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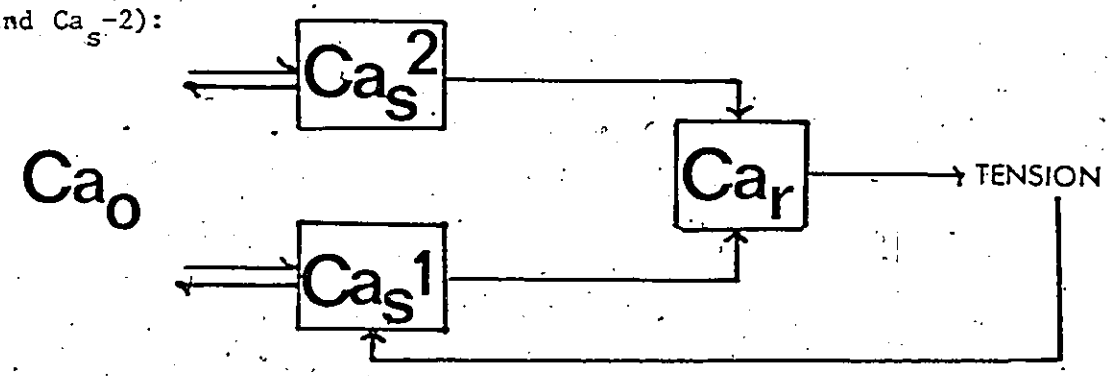
ABSTRACT

The effect of calcium concentration in the bathing medium, $[Ca^{++}]_o$, on the Tension-Frequency (T-F) relationship and post-stimulation potentiation (PSP) has been investigated in the isolated papillary muscle preparation of rat myocardium. Above 2.5 mM $[Ca^{++}]_o$ a negative inotropic T-F response is obtained and PSP is absent. Decreasing $[Ca^{++}]_o$ below 2.5 mM results in a positive inotropic T-F response and PSP is obtainable. The cardiac glycoside Ouabain is inotropically active (1.37×10^{-5} M.) only when effective $[Ca^{++}]_o$ is reduced below 2.5 mM. A decrease in the pH of the Tyrode medium by changing to 20% CO_2 from 5% CO_2 is analogous to a decrease in $[Ca^{++}]_o$ and Ouabain (1.37×10^{-5} M.) becomes positively inotropic at 2.5 mM $[Ca^{++}]_o$.

The effect of $[Ca^{++}]_o$ on the T-F relationship can be interpreted in a model of calcium movement in which a tension determining calcium pool (Ca_r) is recharged between stimuli from two stored calcium fractions (Ca_s-1 and Ca_s-2):



This is based upon analysis of tension recovery between successive rested state contractions (RSC's) in which a double exponential function

was fitted to RSC recovery. This recovery is characterized by a fast phase ($t_{\frac{1}{2}}$-1-0.5 seconds) and a slow phase ($t_{\frac{1}{2}}$ -2=0.5-10 seconds). High $[Ca^{++}]_o$ decreased the half time for transfer of Ca^{++} from Ca_s-2 to Ca_r. Furthermore the proportion of calcium donated to RSC recovery by Ca_s-1 increased along with an increase in $[Ca^{++}]_o$. Although a double exponential could describe RSC recovery our data suggests that a sigmoid shaped relationship between RSC tension and $[Ca^{++}]_o$ may contribute to the double exponential.

The T-F relationship at high $[Ca^{++}]_o$, (> 2.5 mM) can be explained by the function for RSC recovery. However in order to explain the positive inotropism in the T-F response at low $[Ca^{++}]_o$ the model predicts 1) that significant recycling from the myofilaments is dierected to Ca_s-1 and 2) that gating of calcium from the external calcium pool (Ca_o) with each stimulation will result in a redistribution of internal calcium stores, mainly to Ca_s-1.

The analysis of tension during RSC intervals also indicated that ouabain ($1x10^{-6}$M) resulted in an increase in the proportion contributed by Ca_s-1. This suggested that ouabain acts synergistically with calcium in redistributing internal Ca^{++}. Our data also suggests that a Na^+/Ca^{++} competition may be involved in the T-F and PSP response of the rat myocardium/

An ultrastructural complement to the model we have proposed would be where:Ca_r represents terminal cisternae, Ca_s-2 represents sarcolemmal Ca^{++} binding sites, Ca_s-1 represents longitudinal elements of the T system and recycling to Ca_s-1 is performed by the sarcoplasmic reticulum.

TO SANDY

Definitions

Steady-State Tension -- tension or force developed at a constant frequency of stimulation.

Tension - Frequency Relationship (T-F, Force - Frequency, Interval - Strength) - The steady-state tension developed at various frequencies of stimulation.

Negative Staircase - a tension - frequency relationship where steady-state tension falls with an increase in the frequency of stimulation.

Positive Staircase - a tension -- frequency relationship where the steady-state tension increases with an increase in the frequency of stimulation.

Post-Extrasystolic Potentiation (PESP) - an increase in tension following a premature stimulation.

Post-Stimulation Potentiation (PSP) - an increase in tension following a number of contractions at a higher frequency.

Rested State Contraction (RSC) - a contraction which is uninfluenced by events preceding it.

Rested State Contraction Interval - the interval necessary to produce RSC's.

Rested State Contraction Tension (RSC tension) - the tension produced at the RSC interval.

SECTION I

INTRODUCTION

Introduction

It is only in the past five years that a knowledge of the ultrastructural, biochemical and physiological characteristics of cardiac muscle have accumulated to the extent that we can begin to answer the question: "How does cardiac muscle develop tension?" (Katz, 1970; Sonnenblick and Stam, 1969). This knowledge is now being applied to analyze the contractile behaviour of the whole heart.

A most fundamental question posed in clinical cardiology is how to increase the work performed by heart muscle. This is especially true with diseased heart where the load on the myocardium may be increased by intrinsic or extrinsic mechanisms.

There appear to be two mechanisms whereby cardiac muscle contractile force is increased. The first is known as the Frank-Starling mechanism and involves a change in muscle length; it is now believed to involve the stretching of muscle fibers to an optimum sarcomere length (z-z line distance). Thus, an increase in z-z line distance up to about 2.2 μ produces maximum developed tension; while stretching cardiac muscle beyond the 2.2μ sarcomere length will decrease developed force from the muscle. This sarcomere distance is thought to represent myofilament overlap and provide optimum crossbridge interaction between actin and myosin filaments (Katz. 1970; Brady, 1968; Grimm and Whitehorn, 1968).

The second major intervention known to control contractility

is the Woodworth or Bowditch Staircase; in isometrically twitching cardiac muscle, an increase in the frequency of contraction will produce an increase in contractile force. Over a wide range of frequencies, this curve often has three components ; a negative inotropism followed by a positive inotropism and beyond a critical frequency a negative staircase. An example of this is shown in Figure I-1 for Guinea Pig ventricular papillary muscle in vitro.

In vivo, a physiologist would have very little control over the Frank-Starling mechanism, but the Bowditch mechanism can potentially be controlled by pacing to produce the desired frequency of stimulation (Kedem, Mahler and Rogel, 1969; Rogel et al., 1971) and increase the force of contraction, or velocity of tension development.

An understanding of the underlying events producing the Bowditch Staircase would thus be valuable both to the pure physiologist and perhaps to the clinician in choosing an appropriate treatment for a failing heart.

In this thesis a study has been made of the interval-strength relationship in isolated papillary muscle of the rat. Rat heart has been referred to as being atypical with respect to the Bowditch Staircase (Henderson et al., 1969), having a negative staircase instead of the classical positive staircase of other mammalian species. It was an interest in this fundamental difference that prompted the following study.

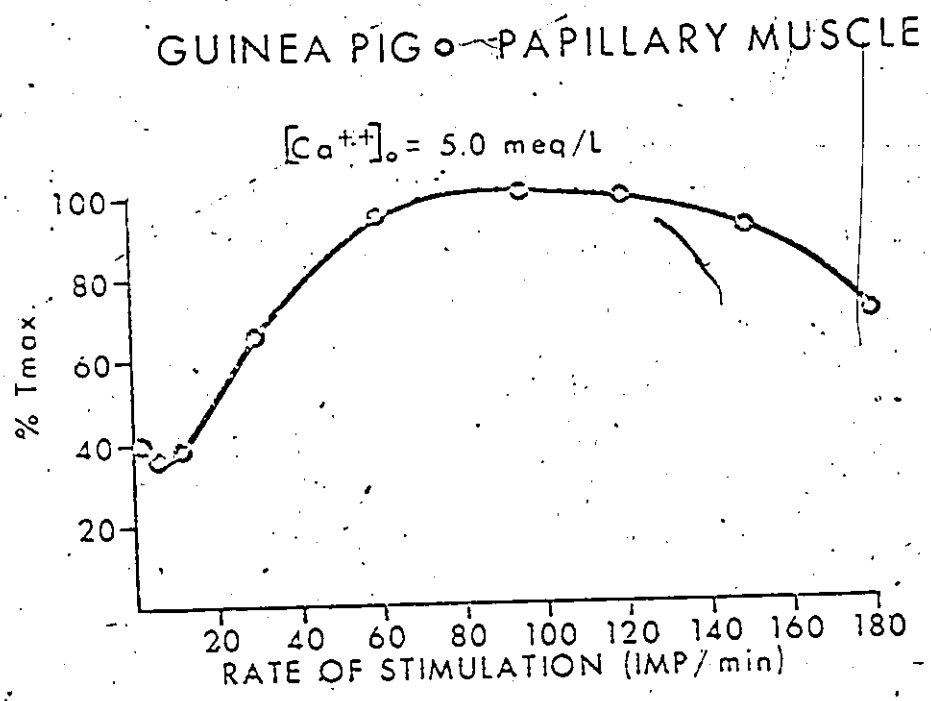


Figure I-1. Tension-Frequency Response in Isolated Papillary Muscle of the Guinea-Pig. $[Ca]_o$ = 2.5 mM; Tmax = .68g.

Ultrastructure of Heart

The structure of typical mammalian heart is shown in Figure I-2. Rat ventricular heart cells are not significantly different from the cells depicted in this diagram. They are cylindrical in shape being on the average about 20 μ x 100 μ in adult heart (Michael, personal communication). They contain all of the normal elements of cardiac muscle: actin and myosin constitute about 48% of muscle volume (Page and McCalister, 1973) and mitochondria occupy about 36% of cell volume indicating the significance of energy transformation in the cardiac cell. The ultrastructure of rat heart has recently been well described by Forsmann and Girardier (1970). The sarcolemma contains invaginations which protrude transversely into the cell; this T system of the rat heart has occasional longitudinal projections to the region adjacent to the sarcoplasmic tubules. At the surface, T tube diameter begins at 1000 Å - 1500 Å, and tapers to a narrow 350 Å at the tips of the longitudinal T system. Horseradish peroxidase (24 x 170 Å) entered the T system including the longitudinal T system but was not present in the sarcoplasmic reticular tubules.

The sarcoplasmic reticulum (SR) is seen as a continuous network of small tubules extending from sarcomere to sarcomere. It appears as if the T system is continuous with the surface membrane (sarcolemma) and forms a network through which the extracellular environment can be extended throughout the fiber. The SR of heart

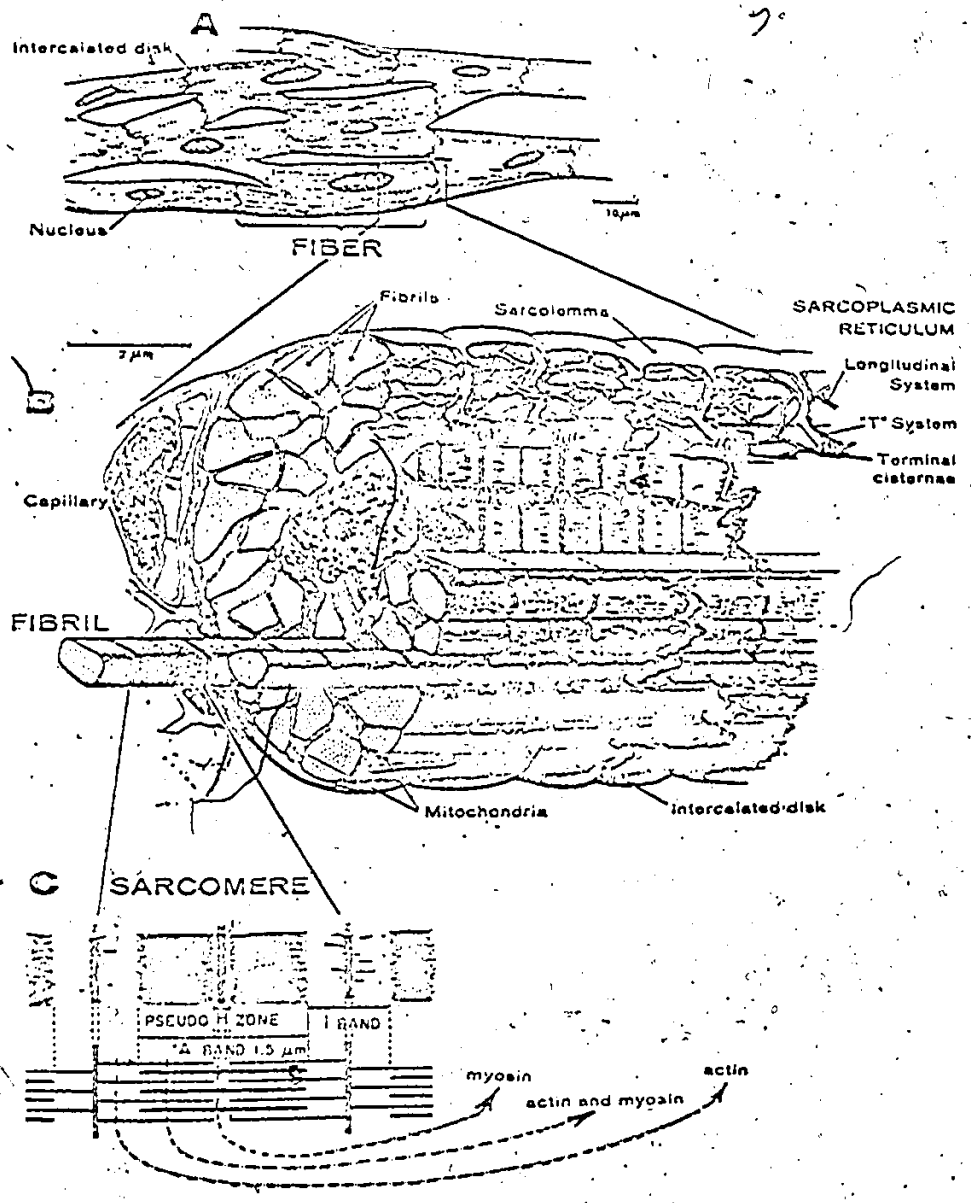


Figure 1-2. Diagram of Heart Muscle Structure.

(After Ghista et al., 1973)

Table I

Composition of Myocardial Cells from Rat Left Ventricles*

Organelle	Percent of Cell Volume	Principal Functions
Myofibrils	47.6 ± 0.7	Contraction and Relaxation
Mitochondria	35.8 ± 0.6	Respiration, Oxidative Phosphorylation
T System	1.0 ± 0.1	Transmission of Electrical Signal at Cell Surface into Interior of the Cell
Sarcotubules	3.5 ± 0.2	Control of Relaxation, Contraction by Regulation of Ionized Ca
Terminal Cisternae	0.35 ± .02	Store Ca^{++} and release it into Sarcoplasm
Rest of Sarcotubular Network	3.15 ± .02	Collect Calcium and return to Terminal Cisternae
Sarcoplasm (cytoplasm) Nuclear and other Structures	12.1	

* After Page and McCalister (1973).

Table I-II

Membrane Areas in Myocardial Cells from Rat Left Ventricles*

Membrane Type	Membrane Area	Units
Plasma Membrane External Sarcolemma	0.30 ± .02	$\frac{\mu M^2 \text{ Membrane area}}{\mu M^3 \text{ Cell volume}}$
External Sarcolemma T system	0.39 ± .02	"
Mitochondrial Cristae	11 ± 1	"
Sarcotubules (± 25%)	1.22 ± 0.05	"
	2.51 ± .08	$\frac{\mu M^2 \text{ Membrane area}}{\mu M^3 \text{ Myofibrillar volume}}$

* After Page and McCalister (1973).

muscle forms junctions with the T system in the region of the Z lines. These areas may constitute points of low resistance between the two tubular networks (T system, longitudinal T and SR) and hence may be important in excitation-contraction coupling.

As well, the SR appears to approach the areas of the close junctions of the intercalated disc, the contiguous area between one muscle cell and another. (Sonnenblick and Stam, 1969; McNutt and Weinstein, 1970). The disc area may also be important functionally in the transmission of the wave of depolarization from cell to cell, since it is considered as a low resistance pathway between cardiac cells (McNutt and Weinstein, 1970). Page and McCalister (1973) estimate that the T system and SR occupy about 5% of the cell volume of rat ventricular cells. It is also estimated that 14% of the external sarcolemmal area is involved in diadic complexes with underlying cisternal areas (Page et al., 1971). The T system (transverse and lateral) accounts for only 1.2% of the cell volume.

Role of Ions in Activation of Cardiac Muscle

At rest, intracellular sodium concentration is kept between 27 - 30 mM in mammalian ventricular tissue, compared to around 140 - 150 mM in the interstitium yielding a $[Na^+]_o$ / $[Na^+]_i$ ratio of about 5. (Conn and Wood, 1959). It now is apparent that this gradient is maintained by an active extrusion of sodium from the cell in spite of a permeability to sodium in the cell at rest (Page, 1962). The active

pump for sodium transport out of the cell has been identified as a membrane ATPase (Schwartz, 1962; Potter, Charnock and Opit, 1966; and Auditore, 1962). The ATPase has been found to be an assymetrical enzyme with the outer surface stimulated mainly by K^+ and the inner surface sensitive to Na^+ (Page, Goeske and Storm, 1964). In red cells the enzyme is also inhibited at the inner surface by Ca^{++}. (Dunham and Glynn, 1961). Page (1965) also demonstrated that cardiac glycosides could inhibit this enzyme only when the drug was applied to the outside of the membrane. A similar specificity is assumed for cardiac cells (Lee and Klaus, 1971).

Sodium transport at rest in cardiac muscle is therefore dependent upon a Mg^{++} dependent Na^+ and K^+ activated enzyme which is also inhibited by Ca^{++} and cardiac glycosides.

The depolarization of the membranes of excitable tissues has been shown to be dependent upon an abrupt increase in Na^+ permeability. This occurs in all excitable tissues such as nerve, (Hodgkin and Huxley, 1952) skeletal and of course in cardiac muscle (Brady and Woodbury, 1960; Johnson and Tille, 1960). This sudden increase in sodium conductance (GNa) is thought to contribute to the spike phase of the action potential. Tetrodotoxin (TTX), which decreases GNa, abolishes the rapid inward current in Purkinje fibers, rat ventricular fibers and guinea pig ventricular fibers (Coraboeuf and Vassort, 1968).

Deck and Trautwein (1964) found that GNa in cardiac Purkinje

fibers declined with a time constant of about 100 msec. during the second phase of the action potential, and that at 300 msec. GNa was still 4 times the resting value; the first rapid increase in GNa may be followed by a slower inward Na^+ movement. Dudel et al. (1966) determined that a slow inward current was measurable after the first rapid depolarization current which was not altered by the absence of calcium nor by the presence of increased calcium in 40X concentration.

The plateau phase of the cardiac action potential has been linked to an inward calcium current, following the first rapid inward sodium ion movement. This work was elucidated in voltage clamp studies in cardiac tissue (Beeler and Reuter, 1970a; Beeler and Reuter, 1970b). In the absence of sodium an inward current was described which was sensitive to changes in $[Ca^{++}]_o$; threshold for tension development was -35mV. Braveny and Sunbera (1968) demonstrated a correlation between action potential duration and the time to maximum tension (TMT) which was affected by calcium concentration. Electrophysiological studies have shown that the mechanical threshold in mammalian heart muscle is dependent upon activation of an inward current which is slow in "turning on" and which can be attributable to calcium.

At this point a word about the voltage clamp technique is in order. The voltage clamp technique is used to measure the current required to hold potential across a prescribed area of a cell membrane at a known value that is uniform with respect to distance and time.

This should allow the instantaneous change of potential at such a rate that the electrical-ionic properties of the membrane under study does not have time to change, allowing identification of the charge carriers across the membrane, and thus the instantaneous voltage-current relationship for the membrane may be determined. Voltage clamping ideally should be carried out on single fibers; however, cardiac tissue presents problems beyond this. 1) cardiac cells are only 10-15μ in diameter making multiple electrode penetrations difficult. 2) the number of fibers in a test area is large. 3) the adult cardiac cells are connected in a syncitium. The technique which has been used in an effort to overcome these anatomical characteristics is the single or more recently the double sucrose gap voltage clamp.

The sucrose gap is used in an attempt to isolate and define an area of membrane with the deionized isotonic sucrose replacing the extracellular fluid and providing a high resistance pathway in the interstitial space.

A recent review article by Johnson and Lieberman (1971) is extremely critical of the results and interpretations obtained with the sucrose gap voltage clamped cardiac tissue. They point out at first the small size of the cardiac cells and secondly the effect of sucrose itself in creating an artificial node.

It appears that a) nodal formation by the sucrose solution may not be uniform from the edge to the center of the fiber, dependent

upon fiber diameter b) hyperpolarization of the membrane in the internode occurs because of a local circuit current between the nodal membrane and the test side of the membrane and c) on increase in the internal longitudinal resistance of the fibers of the gap presumably due to K^+ leakage into the sucrose solution.

In a typical voltage clamp experiment using cardiac tissue (say on rat papillary muscle) one would have a preparation consisting of short cylindrical cells (15-20μ diameter, 100μ long) arranged in long columns which extend the length of the muscle. The frequency and distribution of low resistance connections between the cells, either end-to-end or side-to-side should be sufficient so that if current were injected into the membrane of a single fiber in the current pool of a sucrose-gapped preparation, the current would uniformly distribute and reach all fibers in the test comportment on the other side of the gap. However, Johnson and Lieberman (1971) point out that with the space constants used in typical preparations (Beeler and Reuter, 1970a), it would be difficult to achieve control over membrane potential; as a rule of thumb they suggest a nodal width equal to individual fiber diameter. Rougier et al., (1968) used a nodal width of 100μ in their double sucrose gap method; this is 5-6 times greater than required to achieve perfect spatial and temporal control over the membrane.

Because of these difficulties in technique Johnson and Lieberman argue against the experimental suggestion that the slow

transient inward current in cardiac muscle is due to an inward Ca^{++} movement. Instead they attribute the current-voltage relationship measured with the voltage clamp in a variety of preparations to the lack of spatial and temporal control over the membrane. Furthermore they suggest that this artefact results in a separation of the "control" into a fast response and a slow response; the fast response being due to the "clamp" on the peripheral cells in the cardiac muscle bundle and the slow response, attributed to Ca^{++} movement (Beeler and Reuter, 1970a,b) due to less accessible fibers in the bundle.

Their criticism indicating a lack of control over the membrane for the fast inward current make sense. However, their criticism of the slow second transient are more vulnerable in the light of the experiments of Tarr (1971) which were published after the Johnson and Lieberman article. It appears as if there would be sufficent spatial and temporal control over the membrane to measure the second or slow inward current. Tarr (1971) demonstrated quite convincingly the separation of the fast (Na^{+} carrying) and slow (Na^{+} and Ca^{++} carrying) inward currents by treatment of voltage clamped frog atrial fibers with suitable combinations of TTX, Na^{+}, Ca^{++} and Mg^{++} containing solutions. Although Tarr presents no data to verify a homogeneity of voltage control, these experiments can be interpreted as providing evidence for the separate inward currents. It seems that with the present techniques quantification of the currents is not justified although a qualitative

evaluation is. Furthermore, Mainwood (personal communication) was able to demonstrate a calcium sensitive inward current in voltage clamped rat papillary muscle in sodium free solution; in spite of the short action potential duration (< 50 msec.) calcium inward current is detectable in this species.

The descending phase of the action potential is attributed to depolarization of the cell and outward movement of potassium (Lorber et al., 1962). Direct evidence for K^+ efflux was found by Haas and Kern (1966) in voltage clamped Purkinje fibers; above - 40 mV g_K became significantly greater than at rest and efflux was increased greatly.

The action potential recorded from single cardiac cells can thus be divided into three phases 1) a spike (phase 1) which is the result of a sudden increase in G_{Na}. 2) slow phase 2 which has been shown to be due to an inward calcium current and perhaps a slower Na^+ current and 3) an outward K^+ current (phase 3).

