I-255)7.4;G 7.6
Ø7.5Ø S TR=I;R
Ø7.6Ø T "";S TR=1ØE1Ø;R
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