In the absence of calcium in the medium no mechanical event can be elicited from isolated cardiac cells. Recent evidence suggests that calcium entering during the action potential does not determine contractile strength directly; Beeler and Reuter (1970b) found that Ica was not related to contractile force. Similar studies by New and Trautwein (1972a, b) found no correlation between slow inward current (ascribable to Ca) and contractile force. These data strongly suggest

the presence of a cellular pool of Ca, which supplies calcium to the myofilaments and therefore controls contraction. This calcium may be divided into two classes. 1) that calcium which enters with each A.P. and may trigger the release of 2) a stored calcium pool. Much evidence has been presented for the sarcoplasmic reticulum and the reticular network as a storage site for calcium (Nayler and Merrillees, 1971; Schwartz, 1971; Rothstein, 1968). Indeed, it is now agreed that SR vesicles can reduce intracellular calcium from 10^{-5}M. to about 10^{-7}M. by binding and uptake. It is also agreed that ultrastructural characteristics of mammalian muscle such as a) a negative charged layer of mucopolysaccharide on the lining of the T tubular system b) T tubules of up to 1,000 Å, diameter and distribution of the T system provide clues to the movement, storage and possible releasing sites for calcium (Langer, 1973), from the surface through the branchings of the T and longitudinal T system.

There is an abundance of evidence that indicates that calcium ion plays a significant role in controlling and maintaining muscle contraction both in skeletal and cardiac muscle. Calcium ions are believed to be involved in both the coupling process between excitation and the contraction as well as in the degree of activation of the myofilaments producing tension in individual muscle cells.

Molecular Basis of Contraction

Recent research has accumulated to indicate that the molecular

basis of contraction in both heart and skeletal muscle are similar and differ only quantitatively (Katz, 1970).

The rather simple system proposed by Davies (1963) is now superceded by a more complicated model (Figure I-3). It must be emphasized that this model by Katz (1970) is intended only as a schematic representation and that the precise relationship of the modulatory proteins to the actin and myosin is still a matter of controversy (Huxley, 1973; Ebashi et al., 1973; Drabikowski et al., 1973; Julian et al., 1973). Although all of the details have not been worked out, this system would appear to operate in the following manner: In the top of this figure the situation at rest is obtained when in the absence of Ca^{++} the Actin-Myosin interaction is inhibited by the intact troponin-tropomyosin system. In the presence of Ca^{++}, Ca^{++} binds to troponin and the modulation is abolished; the ATPase and physiochemical reaction between Actin and Myosin are initiated, an active state is established and the muscle shortens. The relationship between the affinity of Ca^{++} for the troponin in the troponin-tropomyosin complex has been well established (Ebashi and Endo 1968) as well as other investigators (Ebashi, Kodama and Ebashi, 1968; Arai and Watanabe, 1968; Fuchs and Briggs, 1968). It is now widely accepted that the relaxation of actomyosin is caused by the binding of MgATP to an inhibitory or relaxing site and that contraction is controlled by the modification of this inhibitory interaction. It is believed that the

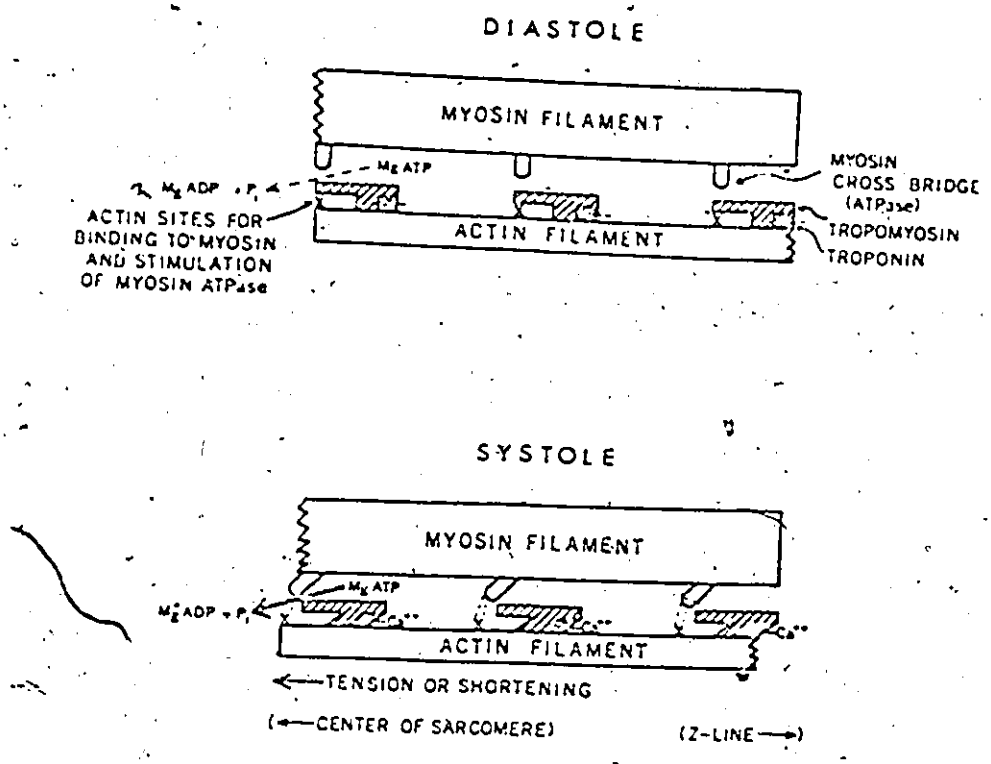


Figure I-3. Molecular Model of Cardiac Muscle Contraction (After Katz, 1970).

troponin-tropomyosin complex without calcium favours relaxation by facilitating MgATP binding to this site, whereas the formation of a calcium troponin-tropomyosin complex has the opposite effect, i.e., it favours contraction by interfering with the binding of Mg-ATP at the relaxing site (Stewart and Levy, 1970).

The maximum shortening velocity of the muscle is believed to be determined by the rate at which the Actin-activated myosin cross bridges hydrolize ATP. This is also supported by early works of Weber and Portzehl (1954) who provided evidence that Actomyosin-ATPase is directly coupled with muscle contraction. There is evidence that the ATPase activity of the Actin-Myosin system (Myofibrillar ATPase) would indicate a)the activity of the interacting sites and b) Ca^{++} activation under conditions where ATP, Mg^{++} and K^{+} are not rate limiting. The activity of the ATPase system is shown in Figures I-4 and I-5.

Maximum tension would be determined by the number of complete interactions between actin and myosin (Katz, 1970).

The movement of calcium between excitation, contraction and relaxation may be called the calcium cycle of the cell. The role of calcium ion in the regulation of contraction and relaxation appears to be the same in both skeletal and cardiac muscle (Fanburg, 1964), but there are differences in the role of calcium in the coupling process between excitation and contraction (Langer, 1973).

In skeletal muscle, it is generally thought that the calcium

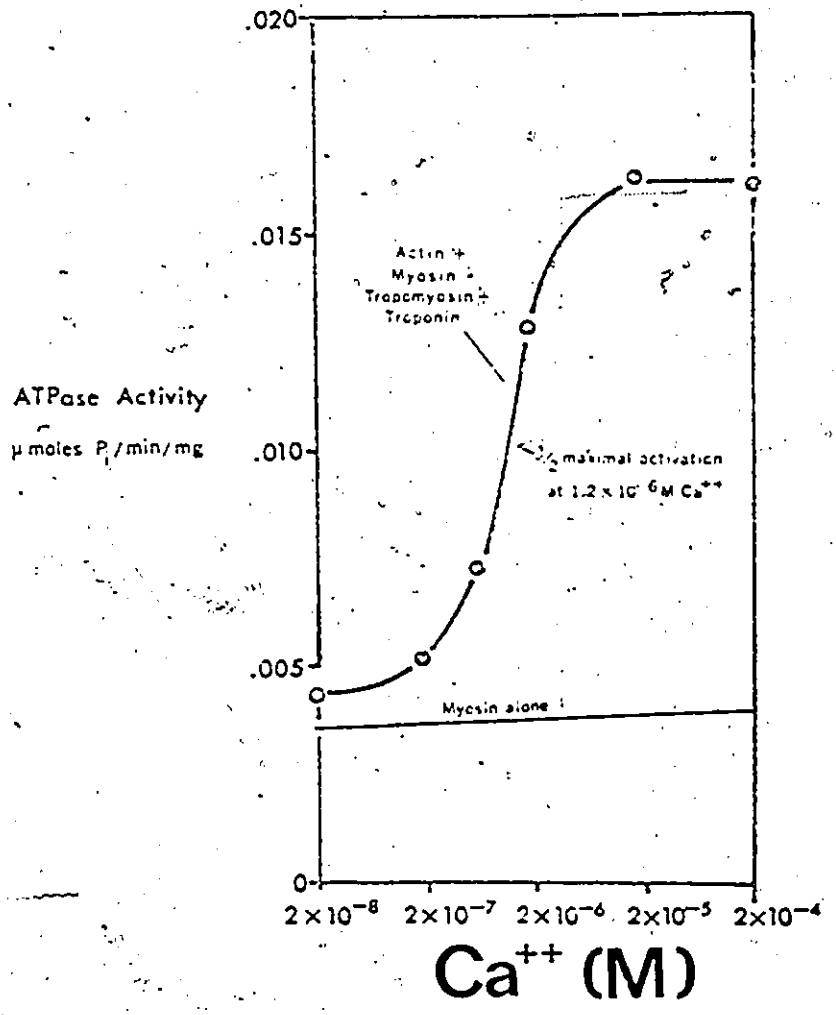


Figure I-4. Effect of Calcium Concentration on ATPase Activity. (After Katz, 1970).

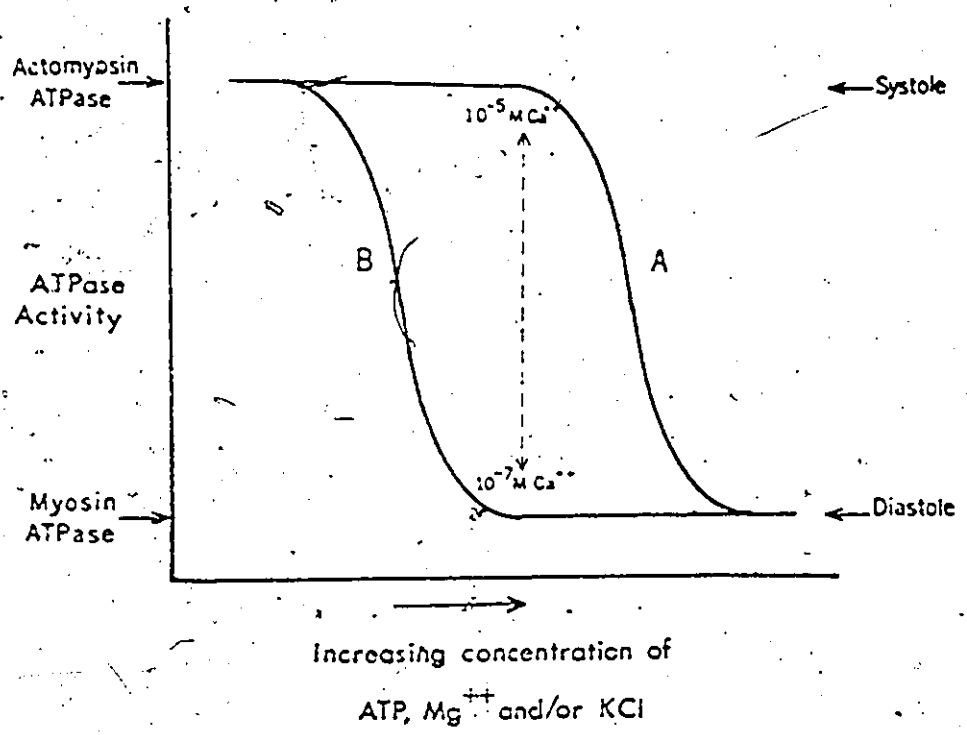


Figure I-5. ATPase Activity of Cardiac Muscle.

responsible for activation lies within the muscle cell in association with the SR and is released upon excitation causing contraction. In cardiac muscle, the cellular calcium also contributes to tension development but calcium influx during the action potential may also contribute to the calcium utilized in tension development (Katz, 1970).

Although the mitochondria occupy about 36% of the cardiac cell volume (Page and McCalister, 1971) and have been shown to have the ability to bind and take up calcium (Patriarca and Carifoli, 1968), this organelle has largely been discounted in providing a dominant role in calcium movements involved in excitation-contraction coupling except as a reserve for calcium over long intervals (Langer, 1973).

In simplest terms, we can see that a calcium cycle in cardiac muscle would involve a movement of calcium from a) the extracellular space to the myoplasm b) release of calcium from the sarco plasmic reticulum to the myoplasm c) chelation of calcium by contractile apparatus d) release of calcium by contractile apparatus and re-sequestration of calcium by the S.R. and e) efflux of calcium from the cell. An understanding of the kinetics of such a cycle would help to elucidate some properties associated with the mechanical characteristics of cardiac muscle.

Calcium Distribution in Cardiac Muscle

The concentration of Ca^{++} in mammalian blood is estimated at 2.5mM/L., while the concentration in mammalian heart is about the

same (Ditmer and Grebe, 1959). However, the distribution of calcium within the heart is not considered to be homogeneous. Evidence for this is provided by calcium exchange studies in guinea-pig atria (Winegrad and Shanes, 1962) in dog papillary muscle (Langer, 1964) and in rabbit ventricular muscle (Shelburne et al., 1967), where at least 3 kinetically definable phases were obtained in washout studies. Table I-III shows the results of Langer's (1969) experiments on dog papillary muscle. Of particular interest is the phase 2 component having a $t_{\frac{1}{2}}$ of 6.0 minutes. In their studies, this fraction correlated well with the change in contractile tension and has been tentatively assigned to a membrane-bound portion (S.R.?).

Since there is abundant evidence that the S.R. can accumulate Ca^{++} in vitro (Inesi, 1972;Palmer and Posey, 1967);Katz & Repke, 1967; Weber et al., 1967) it might be interesting to determine if this organelle is capable of releasing and accumulating enough calcium to support contraction in cardiac muscle. Based upon the computations of Langer (1968) it is possible to estimate calcium exchange in cardiac tissue. Palmer and Posey estimated three types of calcium accumulation in cardiac S.R. representing a) transport and storage b) transport without storage and c) true binding accounting for 40, 5.1 and 0.8 μmoles of Ca^{++}/g. of reticular protein respectively. Weber et al., (1967) estimated Ca^{++} binding at about 0.25 μM/g of muscle. In order to achieve relaxation, Weber et al. (1967) estimate that 0.05 μmoles of Ca^{++} per g

Table I-III

Calcium Exchange in Dog Papillary Muscle

Kinetic Phase	Rate Constant $Min.^{-1}$	$t_{\frac{1}{2}}$ of Exchange Min.	% Tissue Calcium	Probable Origin
0	3.5	0.2	5	Vascular
1	0.59	1.2	25	Interstitial
2	0.116	6.0	25	Membrane-bound (SR)
3	.021	33.0	25	Intracellular
4 Ca	.004	170.0	< 20	Intracellular? Connective tissue?

After Langer (1968)

of tissue would have to be removed from the contractile proteins. This is only 20% of the Ca^{++} accumulating ability of SR determined by Weber et al., (1967).

Langer (1968) also estimates that the energy required for relaxation would only require 6.5% of total energy developed when the S.R. was working at 40-50% efficiency. As far as the time required to accomplish Ca^{++} binding, Ohnishi and Ebashi (1964) estimated that binding within 30 msec. in skeletal muscle SR was sufficient to explain the relaxation process. Since relaxation within 200 msec. is normal in cardiac muscle, a binding system only 15% as rapid as that of Ohnishi's and Ebashi's skeletal system would be required (Langer, 1968). These data suggest that the SR has the major qualities to achieve cardiac relaxation.

In 1955, Neidergerke was able to demonstrate that the direct application of calcium with a micropipette into frog muscle fibers produced muscle contraction. This was followed in 1958 by the classical experiments of Huxley and Taylor who showed that local electrical depolarization of frog skeletal muscle in the region of the line produced a contraction in the single sarcomere, or adjacent sarcomeres. That calcium might have been involved in this process was demonstrated by Podolsky and Costantin (1964) who then applied Ca^{++} solutions directly over several sarcomeres and obtained contractions.

Gillis (1969) demonstrated through iontophoretic application of Ca^{++} that maximum activation was achieved at the level of A-I band junction in glycerinated crab myofibrils and that activation threshold was related to myofilament overlap.

These experiments strongly suggested the possibility that electrical depolarization in the region of the Z lines, and hence in the T system would result in passage of a wave of depolarization down the T system to the region where the T system juxtaposed with the S.R., and releasing enclosed Ca^{++}. This proposal was supported by the experiments of Lee et al., (1966) who were able to show the release of Ca^{++} from both skeletal and cardiac S.R. fragments in vitro by electrical stimulation. Furthermore, Costantin and Podolsky (1967) could obtain contractions in skinned (without sarcolemma) frog skeletal muscle fibers by electrical stimulation. They interpreted their results as the depolarization of internal membranes by the electrical pulses.

These results indicate the S.R. is capable of a) uptake of Ca^{++} b) storage and transport of Ca^{++} c) true binding of Ca^{++} and d) possibly electrically stimulated release.

Active State in Cardiac Muscle

The term active state is generally meant in the purest form to be the force developed by muscle at constant sarcomere or element length. In this condition the duration of the active state is believed to be determined by the time period during which the Ca^{++} level in the

myoplasm is sufficient to maintain the troponin binding sites Ca^{++} saturated (Brady, 1968). Presumably during an isometric twitch, the contractile element length should stay relatively constant, but changes in contractile element length as great as 8 % have been recorded in the isometric condition (Brady, 1968). The problem is to obtain an index of the state of contraction which might reflect directly the events occurring at the Actin-Myosin interaction. If we make several assumptions it is possible to derive one or more indices of contractility (IOC) which may be used in assessing cardiac muscle performance.

We must assume that contractile element length is relatively constant. This can be assumed if we base a study on isometric contractions at the peak of the length-tension relationship. In this condition, the changes induced by the Frank-Starling relationship are removed (Katz, 1970; Brady, 1968). We must also assume that the availability of compounds for contraction are not limiting. This would apply to ATP, Mg^{++}, and to the biochemical transformers involved in energy production and transport. In these conditions we can predict that the active state intensity would be reflected in the peak of the derivative of tension development (dT/dt) the duration of the active state in the time to peak tension (TMT) (Buccino et al., 1967) and the number of activated sites by the peak tension (T) (Katz, 1970). Each of these can be used as an index of the contractility of the muscle keeping in mind the assumptions which have been made. Many attempts

have been made with cardiac muscle to derive a value for pure active state intensity (Brady, 1968).

Among the indices used to determine contractility are a) contractile element velocity (V_{ce}) at peak stress (Gault et al., 1968; Urschel et al., 1968) b) peak V_{ce} (Mirsky et al., 1969) c) V_{ce} at zero stress (V_{max}), (Sonnenblick, 1962; Urschel et al., 1968; Fry et al., 1964; Hugenholz et al., 1970) maximum dP/dt (Mason, 1969) and force (Sonnenblick, 1965). In clinical studies V_{max}, the maximal, no-load velocity for the contractile element was found to be correlatable with the clinical state (Falsetti et al., 1971).

Hugenholz et al., (1970) have shown that the simultaneous plot of dT/dt against Tension (phase plane plot) can be used to estimate the contractile element velocity. The phase plane plot permits the comparison of contractions for discerning the relative magnitude of the active state at a given value of tension (Taylor, 1969). This is also based upon the assumption that the active state magnitude is proportional to the concentration of the activator-muscle complex in the muscle. If we interpret the activator-muscle complex as the calcium activated troponin-tropomyosin, then the activity of this complex is determined by its interaction with the concentration of calcium liberated from storage sites during stimulation. Hence the level of tension and the rate of tension development would be proportional to the calcium released to the myofilaments. This is corroborated by evidence that changes in

the amount of calcium available to the cardiac proteins would modify only the number of active interactions between actin and myosin but not the rate at which each interaction proceeded (which would alter V_{max}). (Katz, 1970; Teicholz and Podolsky, 1970) and hence the maximal developed tension would reflect the number of active interactions between actin and myosin (Barany, 1967).

The Interval-Strength Relationship

Several terms are used in the literature to describe the relationship between the interval between beats and the strength of contractions in cardiac muscle. Among these are Interval-strength relationship, Force-Interval Relationship and Tension-Frequency Relationship. These can be used interchangeably.

Much attention has centered upon the importance of the Interval-Strength relationship of cardiac muscle as a determinant of cardiac performance. As Koch-Weser and Blinks (1963) indicate in their comprehensive review, this fact was recognized as early as 1871 by Bowditch. Since his original publication, the interval-strength relationship has been identified as being very much identical in most mammalian species including the dog (Kruta and Stejskalova, 1960; Siebens et al., 1959) cat (Henderson et al., 1969), guinea-pig (Tuttle and Farah, 1962) and human (Blinks and Koch-Weser, 1961). This relationship is characterized by an increase in contraction force with an increase in the frequency of contraction within certain frequencies, and referred

to as the Bowditch staircase. The rat is an exception; a negative staircase has been said to be present with an increase in frequency of contraction (Kruta and Stejskalova, 1961). Koch-Weser and Blinks attributed two opposing forces within cardiac muscle as being responsible for the triphasic strength - interval relationship (Koch-Weser and Blinks, 1963). These are the negative and positive inotropic effects of activation (NIEA and PIEA). They believed that at rested state contraction intervals both NIEA and PIEA were absent but as the interval was decreased cumulative effects of NIEA and PIEA could be expressed with changes in frequency. Unfortunately, these factors were not identifiable at the time.

It is to be stressed that the changes in contractility in the Bowditch phenomenon are not associated with the Frank Starling mechanism of the heart. (Brady, 1968; Sonnenblick and Stam, 1969; Langer and Serena, 1967; Langer, 1971). Neither total tissue calcium, nor the rate of calcium exchange was found to be affected by alterations in length-tension relations, muscle shortening or work performed (Langer and Serena, 1967).

An earlier comprehension of the interval-strength relationship was limited by an ignorance of the steps in excitation-contraction coupling, and the production of the active state in cardiac muscle. However, due to our increasing knowledge of these phenomena in the last year or so it is now possible to piece together some factors which may

be responsible for this relationship.

It is clear that calcium plays the central role in determining the strength of contraction. It is clear as well that calcium may also be involved in the coupling process. A review by Langer (1968) outlines the various ionic changes thought to occur in cardiac muscle at rest and during an abrupt change in frequency. Two hypotheses are put forward to explain the T-F relationship: a) that a Na^+ / Ca^{++} competition takes place at the sarcotubular membrane. During an abrupt increase in the frequency of contraction more sodium accumulates at this surface due to a lag in the sodium pump. This increase in sarcotubular $[Na^+]$ displaces more $[Ca^{++}]$ leading to an increased tension development.

The final tension (steady state tension) would then be determined by the Na^+ / Ca^{++} ratio established at the membrane where Na^+ exchange takes place. With high frequencies of stimulation more Na^+ would be exchanged and so Ca^{++} exchange would also be expected to increase. This indeed is what Shelborne et al., (1967) found in assessing Ca^{++} exchange in the Bowditch staircase. They found that the staircase was determined by the calcium in phase 2 (SR calcium) prior to an increment in stimulation frequency. Since Langer (1968) proposes that SR $[Ca^{++}]$ is Na^+ dependent, they suggested that an increase in the rate of contraction was associated with a shift of Na^+ inside the membrane displacing Ca^{++} with positive inotropism on successive beats.

Bailey and Downie (1970) found a similar pool in the gas perfused cat heart.

Glitch, Reuter and Scholz (1970) suggest that internal sodium concentration $[Na^+]_i$ influences calcium influx. They found that even a slight increase in $[Na^+]_i$ above the normal 10 - 20 mM in guinea-pig auricles could be expected to increase calcium influx appreciably. They also described a hypothetical Na-Ca carrier system which would keep $[Ca^{++}]_i$ at a low level during normal contraction rates.

These two systems are similar in that a Na^+ / Ca^{++} competition at the sarcolemmal or sarcotubular membrane is involved. Both would lead to an increased $[Ca^{++}]_i$ when $[Na^+]_i$ was increased.

B) Langer (1968) suggested an alternative explanation for Na^+ / Ca^{++} competition in the T-F relationship. He proposed that the sarcolemma would be the site for Na^+ / Ca^{++} competition alone, and that the sarcotubular system would only bind and store calcium during relaxation. Langer has since rejected his previous proposal and believes that the alternate hypothesis is more valid (Langer, 1971):

Calcium in the interstitial space would be the source of calcium on the sarcolemma binding sites and the T system; this calcium is controlled partly by the intracellular to extracellular sodium gradient - an increase causes an elevated influx to the myofilaments. Relaxation would be accomplished by binding and storage of calcium in the sarcotubular system; this stored calcium then exchanges through the

longitudinal T system to the T system proper.

This has also been used as an explanation for the phenomena of postextrasystolic potentiation and post stimulation potentiation (PESP and PSP). Presumably the extra calcium gated in because of the extra systoles or beats (in PSP), would contribute to the contractile calcium pool. Most investigators consider PESP, PSP and the Force-Frequency Relationship to be an intergral part of the same basic mechanism (Henderson et al., 1969; Johnson and Kuohung, 1968; Tuttle and Farah, 1962).

In essence, Langers hypothesis suggests that calcium movement in the cardiac cell is involved in a relatively closed loop. If indeed calcium ion is the primary substance controlling cardiac contractility, it would be appropriate to construct analogue models of calcium movement in cardiac cells.

Models of Calcium Movement

Based upon the concept that calcium within the myocardial cell may move in a definitive circuit, several investigators have attempted to model the characteristics of myocardium such as the Bowditch Staircase, PSP and PESP. (Entman et al., 1969; Bailey and Dresel, 1968; Wood, Heppner and Weidman, 1969; Legato and Langer, 1969).

A recent model is presented by Johnson and Kuohung (1968) based upon the relationship between peak tension development and the pattern of stimulation in the isolated papillary muscle preparation of

rabbit. Their model encompasses four sites or compartments which have varied abilities to bind and store calcium (Figure I-6). Compartment 1 is accessible to $[Ca^{++}]_o$. On stimulation the calcium in 1 increases suddenly by a constant amount and after a delay declines. Calcium in compartment 2 increases on stimulation by an amount equal to that which remained in compartment 1 just prior to the stimulus, and then this calcium declines. On stimulation the calcium in compartment 3 is ejected and determines the peak tension developed. Compartment 4 represents a store of calcium which can be lost from compartment 3, and apparently does not figure in the overall response of the system except in abnormal behaviour of the heart muscle.

The system described by Johnson and Kuohung was able to describe short term changes in interval-strength relationship satisfactorily and was able to mimic postsystolic and post stimulation potentiation.

However, it can be criticized for the following reasons: a) a change in $[Ca^{++}]_o$ was effected solely by increasing the gain of the system. As we know, the T-F relationship in rabbit papillary muscle, (Teiger and Farah, 1968) is very sensitive to $[Ca^{++}]_o$ and changes the shape of the steady state T-F response.

b) We also know that some recycling of calcium takes place within cardiac muscle. This is effected through the resequestration of calcium by the S.R. (Langer, 1971; Sonnenblick and Stam, 1969). This is

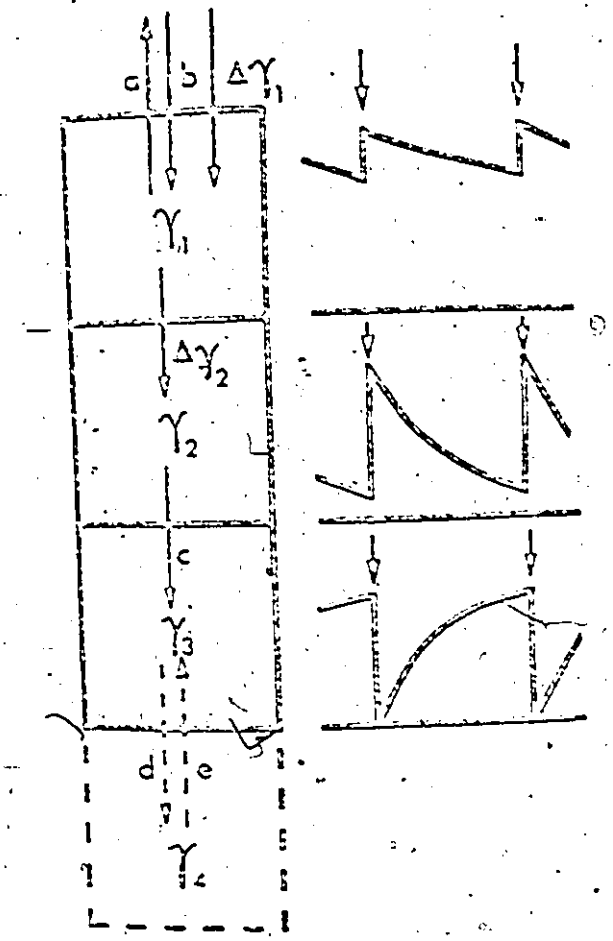


Figure I-6. Model of Calcium Movement in Rabbit Heart. (Johnson and Kuohung, 1968).

not taken into account in the Johnson and Kuohung model of contractility. Although the model appears to work in the sense that it can display certain known characteristics of cardiac muscle, it fails in that the physical analogue in the real cardiac muscle cannot be identified.

A second model has been presented by Manring and Hollander (1971). Figure I-7 gives a schematic diagram of the model. In this model it was assumed that calcium used by the contractile mechanism is released from a single compartment (n_1). A constant fraction f is released when the muscle is stimulated; f is dependent upon the frequency but constant at a given frequency. After release of calcium from the myofilaments, compartment n_2 actively resequesters the calcium, and returns the calcium to n_1. During an action potential, calcium enters n_1 through Ji. Passive calcium influx Jr is balanced by an active calcium pump Jio removing calcium from the cell.

Again, this model was able to predict the interval-strength relationship in guinea-pig atrial muscle. This model was able to describe qualitatively the patterns of stimulation responses corresponding to a) sudden changes in the period of stimulation b) a constant interval between stimulations c) a rest after a regular train of contractions followed by another train of contractions and d) postextrasystolic potentiation and paired-pulse stimulation. While Manring and Hollander were able to duplicate the experimental phenomena mentioned above by the analogue model, using a calcium loop as the only

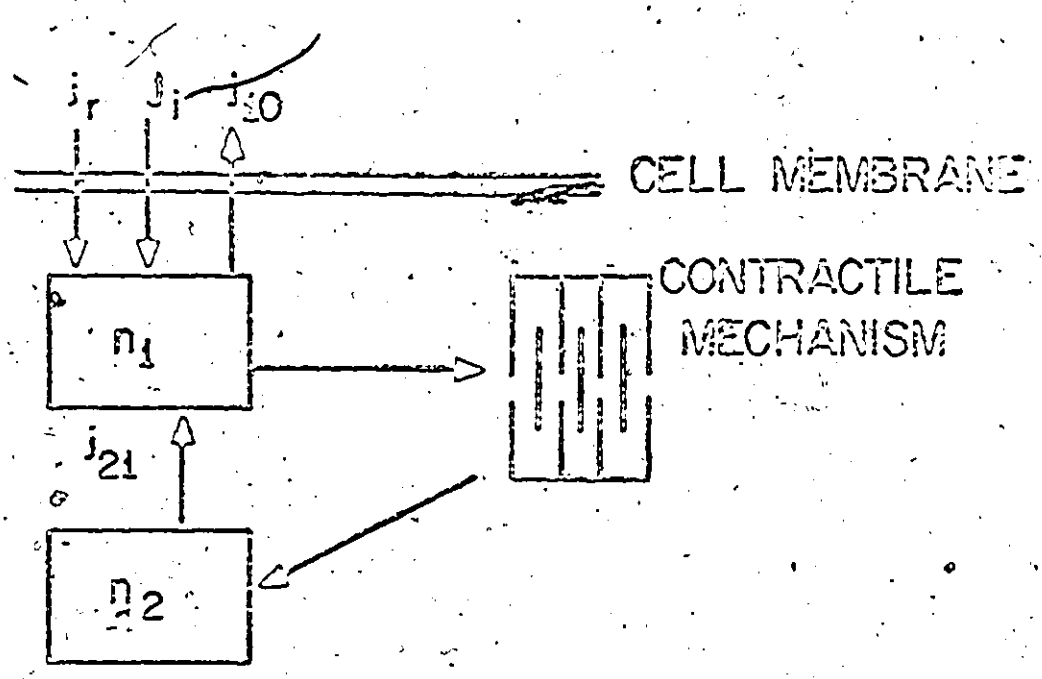


Figure I-7.. Model of Calcium Movement in Guinea-Pig Atrium.
(Manring and Hollander, 1971).

determinant, they are quick to point out that other ions, particularly sodium would affect the kinetics of their proposed model. However, they suggest that the influence of other ions would remain fairly constant in a medium containing constant concentrations. This is rather simplistic since Langer (1968) indicates that ionic changes, particularly $[Na^+]$ may control the T-F responses.

This model therefore does not go beyond the step of utilizing more than one $[Ca^{++}]_o$ in predicting the interval-strength relationship in guinea-pig atrial muscle. It is true that a change in $[Ca^{++}]_o$ alters the $[Na^+]$ / $[Ca^{++}]$ ratio and so might drastically alter the predicted behaviour of this model. (Glitch, Reuter and Scholz, 1970; Langer, 1971a).

Manring and Hollander also point out that the existence of more than a single storage comportment is not justified by ultrastructure. They indicate that while n_1 might correspond to the SR., n_2 has no structural basis; it has a computed decay constant of about 0.5 sec^{-1}.

Thus the Manring and Hollander model is one step closer to matching ultrastructural calcium pool details to an analogue model of the strength-interval relationship in mammalian cardiac muscle.

A similar model is suggested by Bailey et al., (1972) based upon studies of the gas perfused cat heart (Figure I-8). The pathway for calcium in this model involves (a) uptake of calcium from the

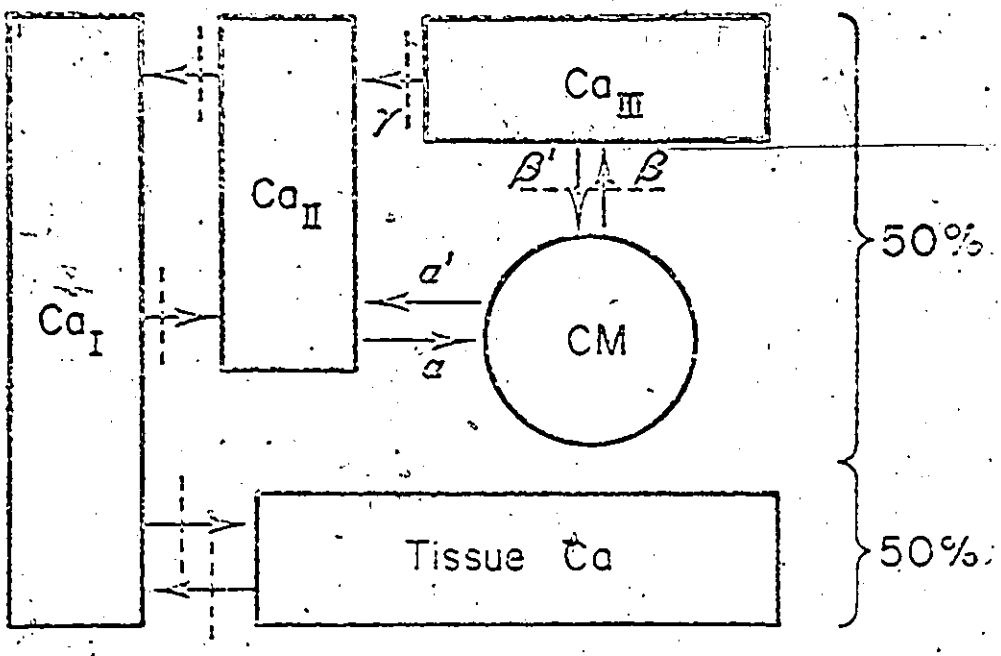


Figure I-8. Model of Calcium Movement in Cat Heart. (Bailey, Ong and Queen, 1972).

vascular space (Ca_1) (b) temporary storage in Ca_{11}, thought to represent the sarcolemmal inner surface and (c) release by depolarization to the contractile elements. Relaxation is accomplished by active accumulation by Ca_{iii} - the S.R., and removal of calcium from the cell. It is to be noted that this model involves a looped movement of calcium in the cell. They determined that Ca_{11} represents contractile calcium since it corresponded to calcium 45 washout from the tissue and can also be called activator calcium since it might contribute to E-C coupling. They determined that the movement of Ca through each step in the cycle would be determined by first-order rate constants for α, B and γ.

Bailey's group has not progressed beyond this model to simulate interval-strength changes in cat cardiac muscle. However, Bailey has determined that Ca_{11} changes in concentration over the positive component of the force-frequency relationship. (Bailey and Downie, 1970).

Statement of the Problem

Preliminary experimentation and a review of the literature had shown that the isolated papillary muscle preparation of the rat appeared to be different from other mammalian species in several important characteristics: Potentiation of force was virtually absent, except at very short intervals (Kruta and Braveny, 1960); the reaction to cardio-active drugs, such as Ouabain, was not positively inotropic (Berforado, 1958); the action potential and the plateau phase were

especially short in duration in the rat (Katz, 1972; Coraboeuf and Vassort, 1968; Lee et al., 1970); and the frequency staircase was found to be negative (Kruta and Stejskalova, 1960; Henderson et al., 1969). All of the evidence cited above tended to indicate that the rat papillary muscle and the rat heart could not be considered as representative of mammalian myocardium, making comparison and extrapolation to other species, especially man impossible.

This raised the following very basic questions: a) is the rat myocardium totally different in its behaviour from other mammalian species?

b) can these differences be attributed to a modification in the Excitation-Contraction Coupling response, or in the handling of the ions known to be responsible for contraction coupling (eg. Ca)?

c) can the mechanical characteristics of the rat heart muscle be modified?

d) can a model of the influences on rat cardiac muscle be proposed from the known and derived properties?

A clue as to what may be responsible for the reported differences is indicated by the fact that in other species:

a) the action potential duration may be modified by $[Ca^{++}]_o$

b) $[Ca^{++}]_o$ appears to be related to the effectiveness of the cardiac glycosides (Lee and Klaus, 1971).

c) the shape of the Bowditch curve may be affected both by glycosides

(Tuttle and Farah, 1962) and $[Ca^{++}]_o$ (Teiger and Farah, 1968).

It therefore seemed possible that calcium ion was in some manner involved in producing the observed characteristics in the rat heart and that an alteration of the calcium mileu would have a profound effect on the isolated papillary muscle of the rat.

Several types of experiments have been utilized to answer the above questions. For the sake of clarity, the results from these experiments have been divided into 3 sections. In Section III, the effect of $[Ca^{++}]_o$ on the Tension-Frequency (T-F) relationship and Post-Stimulation Potentiation (PSP) is described. In Section IV, the interaction between $[Ca^{++}]_o$ and the cardiac glycoside Ouabain, is investigated while Section V presents results from experiments describing the relationship between $[Ca^{++}]_o$ and rested state contraction tension and $[Ca^{++}]_o$ and the recovery of contractility between rested state contractions. Each section also contains a brief discussion of the results in the section. From the results and discussion in each section a tentative model is described which would provide an explanation for the observed characteristics of the rat papillary muscle.

SECTION II

Materials and Methods

MATERIALS AND METHODS

Animals: Male Sprague Dawley Rats (260-350 g. Body Weight) were obtained from BioBreeding Laboratories (Ottawa). The rats were maintained in groups of 4 in plastic cages and stored in the animal quarters of the Faculty of Medicine. All animals were fed standard animal feed and water ad. libitum. Guinea Pigs (300-380 g. Body Weight) were raised in the animal quarters of the Faculty of Medicine and treated as were the rats described above.

Papillary Muscle Preparation

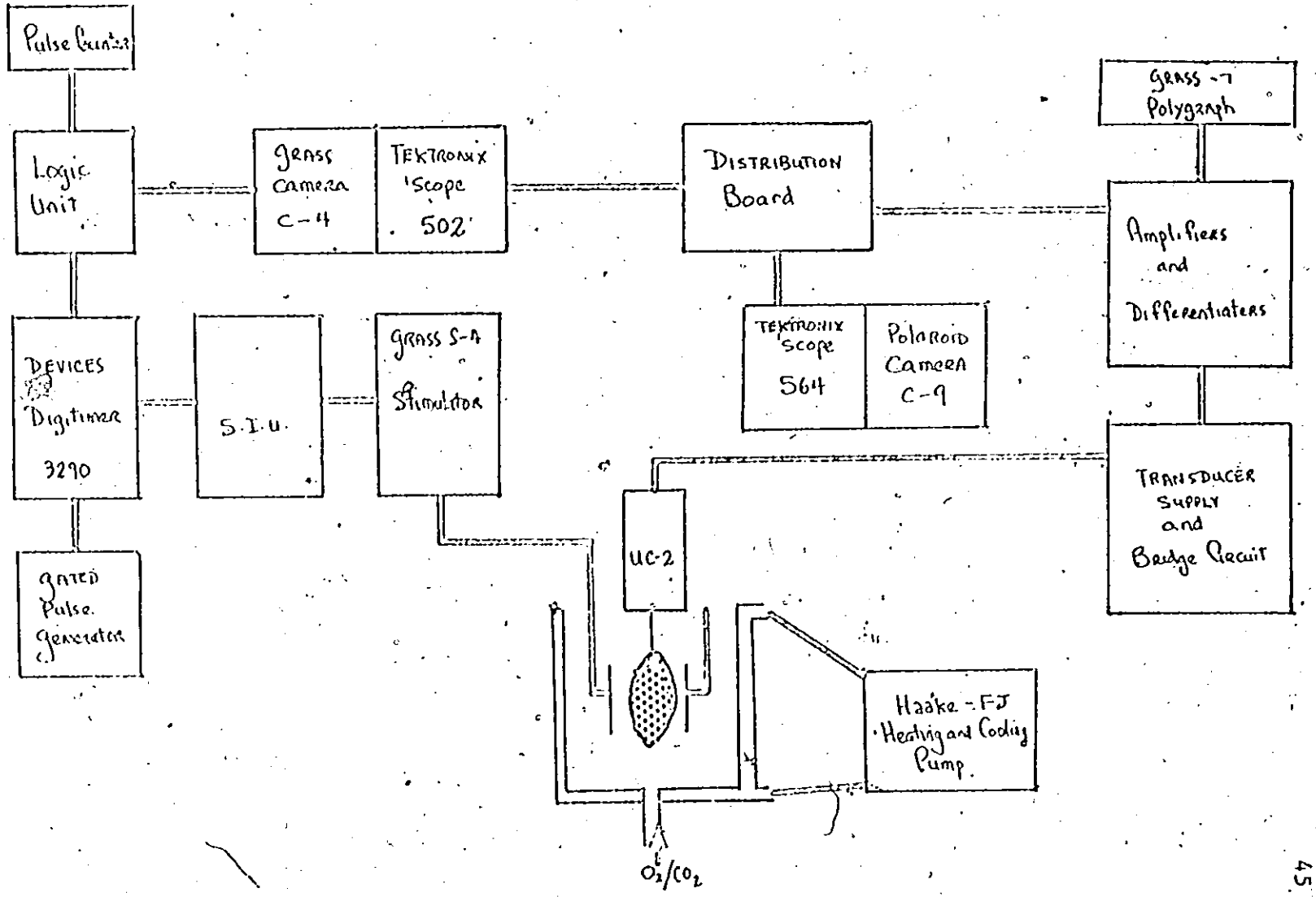
Animals were sacrificed by a sharp blow to the head. The heart was quickly removed from the chest and placed in ice-cold Tyrode solution. The atria were dissected away from the heart and the left ventricle opened to expose the papillary muscles. Using iris scissors, the anterior papillary muscle was dissected free from the ventricular wall and the tendon cut at its attachment to the valvular area. The papillary muscle was then placed in a small dish containing cooled Tyrode solution and carefully trimmed for extraneous tissue.

The muscle was handled as little as possible to avoid damage. The muscle was then clamped in the muscle bath containing Tyrode solution at 26°C and bubbled with 95% O_2/5% CO_2. Figure II-1 shows the experimental set-up.

Tyrode Solution

The normal Tyrode solution used in all experiments was of the

Figure II-1. Schematic Diagram of Isometric Tension Recording System.


Pulse Counter
Logic Unit
Grass Camera C-4
Tektronix 'Scope 502
Distribution Board
Grass -7 Polygraph
Amplifiers and Differentiaters
Devices Digitimer 3290
S.I.U.
Grass S-4 Stimulator
Tektronix Scope 564
Polaroid Camera C-9
Transducer Supply and Bridge Circuit
Gated Pulse Generator
UC-2
Haake - FJ Heating and Cooling Pump
O_2/CO_2

following composition (mM): NaCl 117; KH_2PO_4, 1.2; KCl, 3.5; $MgSO_4$, 3.1; $NaHCO_3$, 28 ; $CaCl_2$, 2.5; and glucose 11.1. Calcium concentration was altered by changing the concentration of calcium chloride. Changes in sodium concentration was performed by replacing NaCl with sucrose to maintain osmolarity. pH in the Tyrode was maintained at 7.40 by bubbling with 95% O_2/5% CO_2. The bath was kept at 26°C by a water jacket attached to a thermostatically controlled circulating pump. (Haake, Model PJ).

Isometric Tension Recording System

The papillary muscle was suspended with plastic cips in the muscle bath. One clip was attached by a stainless steel wire to a Statham UC-2 universal force transducer. The other clip was attached to a rigid plexiglass rod protruding to the bottom of the bath. Platinum plate electrodes were attached to the plexiglass rod and were placed parallel to the muscle. The muscle was stimulated through the electrodes from a Grass (S-4) stimulator maintained at 50% above threshold voltage throughout the experiment. Stimulating square pulses of 5msec. duration were applied. The stimulator was controlled from a Devices 3290 Digitimer through a Devices SI.U used to step-up voltage from 12V. to 30V, so that the S-4 could be triggered.

The tension signal from the force transducer was amplified and differentiated through a circuit with appropriate time constants (Figure II-2-1). Both tension (T) and the rate of tension development

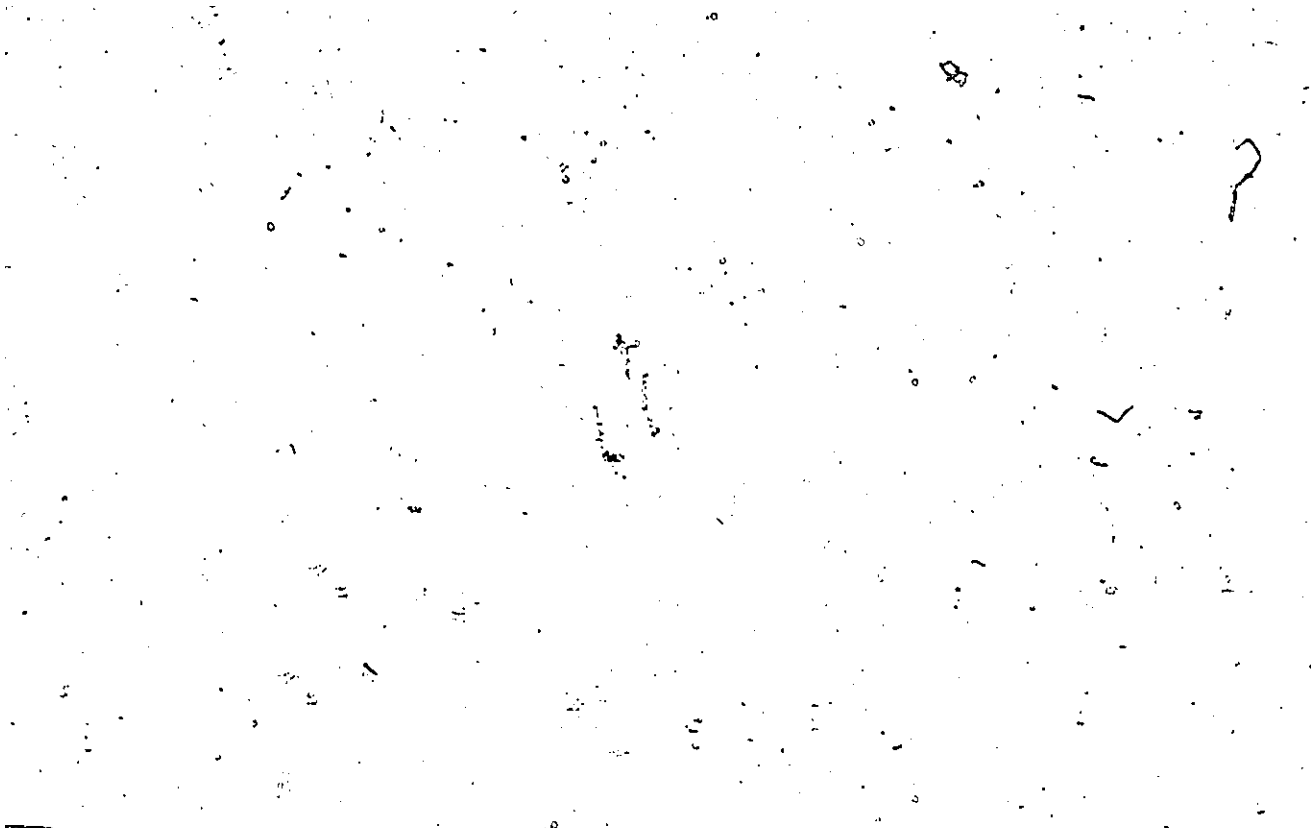
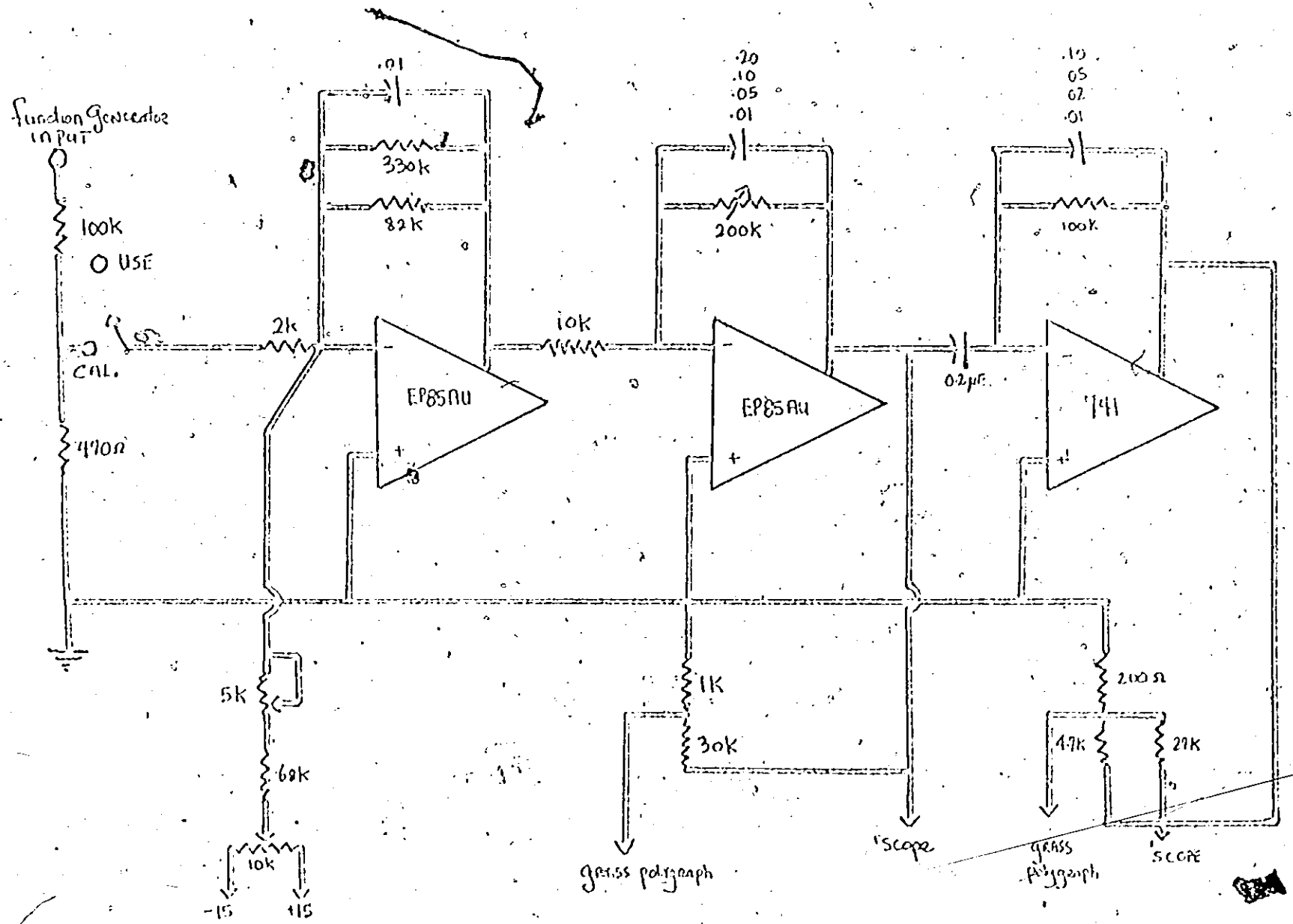


Figure II-2-1. Amplification and Differentiating Circuit used in Recording Isometric Tension.


Function Generator Input
100K
USE
CAL.
470Ω
2k
.01
330k
82k
EP85AU
10K
.20
.10
.05
.01
200K
EP85AU
.02μF
.10
.05
.02
.01
100K
741
5k
6.8k
10k
-15
+15
1K
30k
grass polygraph
scope
200Ω
4.7K
27K
grass polygraph
SCOPE

dT/dt) could be displayed on a storage oscilloscope (Tektronix 564B) or on paper with a Grass Model 7, 4 channel polygraph. The amplification and differentiating circuits were calibrated with a function generator (Hewlett Packard 33 A) using a triangular wave. Gain on the second operational amplifier was adjusted so that 1 g. of force at the UC-2 resulted in an output of 1 volt. (Figure II-2-2).

The record from the storage oscilloscope could be obtained with a Polaroid camera (Tektronix C-9) or alternatively on 35mm film with a Grass Recording Camera (C-4) triggered by the S-4 stimulator through a relay controlled by a logic unit. The relay also advanced a pulse counter to monitor frame number on the camera film.

For Phase-plane tracings, a dual beam oscilloscope was used (Tektronix 502). The time base was disconnected and beam 1 substituted so that one input could be plotted against the second input.

An equilibration period of 90 minutes was allowed for the muscle. Muscle length was adjusted initially so that no resting tension was produced. During the equilibration the muscle was stimulated at a frequency of 10/minute. At the end of the 90 minutes, muscle length was extended to the peak of the length-tension curve, and the stimulus rate was reduced to 2/minute. When rested state contraction tension was obtained (no change in tension from beat to beat) the experimental procedure was initiated.

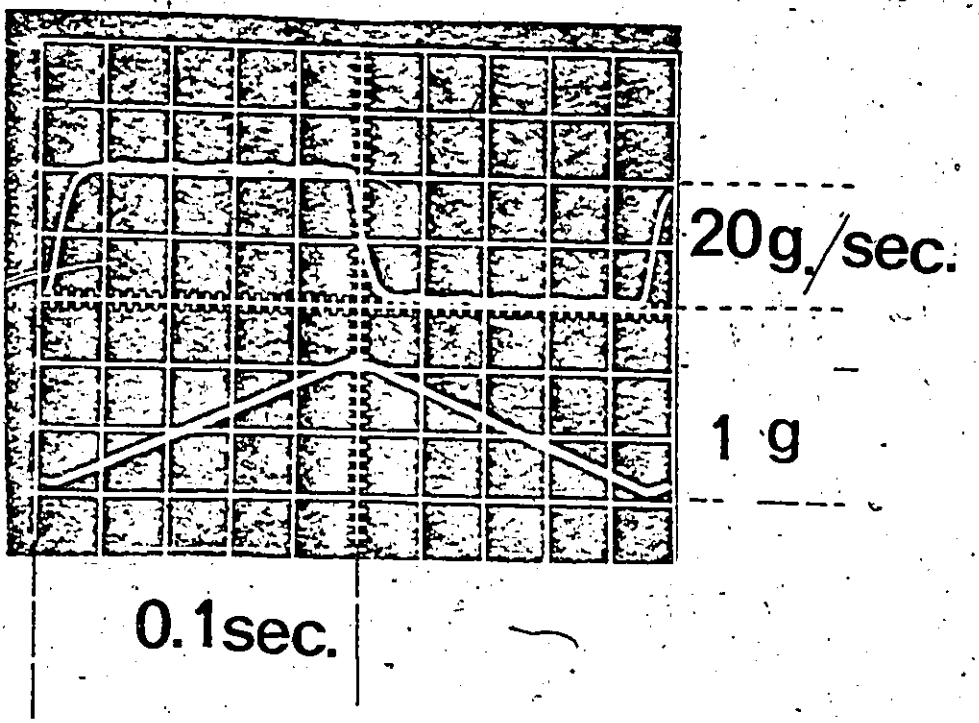


Figure II-2-2. Calibration Signal from Amplifying and Differentiating Circuit used in Isometric Tension Recording. Lower Trace: Triangular Wave Signal from Function Generator Corresponding to Output of 10g./sec. rise time (Time to peak tension). Upper Trace: Differentiated Signal of Lower Tracing. Apparent time constant - approximately 10 mSec.

Calcium Determinations

Calcium concentration was determined by 3 methods.

1. Total Plasma Calcium.

Total plasma calcium was determined by atomic absorption spectroscopy according to the method outlined by Hunt (1970).

2. Ionic Plasma Calcium.

An ion-specific electrode utilizing a flow-through system (Orion Model 9D-03-00) was used to determine ionic calcium in serum. This method required only 200 µl of sample.

3. Total Muscle Calcium

Total calcium in papillary muscles was determined by a method similar to the one devised by Borle and Briggs (1968) using a fluorometric titration.

Muscles were carefully removed from the bath following the experiment, blotted to remove surface fluid, weighed on a torsion balance (Roller-Smith) to nearest 0.1 mg and placed in the bottom of cleaned Vycor (quartz) cuvettes designed especially for the fluorometer and constructed by Expertise Glass (Montreal, P.Q.). The cuvettes were round and flat bottomed, 90 mm long, 7.5 mm bore with 1 mm wall thickness. Samples were dried overnight in the cuvettes in a vacuum drying oven at 80°C, and then placed for 24 hours in a muffle furnace at 600°C to be ashed. After cooling to room temperature the calcium content of each cuvette was determined by the fluorometric titration method.

100 µl of 0.1 N HCl was added to the cuvette and a Teflon stirring rod introduced. The cuvette was placed in the fluorometer and the ash allowed to dissolve in the acid. 50 µl. of fresh Calcein Buffer-KOH was added and total volume in cuvette adjusted to 2.0 ml. with top-quality distilled water. Titration was carried out with 0.25 mM EGTA and extinction of fluorescence followed on a Sargent pen recorder (Model SRL). Each pulse from the Lamda pump was registered on the chart by an event marker triggered from the pump driver. Top quality distilled water was obtained by passing double distilled water from the department's distillation system through two mixed bed resins and a cation exchanging resin connected in series. This water contained in a reservoir was recirculated through the resin system by a centrifugal pump (Cole-Parmer, Model 700).

All chemicals used were of highest quality and obtained from reputable suppliers. Calcein (Fluorescein-methylene-imonodiacetic acid) was obtained from Sigma Chemical Company, St. Louis, Missouri as was Ouabain (Strophanthin-G).

SECTION III

The Tension-Frequency Response and Post Stimulation Potentiation in Rat Papillary Muscle.

Introduction

The review of the literature suggested that calcium ion concentration both within the cardiac muscle and in the surrounding fluid space might affect the mechanical response of the muscle. Hence the affect of $[Ca^{++}]_o$ on the isolated papillary muscle of the rat heart has been investigated. Two indices of contractility have been used in this section: a) peak isometric tension, T, and b) peak dT/dt. The former is thought to represent the number of interactions between actin and myosin (Katz, 1970) while the latter index might represent the intensity of the interaction (Brady, 1968). The value of using these indices as comparable reflections of the active state in cardiac muscle is also indicated in this section through use of the phase plane plot.

The Tension-Frequency (T-F) Relationship in Rat and Guinea-Pig Papillary Muscles.

Figure III-1 shows the T-F relationship of the rat (closed circles) and guinea-pig (open circles) papillary muscles when external calcium concentration, $[Ca^{++}]_o$, is 2.5 mM. Between 2 and 10 stimuli/minute the guinea-pig displays a negative inotropism changing to a positive inotropism between 10 and 100 stimuli/minute and a decrease in tension above a stimulation rating rate of 120/minute. Over the same frequency range the rat muscle preparation has a negative inotropism; maximum tension (Tmax.) was always observed at a stimulation frequency of 2/minute.

The Effect of $[Ca^{++}]_o$ on the T-F relationship

With the rat papillary muscle preparation an increase in $[Ca^{++}]_o$ from 2.5 mM to 5.0 mM yielded a small increase in peak tension at a frequency of stimulation of 2/minute (Table III-I), but the shape of the T-F relationship was identical to that seen at 2.5 mM (Figure III-2-1). A decrease of $[Ca^{++}]_o$ below 2.5 mM changed the shape of the T-F curve dramatically. As $[Ca^{++}]_o$ was lowered below 2.5 mM, peak tension at the steady state interval (rested state contraction) of 30 seconds fell and a positive component to the T-F relationship developed (Table III-I, Figure III-2-1). This positive inotropism peaked around 120 stimuli/minute, close to the peak observed in the guinea-pig papillary muscle at 2.5 mM $[Ca^{++}]_o$. The positive component became increasingly

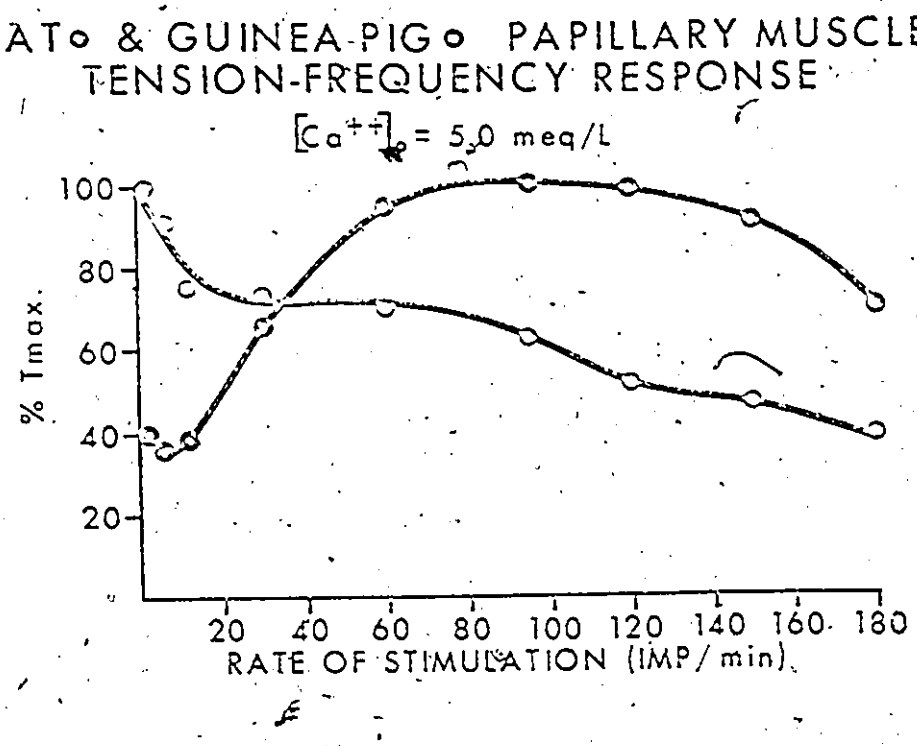


Figure III-1. Tension-Frequency Relationship in Rat (closed circles) and Guinea-Pig (open circles). $[Ca^{++}]_o$ = 2.5 mM.

Table III-1

Effect of $[Ca^{++}]_o$ on the Tension-Frequency Relationship in Isolated Papillary Muscle of the Rat.

$[Ca^{++}]_o$ (mM)	5.0		2.5		1.0		0.5		0.25	
Frequency of Stimulation (Imp/min.)	T_{max} g.	$\%T_{max}$	T_{max} g.	$\%T_{max}$	T_{max} g.	$\%T_{max}$	T_{max} g.	$\%T_{max}$	T_{max} g.	$\%T_{max}$
2	2.85	100	2.80	100	1.73	100		60		44.9
6		97		95.6		83.8		35		20.2
12		95.6		88.2		78		32.5		16.8
30		88.9		79.4		89.5		52.5		22
60		72.2		73.5		88		80		63
95		63.8		67.6		72.2	1.0	100		97
120		61.0		58.8		62.4		82.5	.89	100
150		58.0		50		58.9		72.5		87.6
180		53.8		44.1		49		55.5		67

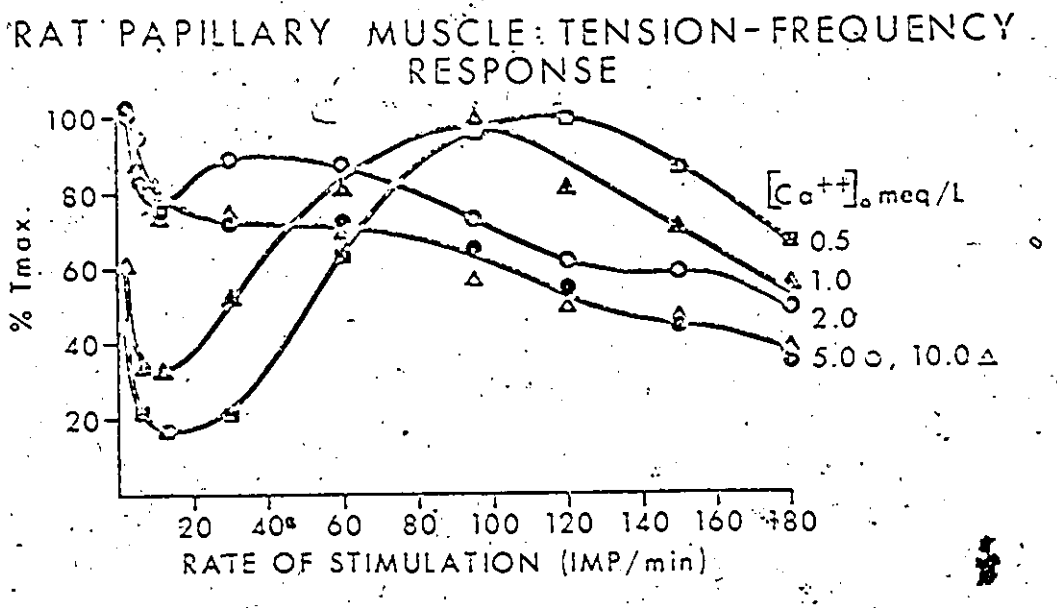


Figure III-2-1 Effect of $[Ca^{++}]_o$ on the Tension-Frequency Response of Rat Papillary Muscle.

prominent as the $[Ca^{++}]_o$ was lowered below 2.5 mM. At 0.25 mM. a response closely matching that observed in the guinea-pig could be elicited in the T-F relationship. The positive inotropic phase of the T-F response could be obtained at any point in the experimental procedure by lowering $[Ca^{++}]_o$ below 2.5 mM. A negative inotropic response was always obtained in rat muscle at a $[Ca^{++}]_o$ of 2.5 mM or greater. Oscilloscope photographs of the T-F relationship in rat papillary muscle at 2.5 mM; 1.0 mm and 0.25 mM $[Ca^{++}]_o$ are shown in Figures III-2-2, III-2-3, III-2-4 respectively.

Post-Stimulation Potentiation (PSP).

Post-stimulation potentiation was tested in several ways with the rat papillary muscle preparation. Figure III-3 shows the effect of a change in $[Ca^{++}]_o$ on dT/dt when the number of stimuli applied in a two second interval were varied. The dT/dt after a two-second rest interval was measured and compared to the rested state contraction dT/dt. This indicated that potentiation of dT/dt was most pronounced when $[Ca^{++}]_o$ was below 2.5 mM. Potentiation was obtainable but not significant at $[Ca^{++}]_o$ above 2.5 mM. At low $[Ca^{++}]_o$ less than 6 pulses actually decrease dT/dt after the two second rest, but a 10 pulses in 2 seconds (200 msec pulse-interval) potentiation of dT/dt is most evident ($[Ca^{++}]_o$ = 0.5 mM). In a sequel to this experiment, Figure III-4 shows that potentiation of T appears to peak about 30 seconds after the application of a rapid stimulus train (10 pulses at 200 m.Sec pulse interval). Based

Figure III-2-2. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 2.5mM. Frequency of Stimulation (Imp./min.).

A) 2

B) 6

C) 12

D) 30

E) 60

F) 95

G) 120

H) 150

Upper Tracing: dT/dt; 1 division = 10g./sec.

Lower Tracing: Tension (T); 1 division = 1.0g.

Time Scale: 1 division = 0.05 sec.

Figure III-2-2. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]$ = 2.5mM. Frequency of Stimulation (Imp./min.).

A) 2

B) 6

C) 12

D) 30

E) 60

F) 95

G) 120

H) 150

Upper Tracing: dT/dt; 1 division = 10g./sec.

Lower Tracing: Tension (T); 1 division = 1.0g.

Time Scale: 1 division = 0.05 sec.

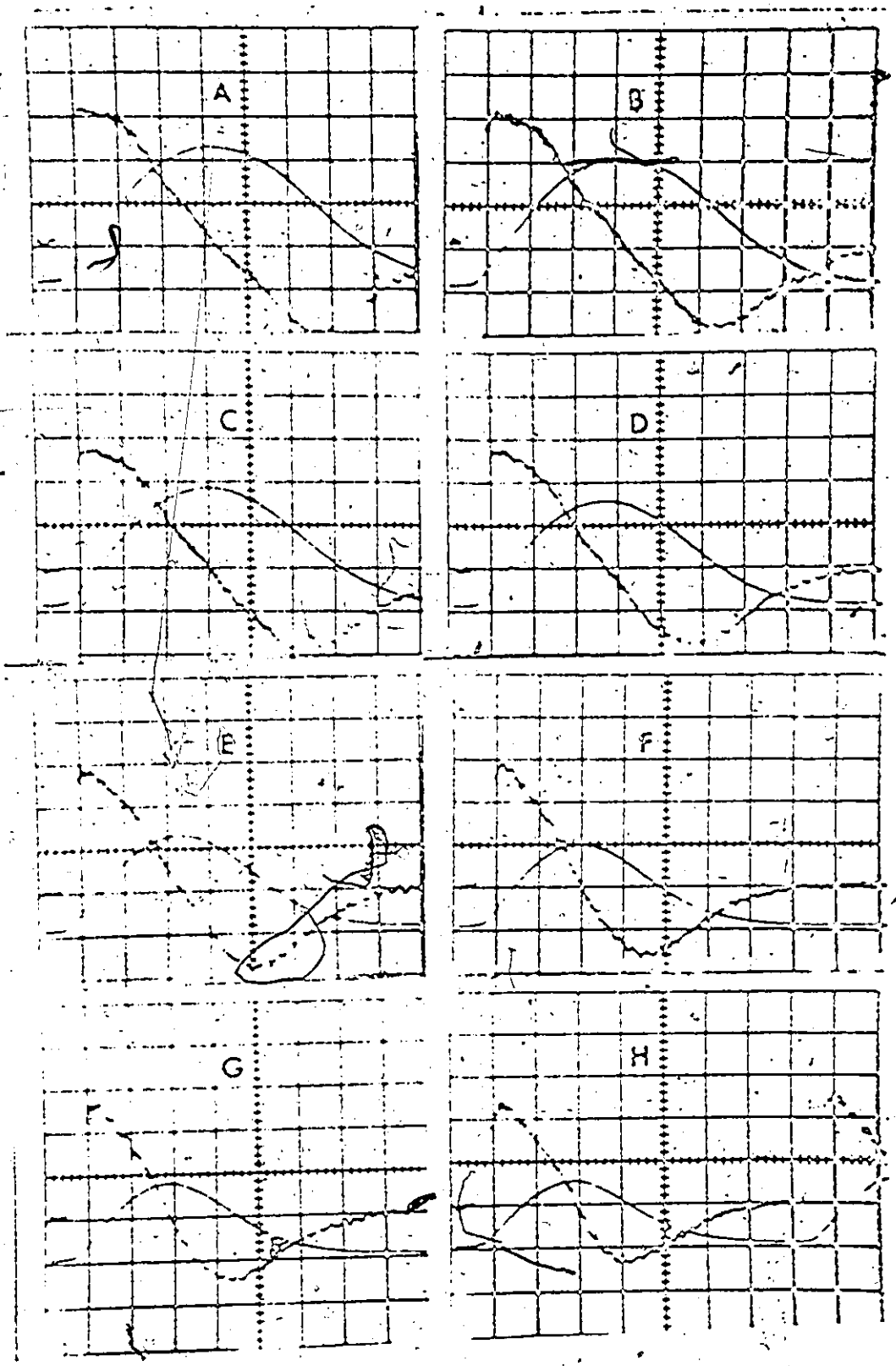

A
B
C
D
E
F
G
H

Figure III-2-3. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 1.0mM. Frequency of Stimulation (Imp./min.).

A) 2 B) 6

C) 12 D) 30

E) 60 F) 95

G) 120 H) 150

Upper Tracing: dT/dt; 1 division = 10g./sec.

Lower Tracing: Tension (T); 1 division = 1.0g.

Time Scale: 1 division = 0.05 sec.

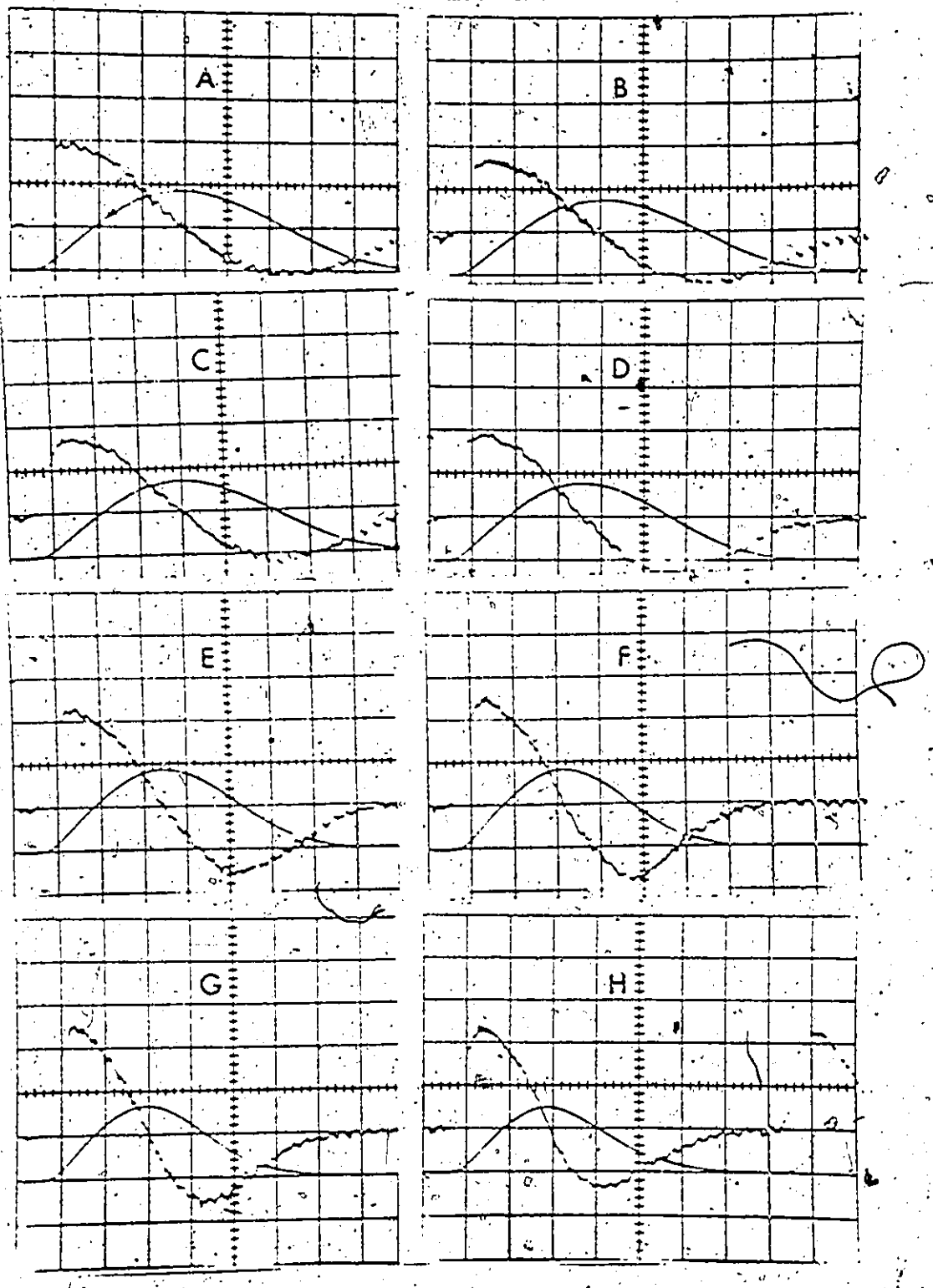

A
B
C
D
E
F
G
H

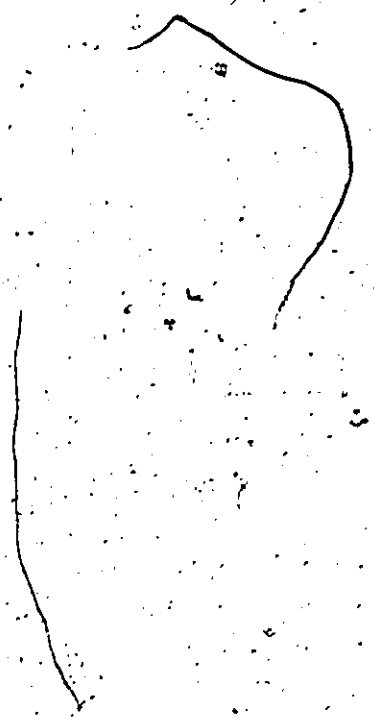
Figure I-2-4. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 0.25mM. Frequency of Stimulation (Imp./min.).

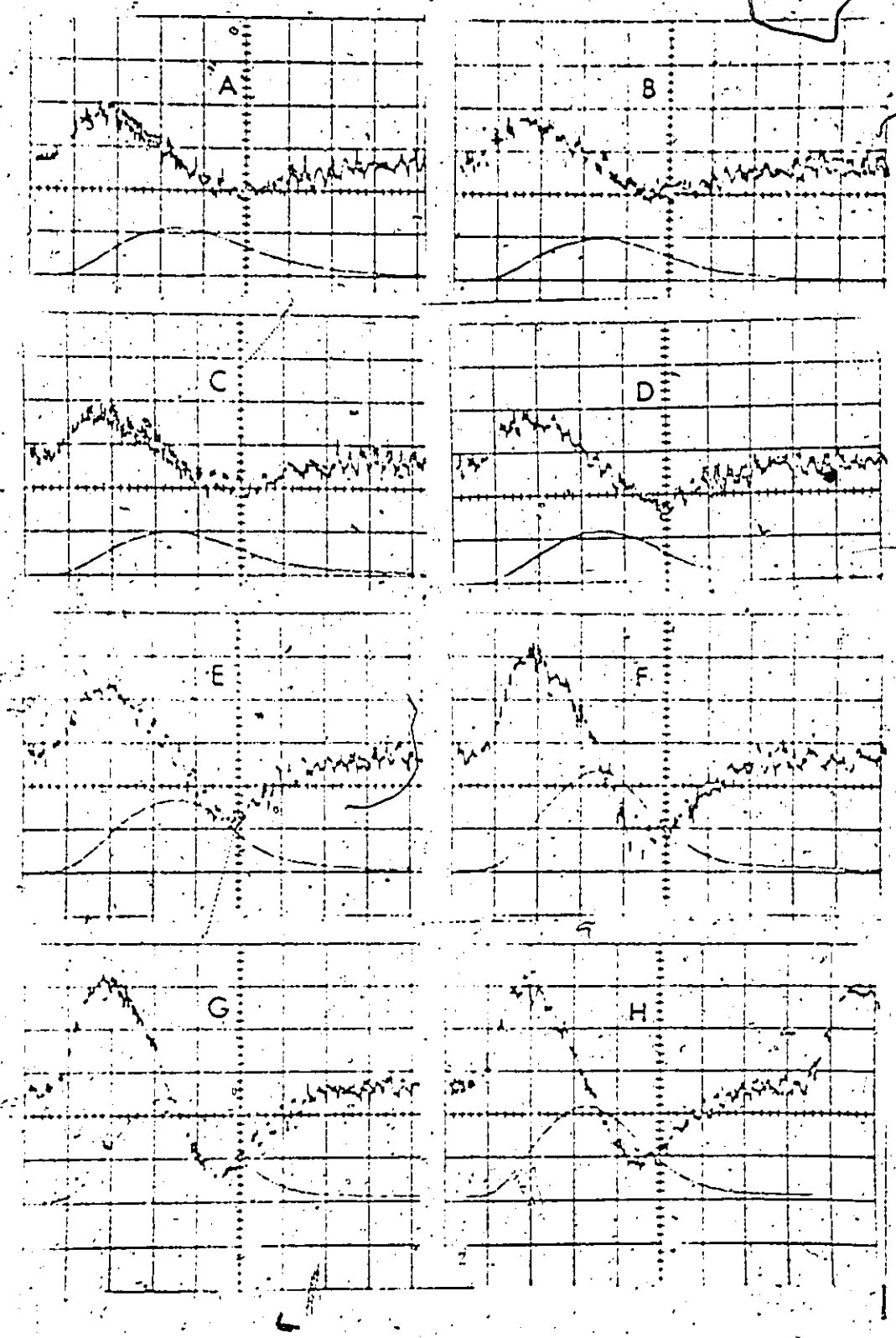
A) 2
B) 6
C) 12
D) 30
E) 60
F) 95
G) 120
H) 150

Upper Tracing: dT/dt; 1 division = 2.5g./sec.

Lower Tracing: Tension (T); 1 division = 0.2g.

Time Scale: 1 division = 0.05 sec.




A
B
C
D
E
F
G
H

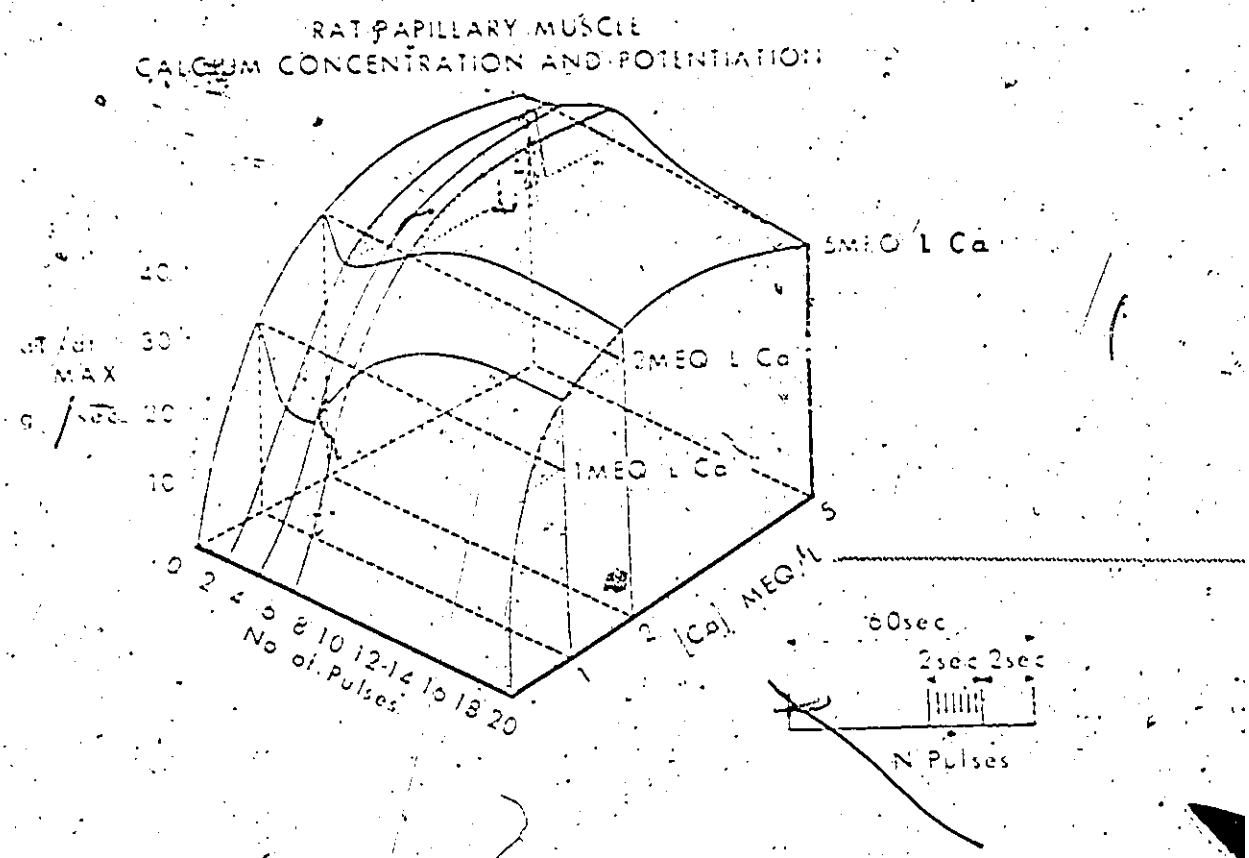
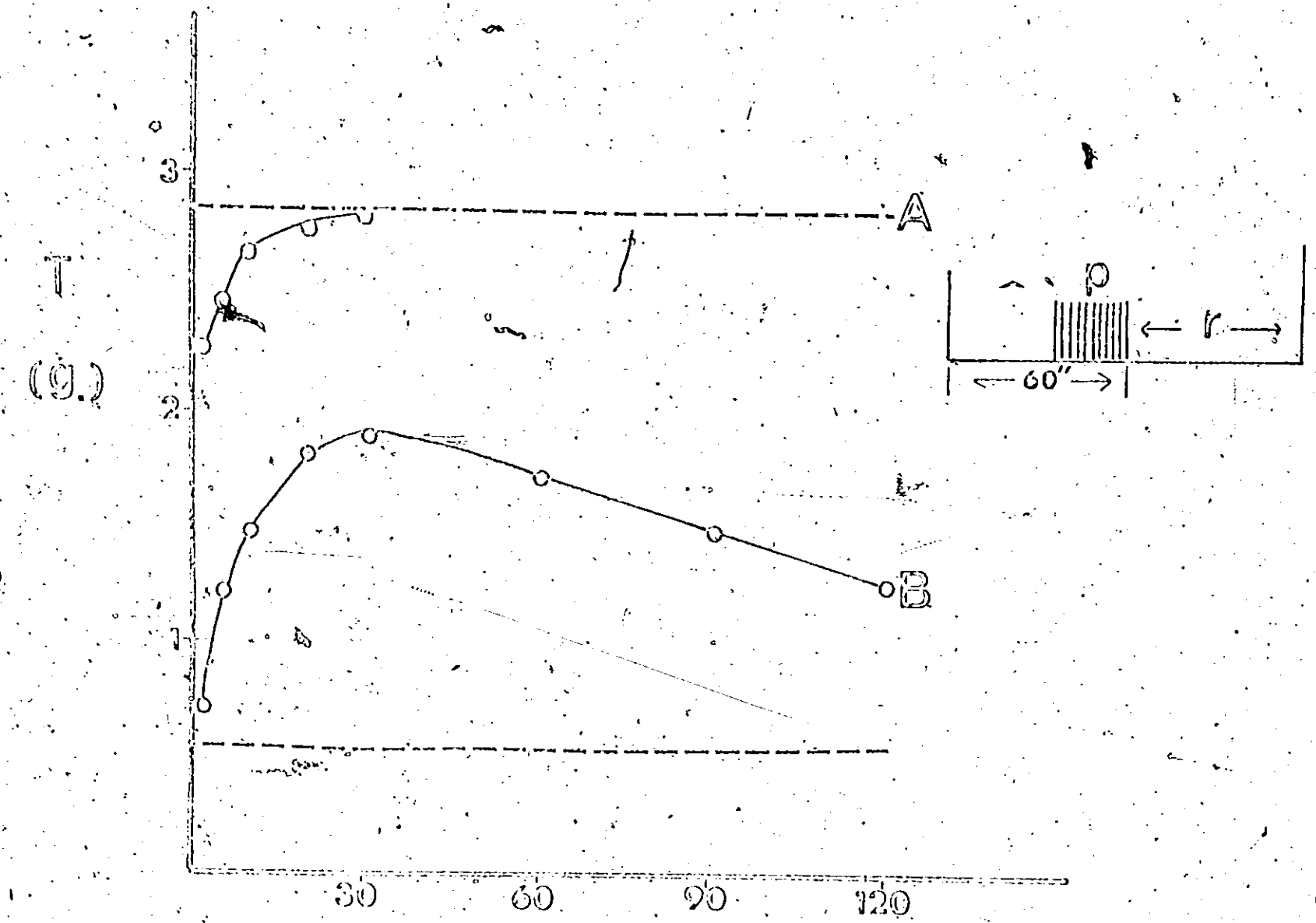


Figure III-3. Post-Stimulation Potentiation in Isolated Papillary Muscle of the Rat. Effect of Pulse Interval in the Conditioning Train and $[Ca^{++}]_o$.

Figure III-4. Effect of Rest-Interval (r) on Post-Stimulation Potentiation (PSP). 10-200msec. pulses introduced (p) followed by r interval. A (●) $[Ca^{++}]_o$ = 2.5mM; B (○) $[Ca^{++}]_o$ = 0.25mM. Broken line indicates Rested State Contraction tension (RSCt).


T
(g.)
3
2
1
A
B
P
r
60"
30
60
90
120
r Interval - sec.

upon these results, a standard test for PSP was instituted. With a basic stimulus interval of 30 seconds a train of 10 pulses was applied at 200 msec. intervals followed by a rest interval of 30 seconds and a single stimulus. The tension and/or dT/dt measured at the end of the rest period was compared to the control tension and/or dT/dt developed before the pulse train; this is called $PSP_{30"}$.

Table III-II shows the effect of $[Ca^{++}]_o$ on $PSP_{30"}$ in a rat papillary muscle preparation. The % $PSP_{30"}$ increases as $[Ca^{++}]_o$ is decreased below 2.5 mM, and $PSP_{30"}$ disappears above $[Ca^{++}]_o$ = 2.5 mM. That the limit to obtainable potentiation is coincident with the tension obtainable at $[Ca^{++}]_o$ around 2.5 mM is best illustrated in the following experiment.

$PSP_{30"}$ was tested over a wide range of external calcium concentrations and with a wide number of stimuli preceding a 30" rest period. These results are grouped in Table III-III and illustrated in Figure III-5. It is clear that the limit of potentiation obtainable from isolated rat papillary muscle is practically the same as the maximum tension achieved by increasing external calcium concentration in the bathing fluid. The greatest proportional increase of tension with $PSP_{30"}$ is obtained when $[Ca^{++}]_o$ = 0.2 mM.

The Phase-Plane Plot and Post Stimulation Potentiation

In the preceding results, peak isometric tension (T) and the peak derivative of tension development, dT/dt, have both been

Table III-II

Rat Papillary Muscle

Effect of $[Ca^{++}]_o$ on $PSP_{30"}$ *

$[Ca^{++}]_o$ mM	T g.	$PSP_{30"}$ g.	%PSP	dT/dt g./sec.	PSPdT/dt g./sec.	% Control
5	2.8	2.5	89	32.5	30.0	92
2.5	3	2.85	95	36.0	35.0	97.0
1.0	1.7	2.7	159	20.	31.0	155
0.25	0.4	1.95	488	5.0	21.5	430

* 10 pulses at 200 Msec. pulse Interval - 30 second rest period.

Table III-III

Effect of $[Ca^{++}]_o$ and Preconditioning Pulses on $PSP_{30''}$.

$[Ca^{++}]_o$ mM	Tension g.	# Pulses	PSP g.		% PSP
	.070	1			
	.070	2	.075		107
	.070	4	.10		143
0.1	.070	8	.12		171
	.08	15	.13		186
	.080	25	.155		221
	.075	50	.19		271
	.07	100	.27	*	386
	.335	1			
	.340	2	.370		100
	.340	4	.495		146
0.2	.330	7	.705		214
	.320	15	1.01		316
	.310	25	1.330		415
	.280	50	1.35		421
	.250	100	1.40	*	437
	.83	1			
	.83	2	91		110
	.83	4	1.34		161
0.3	.85	8	2.25		265
	.75	15	2.70		360
	.75	25	2.70	*	360
	.70	50	2.45		350
	.60	100	2.32		361
	1.15	1			
0.4	1.10	2	1.23		112
	1.12	5	2.28		204
	1.05	8	2.80		267
	1.05	15	3.20	*	305
	1.07	25	2.95		276

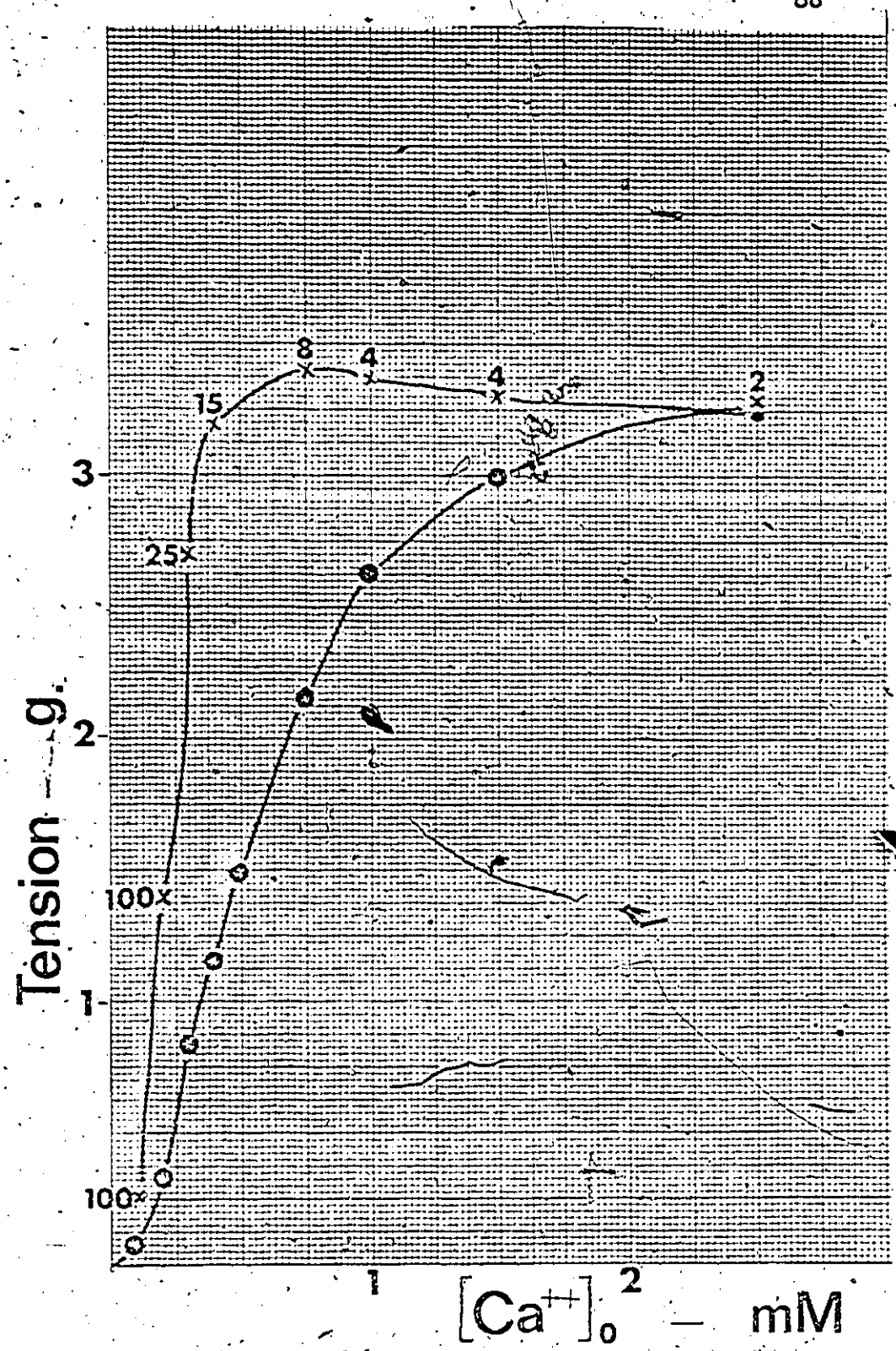
* Maximum potentiated tension.

Table III-III (cont'd)

$[Ca^{++}]_o$ mM	Tension	# Pulse	PSP g.		% PSP
	1.5	1			
0.5	1.5	2	1.78		119
	1.32	4	2.30		174
	1.30	8	3.15	*	242
	1.30	15	3.13		241
	1.30	25	3.07		236
	1.20	50	2.92		225
	2.15	1			
.75	2.17	2	2.50		115
	2.17	4	3.12		144
	2.17	8	3.40	*	157
	2.17	15	3.30		152
	2.63	1			
	2.63	2	2.95		112
1.0	2.63	4	3.35	*	127
	2.48	8	3.25		133
	2.50	15	3.30		132
	2.33	25	3.15		135
	2.35	50	2.87		122
	2.95	1			
1.5	2.95	2	3.2		108
	2.98	4	3.29	*	110
	2.97	8	3.25		109
	2.95	15	3.17		107
	3.20	1			
2.5	3.20	2	3.29	*	103
	3.22	4	3.22		100
	3.23	8	3.19		99

* Maximum potentiated tension.

Figure III-5. Post-Stimulation Potentiation in Isolated Papillary Muscle of the Rat (X). Numbers next to X's indicate number of stimulating pulses to produce potentiation; (●) indicates RSC tension for each $[Ca^{++}]_o$.

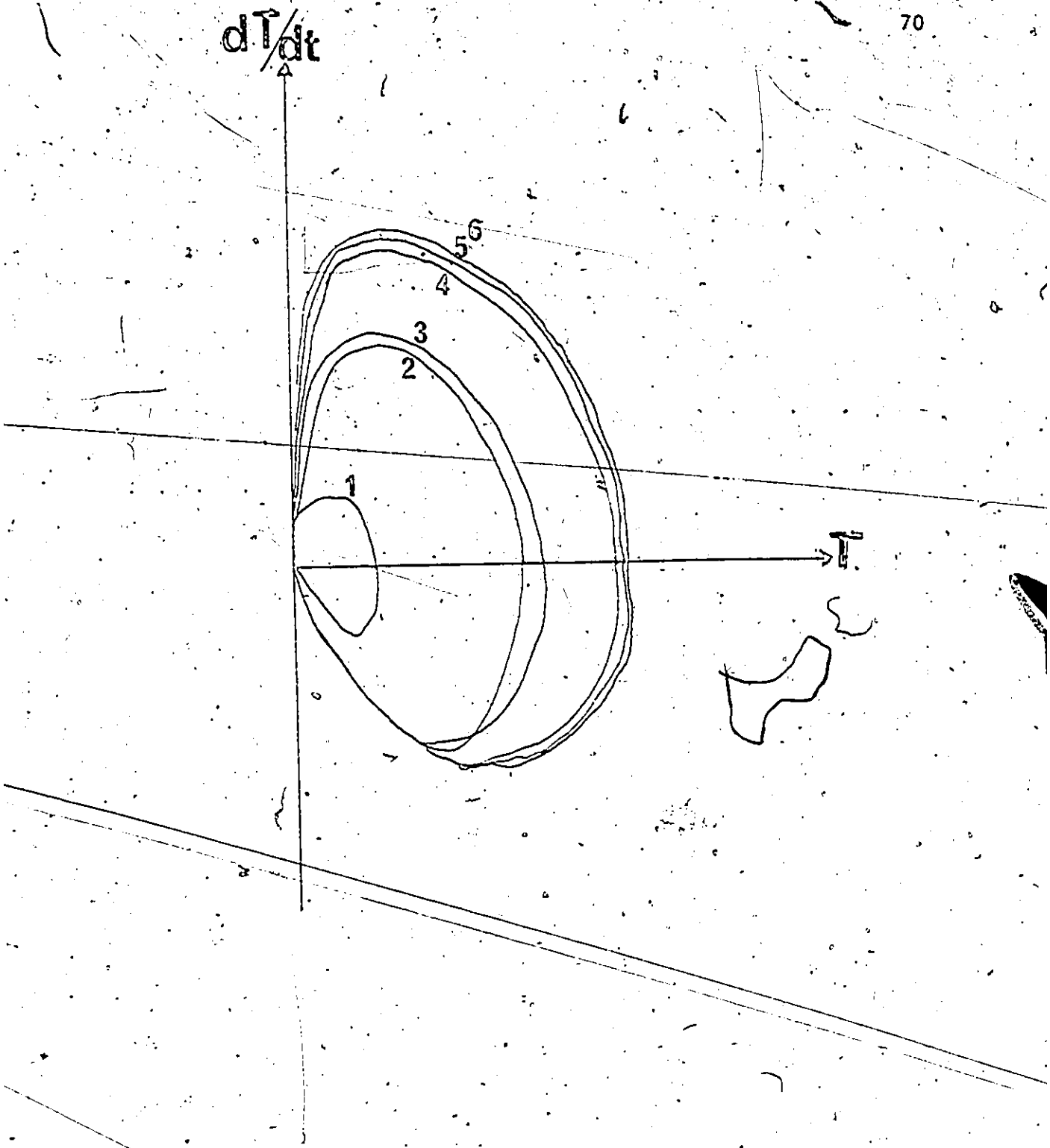

Tension — g.
3
2
1
8
4
4
2
15
25×
100×
100×
1
$[Ca^{++}]_o^2$ — mM

utilized as indices of contractility interchangeably. There is some indication that both of these indices change in a similar way with potentiation (Table III-II). However, a change in peak isometric tension can be arrived at through several routes. For example, if one observes a diminution of the peak isometric force by 50%, it is possible that a) when there is little elasticity in the muscle, the rate of tension development will not change from that observed at the former condition. b) when there is moderate elasticity the rate of tension development will lag in time and c) in the condition where the active state develops slowly peak tension might still be reached but peak dT/dt would be very small. Therefore a change in the active state can properly only be assessed by measuring the rate of change of dT/dt at each value of T. The phase plane plot allows comparison of contraction in these circumstances. Therefore if we can demonstrate that the interventions of calcium ion concentration, $[Ca^{++}]_o$, and PSP maintain the temporal relationship in the phase plane plots of dT/dt versus T, we would have a proof for utilizing the contractility indices of peak isometric tension and peak dT/dt as mutual indicators of contractile function.

The simultaneous display of the derivative of tension development dT/dt versus tension (T) in the analysis of potentiation shows clearly that PSP is comparable to an increased calcium load on the papillary muscle preparation. Figure III-6 shows the phase-plane

Figure III-6. Phase-Plane Tracing of Isometric Contractions in Isolated Papillary Muscle of the Rat. Effect of $[Ca^{++}]_o$ on PSP_{30}''.

1. RSC at 0.25mM $[Ca^{++}]_o$.
2. PSP_{30}'' at 0.25mM $[Ca^{++}]_o$.
3. RSC at 1.0mM $[Ca^{++}]_o$.
4. PSP_{30}'' at 1.0mM $[Ca^{++}]_o$.
5. RSC at 2.5mM $[Ca^{++}]_o$.
6. PSP_{30}'' at 2.5mM $[Ca^{++}]_o$.


dT/dt
6
5
4
3
2
1
T

plots for rested state contractions and potentiated contractions at 2.5, 1.0 and 0.25 mM external calcium concentrations. Clearly the potentiated contraction at 0.25 mM $[Ca^{++}]_o$ is close to that of the rested state contraction at 1 mM $[Ca^{++}]_o$ while the potentiated contraction at 1 mM superimposes upon the phase-plane plot of the rested state contraction at 2.5 mM $[Ca^{++}]_o$. The potentiation of tension at 1.0 mM is shown again in Figure III-7 with the contractions at 30, 60, and 90 seconds after the pulse train. Figure III-8 shows the phase-plane plots of the same procedure at 0.25 mM $[Ca^{++}]_o$.

Effect of Frequency of Stimulation on Diminution of PSP.

PSP was produced in the papillary muscle preparation and the decay of potentiation was followed at various frequencies of stimulation following the initial 2 second pause subsequent to the pulse train ($PSP_{2''}$). At 2.5 mM $[Ca^{++}]_o$ there is no potentiation observable and the "potentiated" contraction is below the control value until the interval is 10 seconds (Figure III-9). At intervals less than 10 seconds, the tension falls to levels characteristic for that frequency according to Figure III-2-1. However, in the experiments where $[Ca^{++}]_o$ is below 2.5 mM, and potentiation of tension is produced in the 2 second interval between the pulse train and the PSP pulse, an interesting phenomenon is observed as the $[Ca^{++}]_o$ is lowered. It appears as if the decay of potentiation becomes independent of frequency; that is the 2nd, 3rd and 4th contractions are about equal in tension at frequencies of

Figure III-7. Phase-Plane Tracing of Isometric Contractions in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 1.0mM.

1. Control
2. $PSP_{30"}$
3. $PSP_{30"} + 30"$
4. $PSP_{30"} + 60"$

Loop is traced clockwise beginning at origin.

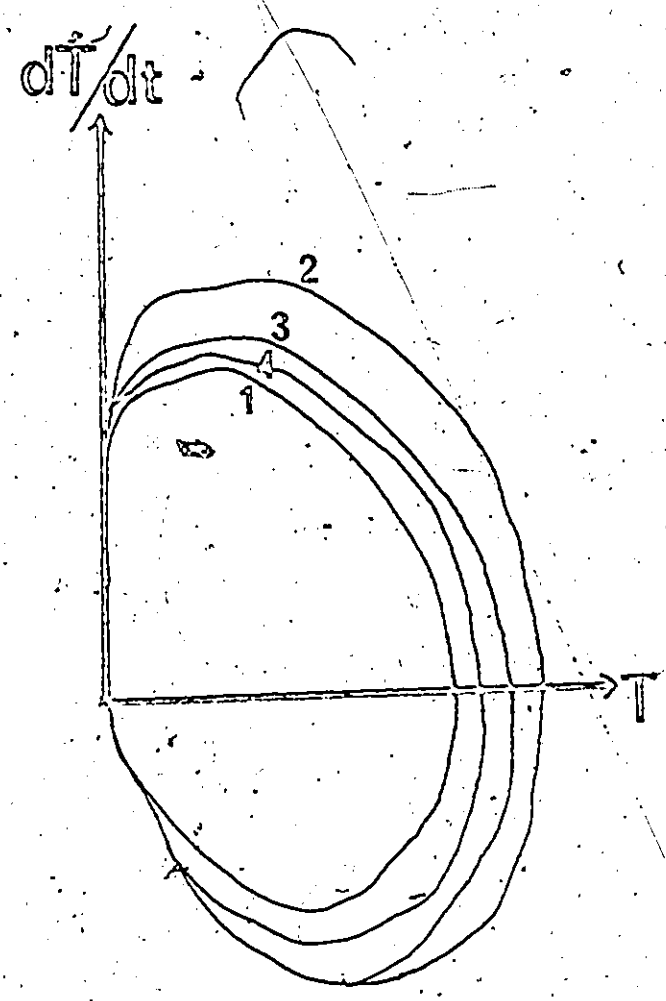

dT/dt
T
1
2
3
4

Figure III-8. Phase-Plane Tracing of Isometric Contractions in Isolated Papillary Muscle of the Rat. Ca_o = 0.25mM.

1. Control
2. $PSP_{30"}$
3. $PSP_{30"}$ + 30"
4. $PSP_{30"}$ + 60"

Loop is traced clockwise beginning at origin.

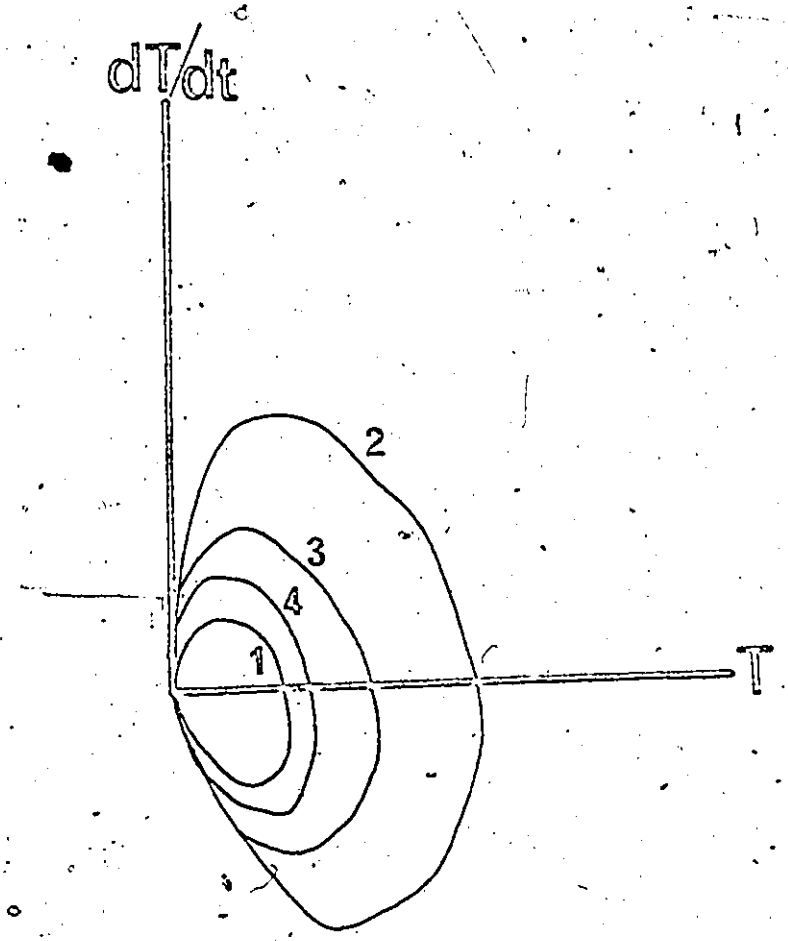
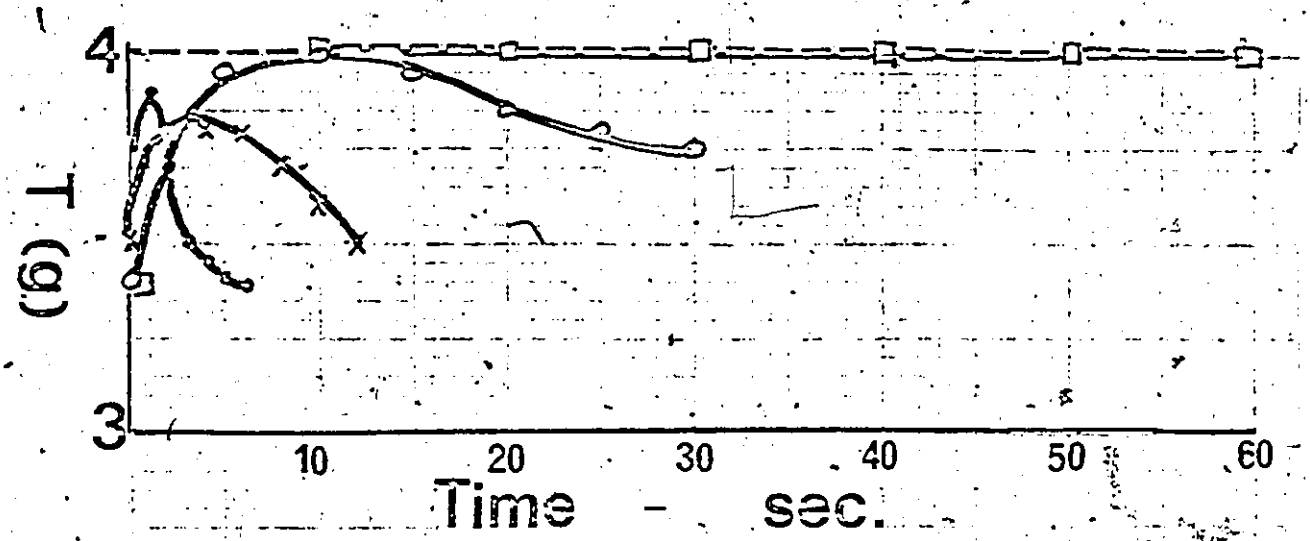

dT/dt
T
1
2
3
4

Figure III-9. Decay of PSP_2 in Isolated Papillary Muscle of the Rat. Effect of Frequency of Stimulation (Imp/sec.) (●) 1; (X) 0.5; (0) 0.2; (□) 0.1.

Broken line indicates RSC tension. $[Ca^{++}]_o$ = 2.5mM.



1, 2, 5 and 10 second pulse intervals. This is most evident at 0.25 mM $[Ca^{++}]_o$ but still observed at 0.5 mM and 1.0 mM (Figures III-10, III-11 and III-12).

Discussion

These experiments clearly show that external calcium concentration plays an important if not a direct role in determining the shape of the Tension-Frequency relationship. They also demonstrate that the negative T-F relationship normally associated with rat ventricular muscle (Henderson et al., 1969, Hoffman and Kelly, 1959; Kelly and Hoffman, 1960) can be modified to appear similar to that of other mammalian species and show a positive inotropic component (Koch-Weser and Blinks, 1963). By lowering the external calcium concentration below 2.5 mM we have been able to show the appearance of the positive inotropic component at frequencies of stimulation between 20 and 100 shocks per minute, with the peak in tension occuring at the latter frequency. This peak is similar to that obtained with guinea-pig (Figure III-1) and cat (Henderson et al., 1969). That calcium ion plays a dominant role in the T-F relationship was also demonstrated by Teiger and Farah in rabbit heart muscle where lowering $[Ca^{++}]_o$ increased the slope of the positive inotropic response and increased calcium concentrations caused a decrease in the slope of the positive compcnent of the T-F relationship. In rabbit muscle the negative inotropic T-F response was obtained when $[Ca^{++}]_o$ approached 7.2 mM with the greatest

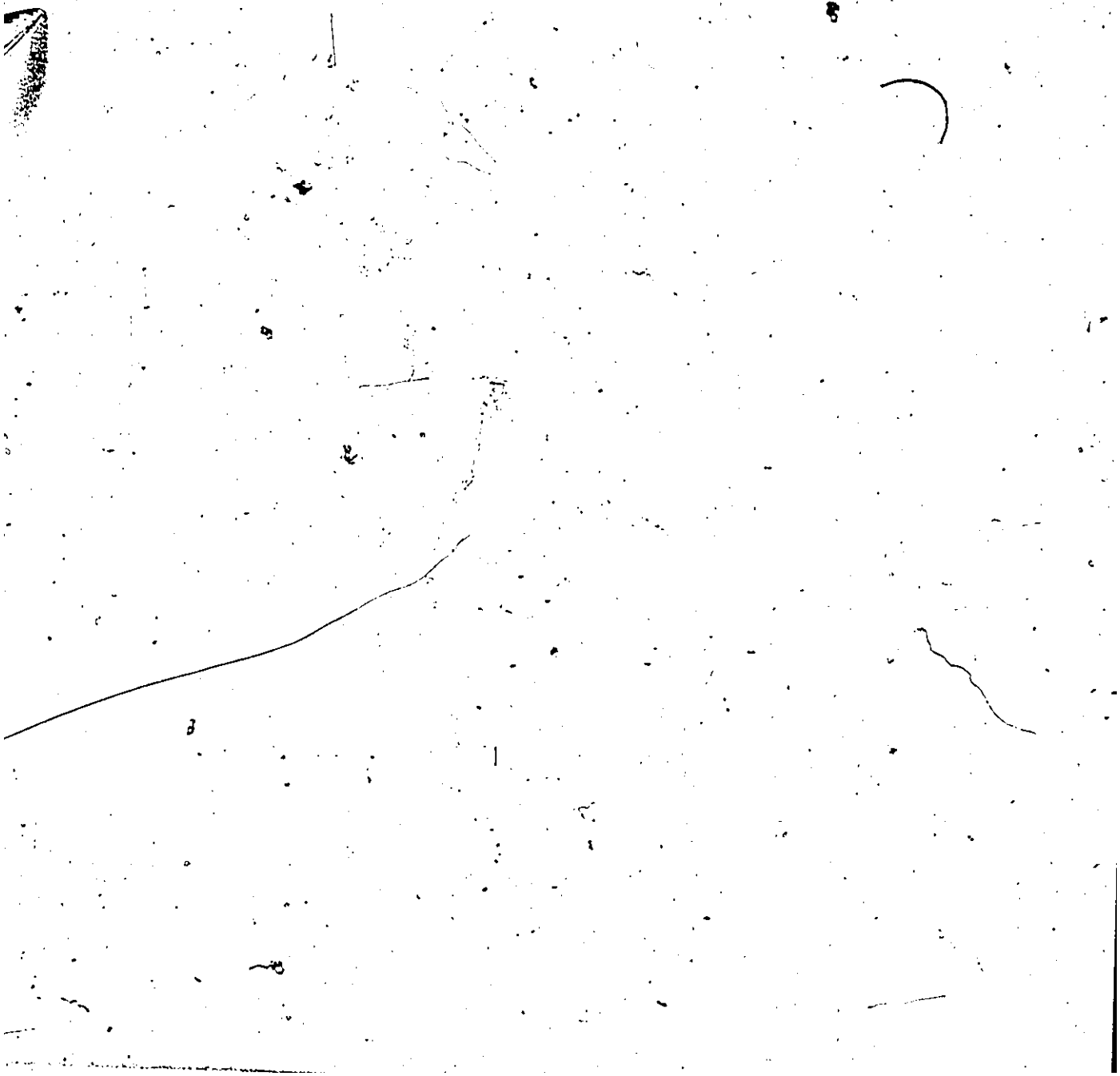


Figure III-10. Decay of PSP_{2n} in Isolated Papillary Muscle of the Rat. Effect of Frequency of Stimulation. (Imp./sec.) (●) 1; (X) 0.5; (0) 0.2; (□) 0.1. Broken line indicates RSC tension. $[Ca^{++}]_o$ = 1.0mM.

60
70
80
Time sec
-1
-2
-3
T (a)

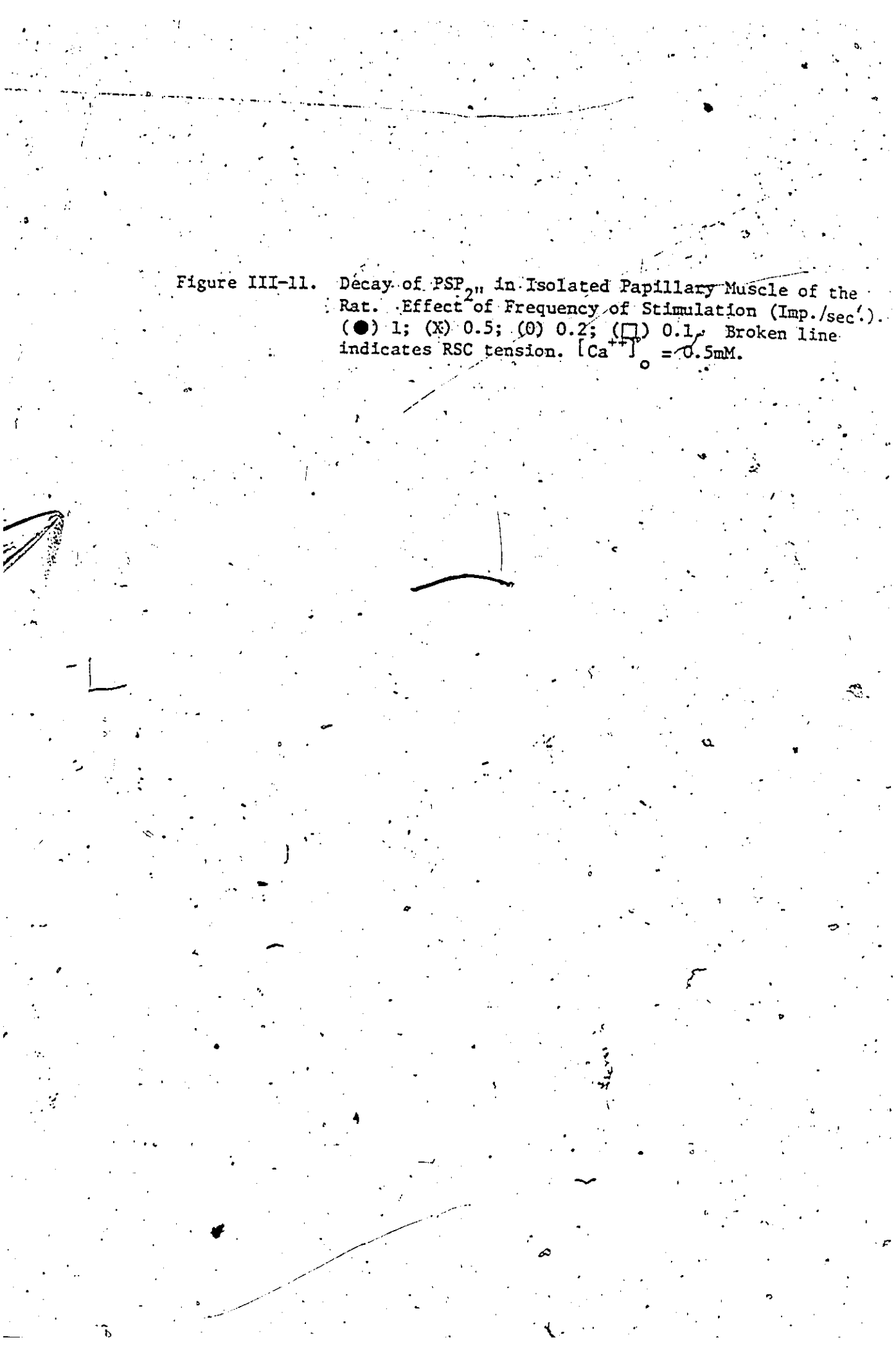


Figure III-11. Decay of PSP_2" in Isolated Papillary Muscle of the Rat. Effect of Frequency of Stimulation (Imp./sec.). (●) 1; (X) 0.5; (0) 0.2; (□) 0.1. Broken line indicates RSC tension. $[Ca^{++}]_o$ = 0.5mM.

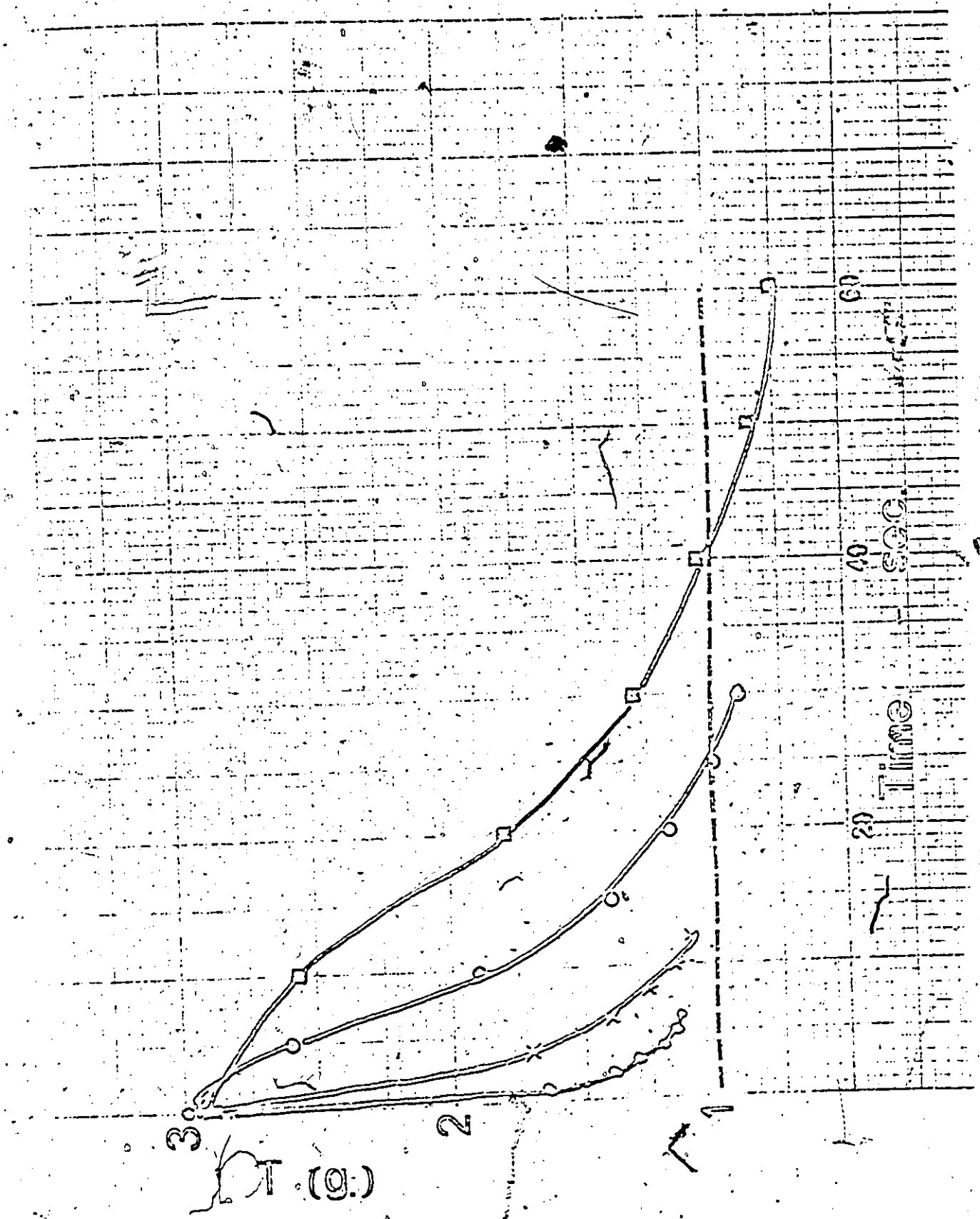
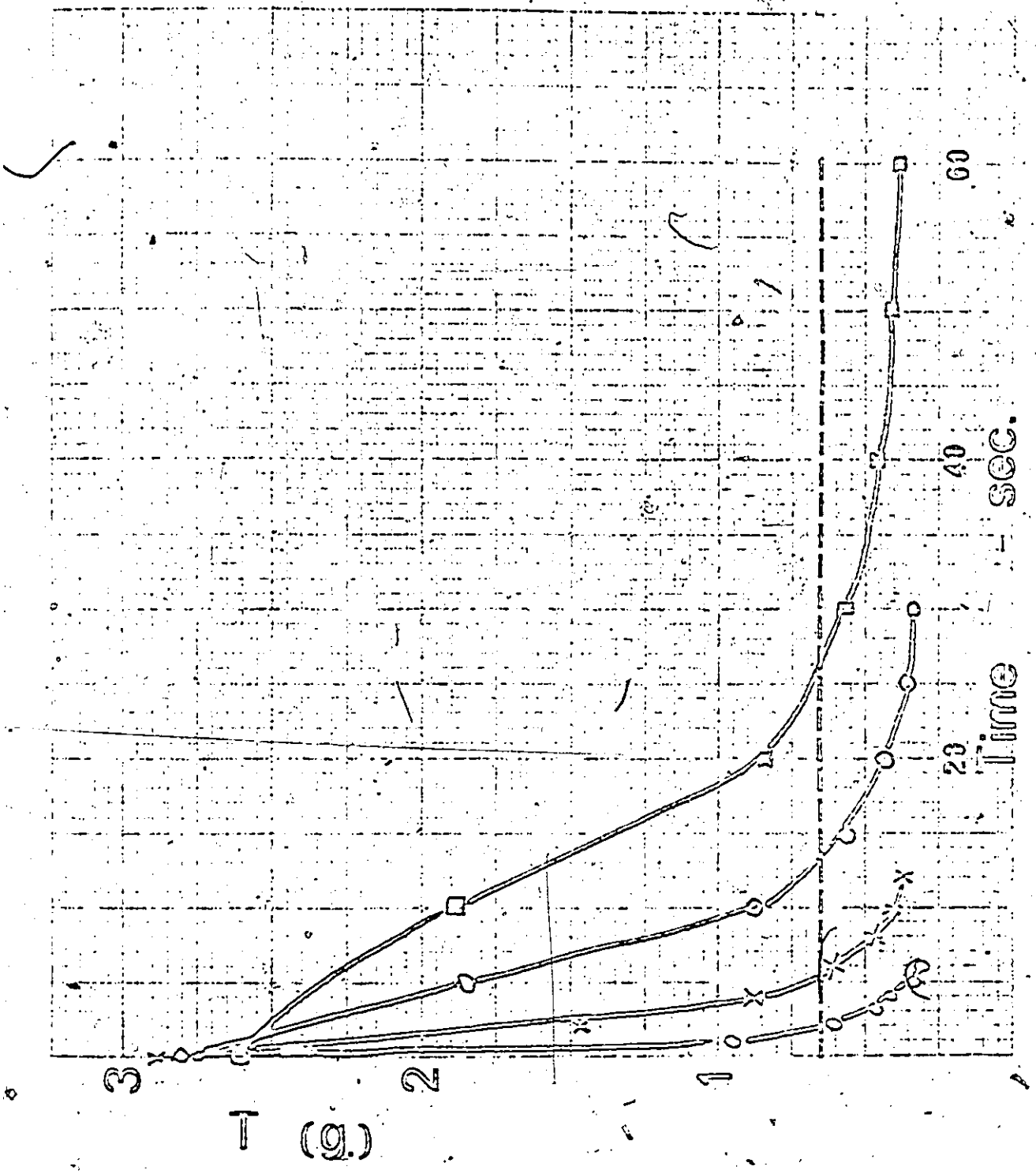

60
40
20
Time (sec)
3
2
1
T (g.)

Figure III-12. Decay of PSP_2 in Isolated Papillary Muscle of the Rat. Effect of Frequency of Stimulation. (Imp./sec.) (●) 1; (X) 0.5; (0) 0.2; (□) 0.1. Broken line indicates RSC tension. $[Ca^{++}]_o$ = 0.25mM.


60
40
sec.
20
Time
3
2
1
T (g.)

positive inotropic slope occurring at $[Ca^{++}]_o$ = 1.8 mM. Our data with rat heart indicates that we are dealing with a similar effect although the range of calcium concentration required to reverse the positive inotropism of the T-F relationship is less in the rat than the rabbit. As indicated in the review of Mommaerts et al., (1960), the relationship between the stimulus rate and the force of contraction is thought to encompass the Frequency-Force curve as well as phenomena such as post-extrasystolic potentiation and post-stimulation potentiation. Our results indicate conclusively that PSP can be obtained from rat heart muscle and that the degree of potentiation is related to the external calcium ooncentration (Table III-II). A similar conclusion was reached by Teiger and Farah (1968) with rabbit heart. Meijler et al., (1962) and Meijler (1962) demonstrated both the positive staircase and post-extrasystolic potentiation in isolated isotonically contracting rat heart, but did not attribute these phenomena to calcium ion. Nieuwendijk et al., (1965) however, suggested that contractility changes originated by interval changes are regulated by calcium ion concentration. Our results indicate that in rat papillary muscle 1) potentiation only occurs when $[Ca^{++}]_o$ is lowered below 2.5 mM. 2) the maximum potentiated tension attainable is the same as the maximum rested state contraction tension (Figure III-5), and 3) that potentiated tension is related to a) number of potentiating pulses b) the time interval from the potentiating influence.

The last factor appears to be the most critical. Even with a large number of pulses, if the rest period following this is not sufficiently extended, potentiation will not be obtained (Figures III-3, III-4). Therefore, whatever mechanism or factor is involved in potentiation, the requirement appears to be that a time dependency is included. At the lowest calcium concentration used (0.25 mM) a time course of approximately 15seconds is needed to express the maximum effect of the preconditioning pulse train and thereafter the potentiated state is maintained for a fairly long interval decaying slowly compared to the buildup of potentiation. The "factor" buildup during potentiation must therefore exist in a relatively stable fraction (without stimulation) to be mobilized as long as 2 minutes after the pulse train. However, the intervention of stimulating pulses at various frequencies following the buildup during the potentiating period rapidly depletes this factor. At lower calcium concentrations it becomes apparent that the removal of this factor by stimulation at various frequencies is frequency independent, each stimulus removing a fraction of the builtup contractile tension. A similar result was obtained by Wood, Heppner and Weidmann (1969) in voltage-clamped sheep heart muscle where inward current during the plateau phase was modified to create various inotropic states. The decay of the inotropic state created by normal stimulation was found to be frequency independent. They attributed this to contractile calcium concentration in the tissue. That we are moving up and down within a

similar family of contractile phenomena with the change in external calcium is best illustrated in the phase plane tracings. The fact that a potentiated contraction can fit directly over a loop traced at a higher calcium concentration indicates that there has been no disturbance in the overall mechanics of the muscle in time, and suggests that we are dealing with a similar muscle at high calcium and at low calcium potentiated with our technique. These data suggest strongly that changes in external calcium concentration are mirrored in internal changes of the same ion in the rested state and that potentiation and the Tension-Frequency relationship are in turn reflections of the same changes over interval dependent periods.

The observation that the phase plane plot of a potentiated contraction superimposes on a plot of a contraction at a higher calcium concentration may merely be coincidental. However, the phase plane plots appear to reflect a system where the perimeter of the phase plot is determined by the gain in the system; i.e., a greater gain produces a larger plot with the overall temporal relationship between T and dT/dt maintained. Again, this would suggest that the interventions we have used in altering the contractile behaviour, PSP and $[Ca^{++}]_o$, have identical effects on internal mechanisms of the cardiac muscle - at least as far as can be ascertained by the phase plane plot. As with other heart tissues, the inotropic effect of increased $[Ca^{++}]_o$ is elicited to a maximum - 2.5mM in the isolated rat preparation - at

which point both potentiation and the positive component to the T-F relationship are no longer obtainable. As a sequel to this we investigated the calcium concentration in arterial blood of 4 rats. Using atomic absorption analysis, rat plasma was found to contain calcium at 2.50 mM - considered as total calcium - while calcium concentration measured with a flow - through ion - specific electrode indicated an ionic concentration of 1.31 mM, about half of the total calcium level. This is comparable to the ionized calcium level found in other species (Ganong, 1969). Most studies on the isolated papillary muscle of the rat have been conducted at bath calcium concentrations above this ionized level (Henderson et al., 1969, Kelly and Hoffman, 1960). Presumably, the level which tended to give the greatest tension for the study of the mechanical activity was used and carried over from experiment to experiment. If one were to use the 1.31 mM in the bath as a model of the blood's concentration of calcium one would then have the beginning of a positive component to the T-F response as well as the presence of potentiation in rat heart muscle.

It must therefore be emphasized that for the comparable data to be obtained from isolated preparations of rat papillary muscle a calcium concentration of close to 1.31 mM should be used. Preferably, a wide range of $[Ca^{++}]_o$, between 0.25 to 2.5 mM, should be utilized in comparing *in vitro* with *in vivo* studies.

A Model to Explain the T-F response and PSP in Rat Heart Muscle

These experimental results indicate that external calcium

concentration can determine the shape of the T-F response and the extent of PSP in isolated rat heart muscle. It is therefore possible that internal Ca^{++} may also be involved in these processes.

If we are to consider that calcium ion may be responsible for the changes observed in rat heart muscle by altered concentrations within myocardial cells, then a simple model may be proposed. Lee et al., (1970) suggested a model of calcium movement within rat heart tissue based upon their studies with paired-pulse stimulation. It might therefore be interesting to use their model as a base for more interpretative studies and modification. Their model shown in Figure II-13. Lee explained their model in the following manner:

> "The action potential triggers off the release of a rapidly releasable calcium fraction. This fraction (Ca_r) is completely released by the brief action potential and no further release can occur until the fraction is recharged from another calcium reservoir (Ca_s). The recharging is accelerated by an increased calcium level and also by epinephrine and by an increased temperature".

This model might also be appropriate to explain the results we have obtained concerning the T-F relationship and potentiation in rat cardiac muscle.

If Ca_r were the rapidly releasable pool containing a fraction of the total stored calcium (Ca_s) at rested state contraction, then at low rates of stimulation, say 2/minute, sufficient time would elapse to recharge Ca_r from Ca_s completely between contractions. As the interval between beats is reduced, less recharge time is allowed and hence Ca_r

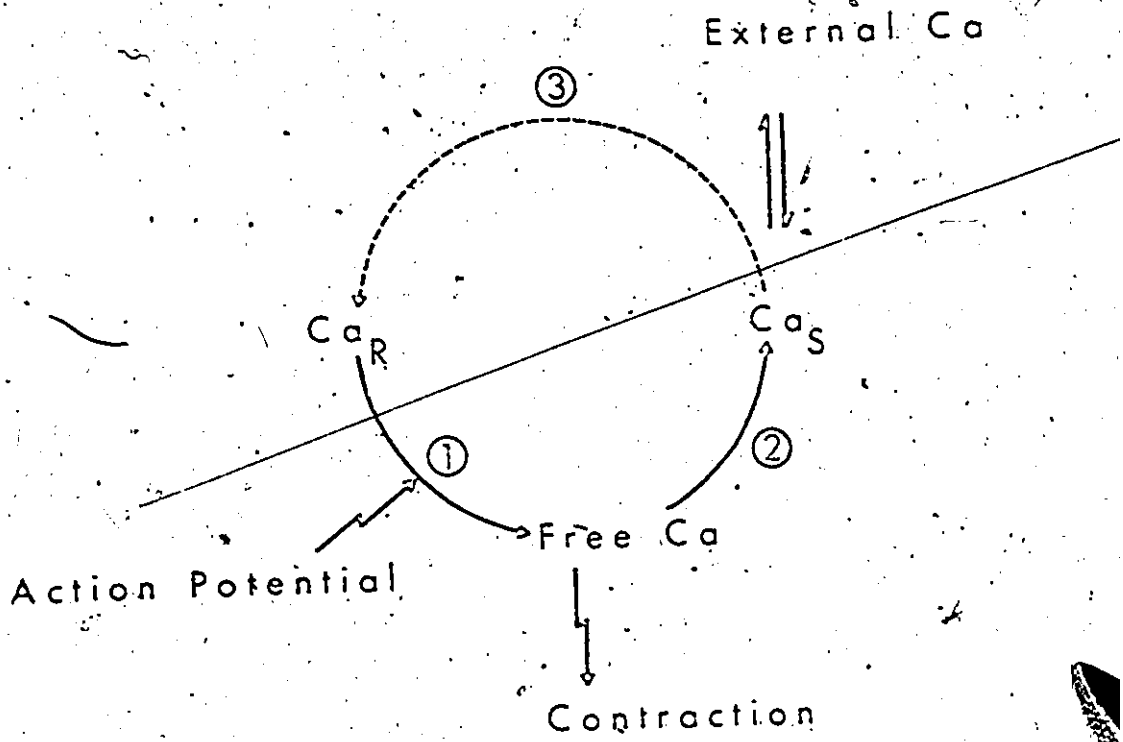


Figure III-13. Model of Calcium Movement in Rat Myocardium. (After Mainwood and Lee, 1969).

would be reduced at the higher frequencies. If, at a constant $[Ca^{++}]_o$ the same amount of calcium is gated into Ca_s during the brief action potential (gated calcium) the net result would still be a negative inotropism with an increase in frequency. If we suppose that at high $[Ca^{++}]_o$ (> 2.5 mM) that the system (Ca_s Ca_r) would be saturated; rested state contractions would produce the maximum obtainable tension. However, if we suppose that a lowering of the external calcium concentration reduces internal calcium stores (Ca_s) and conceivably the releaseable fraction (Ca_r), rested state contraction tension would be reduced, but we would now have a reserve capacity based upon the level in Ca_s at high calcium minus the level at low calcium. Similarly a reserve capacity would exist in Ca_r between the levels at high and low calcium. This reserve capacity would represent as well the capacity to potentiate tension in the muscle. For the T-F response at low calcium, a rested state contraction allows for the recharge of Ca_r from Ca_s. An increase in frequency now also results in an initial diminution of tension due to the shortening of the recharge interval. But now, due to 1) the reserve capacity in the system, 2) the gated calcium, 3) the recycling of calcium, a positive inotropic component will appear. That this may occur is shown in Figure II-14, illustrating the beat to beat changes occuring at high (2.5 mM) and low (0.50 mM) $[Ca^{++}]_o$ during a T-F experiment. At high calcium, an increase in frequency of stimulation always results in a decrease from beat to beat, indicating a negative

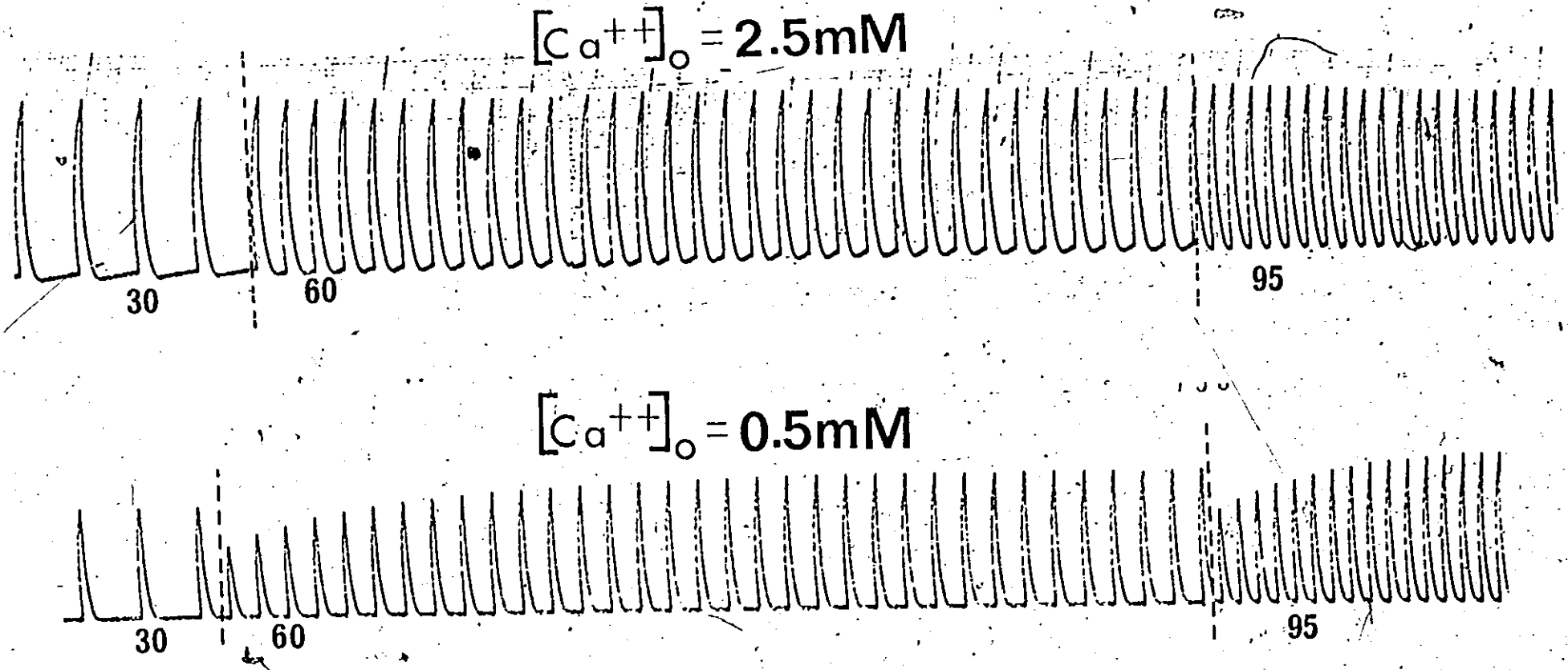


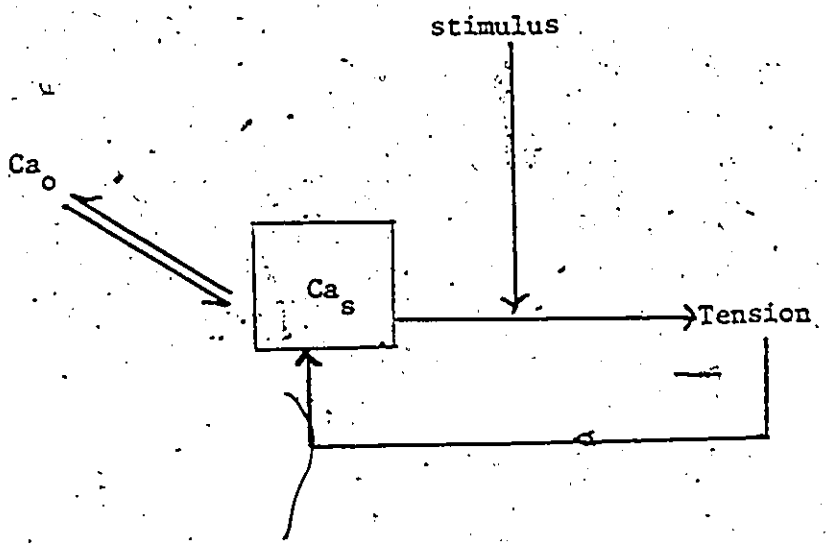
Figure III-14. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. Upper Record: Isometric Tension Record at 2.5 mM $[Ca^{++}]_o$. Lower Record: Isometric Tension Record at 0.25 mM $[Ca^{++}]_o$. Frequency of Stimulation is increasing from 30-60-95 Impulses/Minutes.

inotropism in attaining the steady state. At low calcium, the negative component of the T-F response is illustrated by a fall in tension during the beat to beat response during the frequency change while the positive component shows an increase in tension from beat to beat. This suggests that in attaining the steady state response with the muscles at low calcium we are dealing with a beat to beat increase of the potentiating factor, conceivably calcium, while the opposite occurs at high $[Ca^{++}]_o$.

This model would also explain potentiation in these conditions. At low external calcium the model predicts a reserve capacity in calcium stores. Potentiation of tension is produced by rapidly gating in calcium to Ca_s (within the cell) and then allowing sufficient time for the increase in Ca_s to be expressed as the recharge of Ca_r. Hence the 30 second interval requirement to observe peak potentiation after the pulse train. The relatively slow decay of tension following potentiation suggests that the releaseable fraction (Ca_r) exists in a relatively stable condition once it has been filled. Ca_s would have to be stable as well.

The experiments on the decay of potentiated tension suggests that a fraction of the potentiating factor is removed with each stimulus. Furthermore, it indicates that this fraction appears fixed and independent of the frequency of stimulation. An appropriate model for this condition would be where calcium would be removed from a large reservoir - a fixed fraction with each stimulus. If we maintain the

terminology of the model in Figure III-13, the situation obtained would appear as:



This model eliminates the necessity for a rechargeable pool; the tension determining calcium is removed from Ca_s (stored calcium pool) directly by a stimulus as a fraction of the calcium contained in Ca_s. Two facts tend to disprove this model; a) an interpolated beat a short interval after the first beat results in very little tension, at all levels of $[Ca^{++}]_o$ and b) at low $[Ca^{++}]_o$ it is necessary to wait sufficient time to obtain maximum potentiation after a pulse train and at high Ca^{++} to return to RSC tension.

If we assume that the coupling system between excitation and contraction is intact at all of the pulse intervals studied, these data would suggest that we are indeed dealing with a system that can be modelled as in Figure III-13 with a rechargeable process between the stored calcium (Ca_s) and rapidly releaseable calcium (Ca_r) (process 3).

Intuitively, this system must also incorporate at some point a non-linear portion so that both potentiation, and the positive T-F response can be obtained, i.e. if all portions of the model were linear we would either have a constant tension at all frequencies of stimulation or a negative inotropic linear relationship with a decrease in the interval between stimuli.

The non linearity might be between Ca_s and Ca_r, although it may be placed elsewhere. If this were so an increase in Ca_s due to the gating of calcium from Ca_o and the recycling of calcium from the myofilaments would be linear but due to a non-linearity of process 3

in Figure III-13, it would be possible to obtain both a negative and positive inotropic response with an increase in the frequency of contraction depending upon the level of Ca_o.

If we accept that a recharge process is needed to explain our model, the results on the decay of potentiation may be explained in the following manner. In the potentiated state Ca_s is built up to a level higher than normal for the particular Ca_o by the gating of calcium. This would mean that the driving force of calcium from Ca_s to Ca_r is likewise increased. Since we show that the buildup of potentiated tension takes up to 30 seconds, this would be maintained over a fairly long interval. In the stimulated decay condition we would remove the increased calcium in Ca_s through Ca_r.

The model now must be tested experimentally to see if it can quantitatively explain some characteristics of rat papillary muscle.

Section IV

The Effect of Ouabain, $[Na^+]_o$ and pH on Isolated Rat Papillary Muscle.

Introduction

The previous section reported on the effect of $[Ca^{++}]_o$ on the tension-frequency response and post-stimulation potentiation in rat papillary muscle. The results suggested that the responses of the rat cardiac muscle might be controlled by calcium movement within the muscle. Three interventions which may affect calcium movement or content in contracting cardiac muscle are a) the cardiac glycosides, b) external sodium ion concentration and c) external pH.

It has been reported that rat heart is inotropically insensitive to the administration of cardiac glycosides such as Ouabain even in pharmacologic doses (Lee and Klaus, 1971). Recent reviews (Lee et al., 1970; Lee and Klaus, 1971) suggest that the positive inotropic effect of Ouabain is caused by an increase in the level of intracellular free calcium. However, previous studies on the sensitivity of rat heart to pharmacologic doses of Ouabain (10^{-7}M. - 10^{-5}M.) were performed in the presence of relatively high calcium concentrations (> 1.8 mM.). As we demonstrated in the previous section, maximum tension appears at around 2.5mM $[Ca^{++}]_o$. Therefore, a decrease of the calcium concentration might reveal a sensitivity to this inotropic agent.

Langer (1968, 1970) has suggested that Na^+ ions compete for myocardial membrane sites with Ca^{++} ions. He proposed that an increase in intracellular Na^+ would result in a displacement of Ca^{++} available for tension development producing a positive inotropism. Glitch, Reuter-

and Scholz (1970) suggested a similar response; $[Na^+]_i$ had a profound influence on calcium influx during contraction of guinea-pig atria.

A similar competition is suggested between H^+ ions and Ca^{++} ions (Bass and Moore, 1966; Carvalho, 1966) on membranes.

Therefore the effect of Ouabain, $[Na^+]_o$ and pH has been studied on the isolated papillary muscle preparation of the rat to determine if these interventions play a role in the mechanical response of this cardiac tissue.

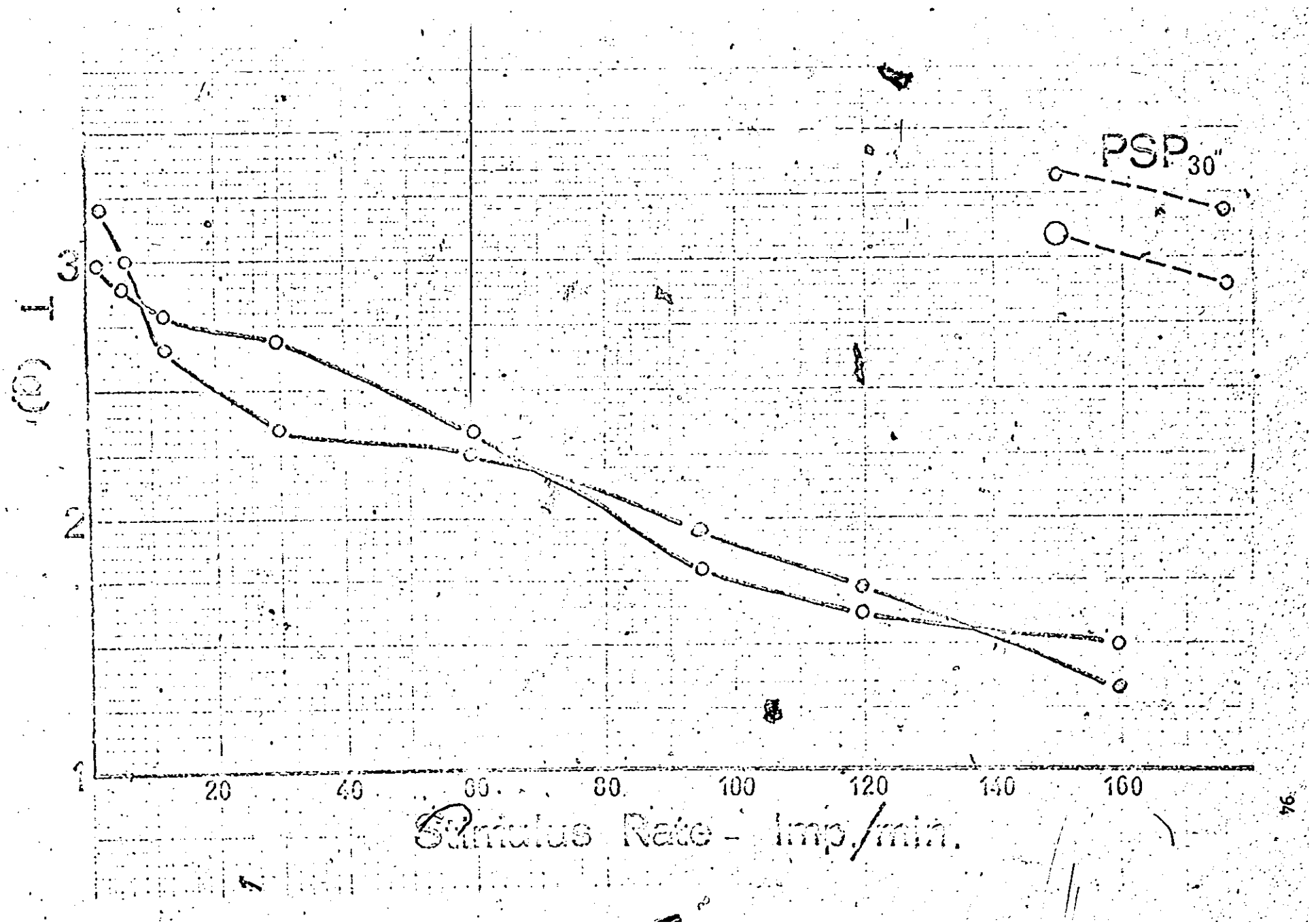
Results

Effect of Ouabain in Rat Papillary Muscle.

The addition of ouabain (10^{-7} - 10^{-5} M) to the bath containing Tyrode solution with 2.5 mM calcium had no effect on the tension developed by rat papillary muscles up to 30 minutes exposure to the drug. After approximately 50 minutes however, a slight decrease in the rested state contraction developed. Figure IV-1 indicates the T-F relationship and $PSP_{30''}$ of a papillary muscle exposed to ouabain (1.37×10^{-5} M.) for 55 minutes. The T-F relationship is not significantly altered by the treatment with ouabain and a negative inotropism is observed over the frequencies tested. Similarly, "$PSP_{30''}$" does not appear to have been changed by the treatment with ouabain although RSC tension had fallen slightly; the slope of the $PSP_{30''}$ response after treatment with ouabain is similar to the slope without ouabain treatment.

On the other hand, the addition of ouabain to a Tyrode

Figure IV-1. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. (●) $[Ca^{++}]_o$ = 2.5 mM. (○) $[Ca^{++}]_o$ = 2.5 mM 10^{-5}M. Ouabain. Broken Line indicates $PSP_{30''}$.


PSP30"
(c) I
3
2
1
20
40
60
80
100
120
140
160
Stimulus Rate - Imp./min.
94

solution containing low (0.25 mM) calcium resulted in a profound inotropic effect. Figure IV-2 traces the tension in a papillary muscle stimulated at 2/minute after exposure to ouabain (1.37×10^{-5}M.).

Within 2 minutes there is a marked increase in developed tension. In a 14 minute period (2 - 16 minutes) tension rose from 0.3 g. to 1.35 g., an increase in the absolute tension of 450%. Only after 50 - 60 minutes exposure to the drug did tension begin to decline and then only at a slow rate. The effectiveness of the drug therefore is optimum within 15 to 50 minutes after administration. This depended upon the relative size (cross sectional area) of the papillary muscle used. In fiber muscles, tension peaked as early as 7 minutes and as late as 70 minutes after ouabain administration (1.37×10^{-5}M.). In muscles which showed a slow inotropic response to ouabain administration, the increased tension was maintained over a correspondingly long period.

Figure IV-3 illustrates the effect of ouabain (1.37×10^{-5}M.) on RSC tension and $PSP_{30"}$ in rat papillary muscle. At $[Ca^{++}]_o$ = 2.5 mM, RSC is obtained without ouabain (point a); potentiation is absent. When $[Ca^{++}]_o$ is decreased to 1.0 mM, RSC tension falls to 1.70 g. (-35%) and $PSP_{30"}$ is 124%. At 0.25 mM $[Ca^{++}]_o$, tension falls to 0.3 g. (point c), 11% of the tension at 2.5 mM. $PSP_{30"}$ is now increased to 1.25 g. (416%). The addition of ouabain (1.37×10^{-5}M.) now caused an increase in the RSC tension to 1.20 g. (+400%) after 20 minutes of exposure. By 30-45 minutes (point d) RSC tension stabilized at 1.45 g. (+483%) but $PSP_{30"}$

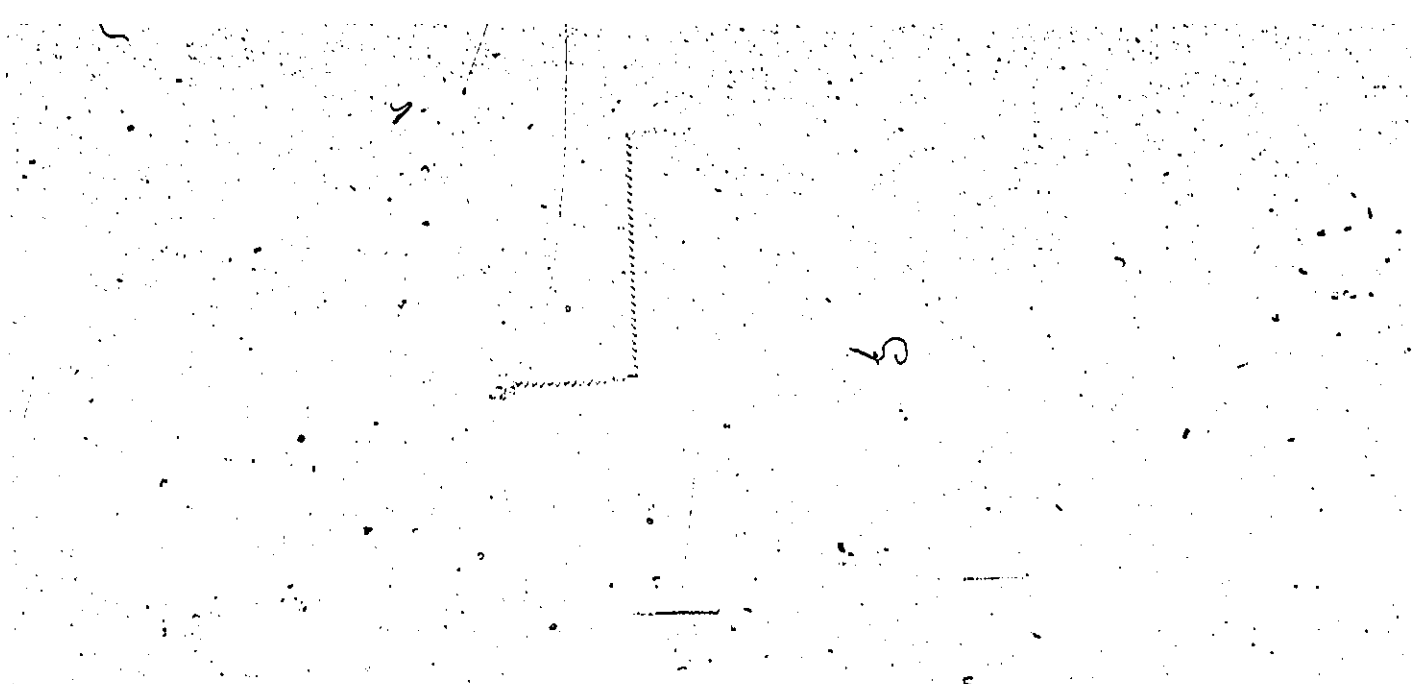


Figure IV-2: Effect of Ouabain (1.37×10^{-5}M.) on Isometric Tension in the Isolated Papillary Muscle Preparation of the Rat. $[Ca^{++}]_o$ = 0.25mM.

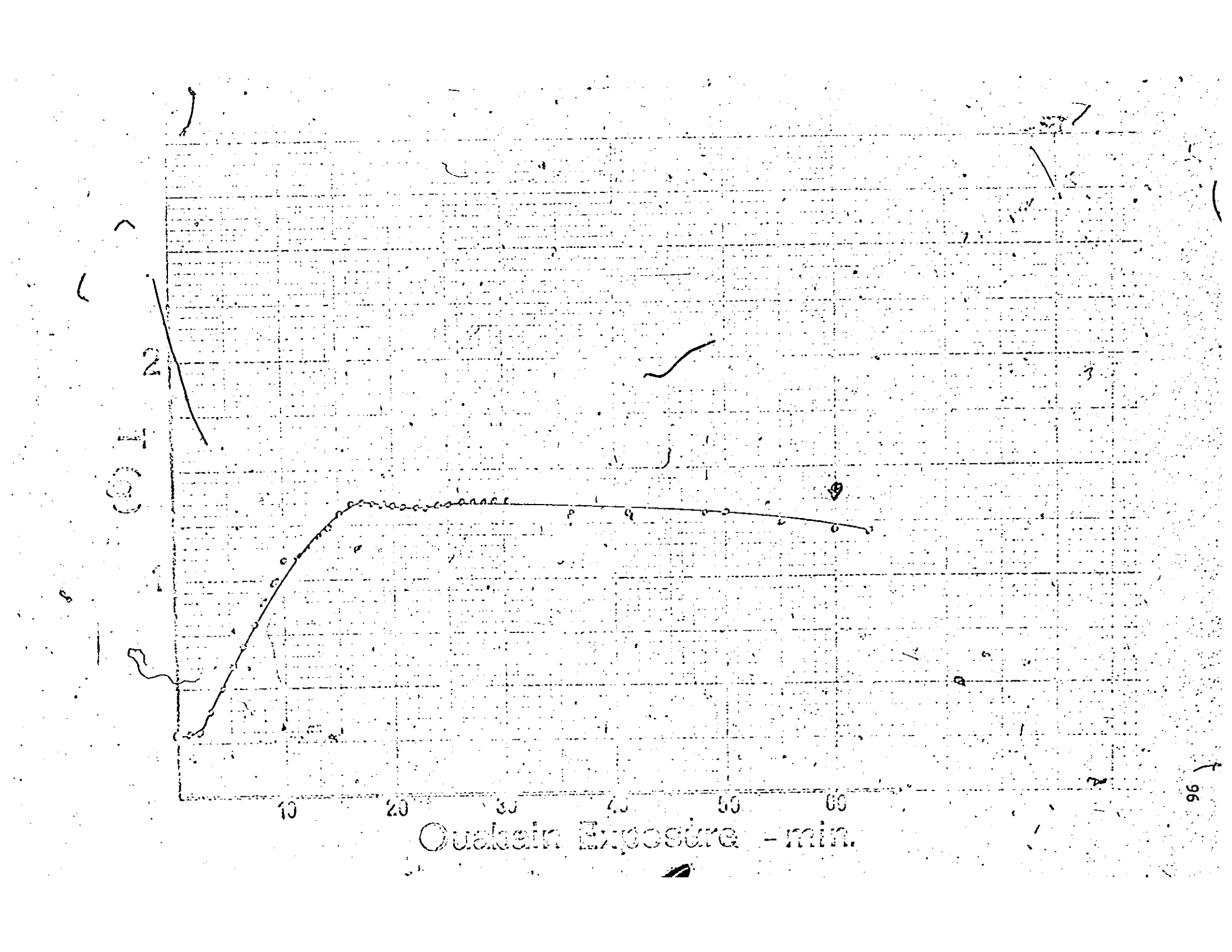

2
1
10
20
Oudatin Exposure - min.

Figure IV-3. Effect of Ouabain on Post-Stimulation Potentiation in the Isolated Papillary Muscle of the Rat.

a) $[Ca^{++}]_o$ = 2.5mM.

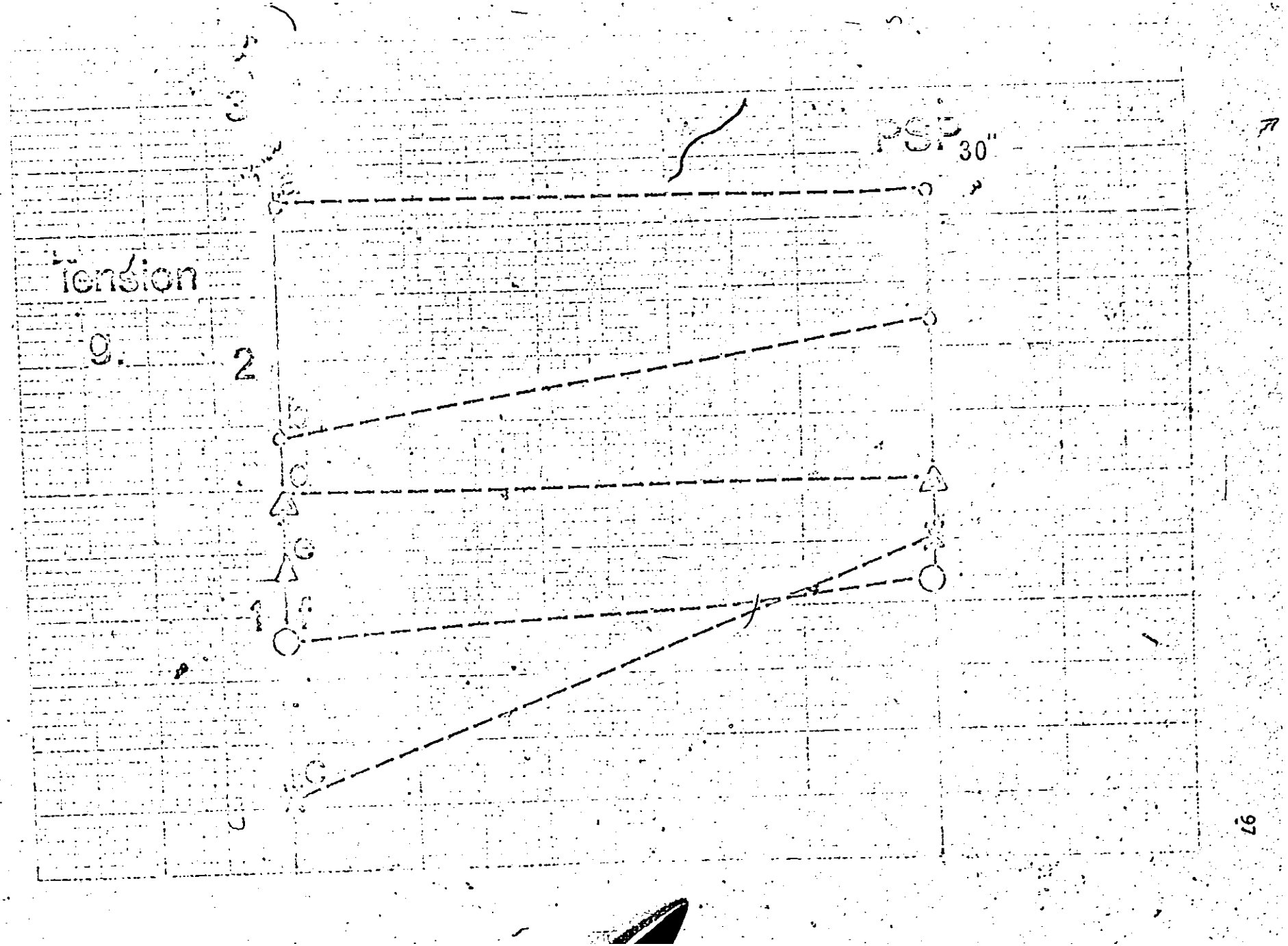
b) $[Ca^{++}]_o$ = 1.0mM.

c) $[Ca^{++}]_o$ = 0.25mM.

d) $[Ca^{++}]_o$ = 0.25mM 1.37×10^{-5}M. Ouabain Exposure Time = 30 minutes.

e) $[Ca^{++}]_o$ = 0.25mM 1.37×10^{-5}M. Ouabain Exposure Time = 60 minutes.

f) $[Ca^{++}]_o$ = 0.25mM. Ouabain washout time, 24 minutes.


PSI
30"
Tension
0.
2

not obtainable. In this experiment RSC tension in the presence of ... started to fall after 55 minutes (point e).

A washout with ouabain-free Tyrode ($[Ca^{++}]_o$ = 0.25 mM) reduces ... tion to 0.9 g. (point f) after 24 minutes a small reappearance of $PSP_{30''}$ is seen. This appears to be a reversible phenomenon, with ... appropriately long washout reestablishing the characteristics seen without the presence of ouabain in the bathing fluid.

Table IV-I and Figure IV-4 show the effect of ouabain (1.37×10^{-5} M.) on the T-F relationship at 1.0 mM $[Ca^{++}]_o$. After 60 minutes ... there is an increase in the rested state contraction tension ... from 2.60 to 3.05 g. (+17%) (Table IV-I). The addition of ouabain results in a flattening of the T-F curve (curve b, open circles); the ... slope of the positive component between 30 and 100 stimuli/minute is ... reduced. After washout with ouabain-free tyrode containing 0.25 mM calcium, the T-F relationship was again tested. The rested state contraction tension fell to characteristic low levels and the T-F curve ... had a steep positive slope between 30 and 120 stimuli/minute, (curve ..., open triangles). Tmax is reached between 95 and 120 stimuli/minute. ... The addition of ouabain at this point (1.37×10^{-5} M.) resulted in a ... slight elevation in the rested state contraction tension, and the ... positive slope of the T-F response was reduced (curve d, solid triangles).

In a similar experiment $PSP_{30''}$ was tested at 1.0 mM and 0.25 mM ... with and without ouabain addition (Figure IV-5). At 2.5 mM, $PSP_{30''}$

Table [illegible]

Effect of Ouabain (1.37×10^{-5} M.) on the Tension-Frequency Response of Isolated Papillary Muscle of the Rat.

Treatment	Freq/Stim. Imp./Min.	T (g.)	%Tmax	Treatment	Freq/Stim. Imp./Min.	T (g.)	%Tmax
$[Ca^{++}]_o$=1.0mM	2	2.65 *	100*	$[Ca^{++}]_o$=1.0mM	2	3.05*	100
	6	2.[illegible]8	83.8		6	2.60	85.2
	12	2.[illegible]6	80.8		12	2.25	73.7
	30	2.08	80.0	Ouabain	30	2.15	70.5
	60	2.[illegible]5	82.7	1.37×10^{-5}M.	60	2.25	73.8
	95	2.[illegible]0	84.6		95	2.20	72.1
	120	2.0	76.9		120	2.125	69.7
	150	1.70	65.4	60 minutes	150	1.80	59.0
	180	1.[illegible]0	50	exposure	180	1.50	49.1
$[Ca^{++}]_o$=0.25mM Ouabain washout	2	0.5	90.9	$[Ca^{++}]_o$=0.25mM Ouabain 1.37×10^{-5}M.	2	.525*	100
	6	0.30	54.5		6	.450	85.7
	12	.265	48.2		12	.225	42.9
	30	.250	45.4		30	.150	28.6
	60	.40	72.7		60	.225	42.9
	95	.55*	100		95	.30	57.1
	120	.55*	100		120	.275	52.4
	150	.40	72.7		150	.250	47.6
	180	.30	54.5		180	.200	38.1

* T_{max}

Figure 10-4. Twitch Frequency Response in Isolated Papillary Muscle of the Rat.

(a) (□) $[Ca^{++}]_o$ = 1.0mM.

(b) (○) $[Ca^{++}]_o$ = 1.0mM 1.37×10^{-5}M Ouabain. (60')

(c) (△) $[Ca^{++}]_o$ = 0.25mM. (ouabain washout).

(d) (▲) $[Ca^{++}]_o$ = 0.25mM 1.37×10^{-5}M. Ouabain.

Figure 17-8. Isometric Tension in Isolated Papillary Muscle of Cat.

(a) $[Ca^{++}]_o$ = 2.5 mM.

(b) $[Ca^{++}]_o$ = 1.0 mM.

(c) $[Ca^{++}]_o$ = 1.0mM + 1.37×10^{-5} M. Ouabain
Exposure = 19, 42 minutes.

(d) $[Ca^{++}]_o$ = 0.25 mM + 1.37×10^{-5} M. Ouabain
Exposure = 45 minutes.

(e) $[Ca^{++}]_o$ = 0.25mM + 1.37×10^{-5} M. Ouabain
Exposure = 56 minutes.

(f) $[Ca^{++}]_o$ = 0.25mM. Ouabain washout, 30 minutes.

30"

was not evident (a) while at 1.0 mM $PSP_{30"}$ was 136%. Addition of ouabain (1.37×10^{-5} M.) for 19 minutes resulted in an increase in rested state contraction tension (point c, 35% over control) and a decrease in the slope of the $PSP_{30"}$ response (%PSP = 106). An identical response was obtained after 42 minutes of exposure to ouabain.

After 30 minutes washout in ouabain-free tyrode containing 0.25 mM calcium, RSC tension fell to 0.12 g. and $PSP_{30"}$ was most evident (>00%). After exposure to ouabain (1.37×10^{-5} M.) for 56 minutes, rested state contraction tension had increased to w.75 g. but $PSP_{30"}$ was reduced to 200%

Figure IV-6 shows oscilloscope pictures of the development and decay of $PSP_{30"}$ in rat papillary muscle at 1.0 mM $[Ca^{++}]_o$. $PSP_{30"}$ is well developed by 30 seconds after the pulse train (Figure IV-6-B). Subsequent contractions elicited at 30" intervals gradually reduced the potentiated tension. By 2.5 minutes after the stimulus train and stimulation at 2/min. (Figure IV-6-F), the tension has still not fallen to the level of the rested-state contraction (Figure IV-6-A). Figure IV-7 traces the development of the increase in tension after the addition of ouabain (1.37×10^{-5} M.) at $[Ca^{++}]_o$ = 1.0 mM. By 7 minutes a definite inotropism is seen (Figure IV-7-B). At 20 minutes (Figure IV-8-A) tension had increased significantly in the rested state contraction and $PSP_{30"}$ was reduced compared to the ouabain-free test (Figure IV-8-B).

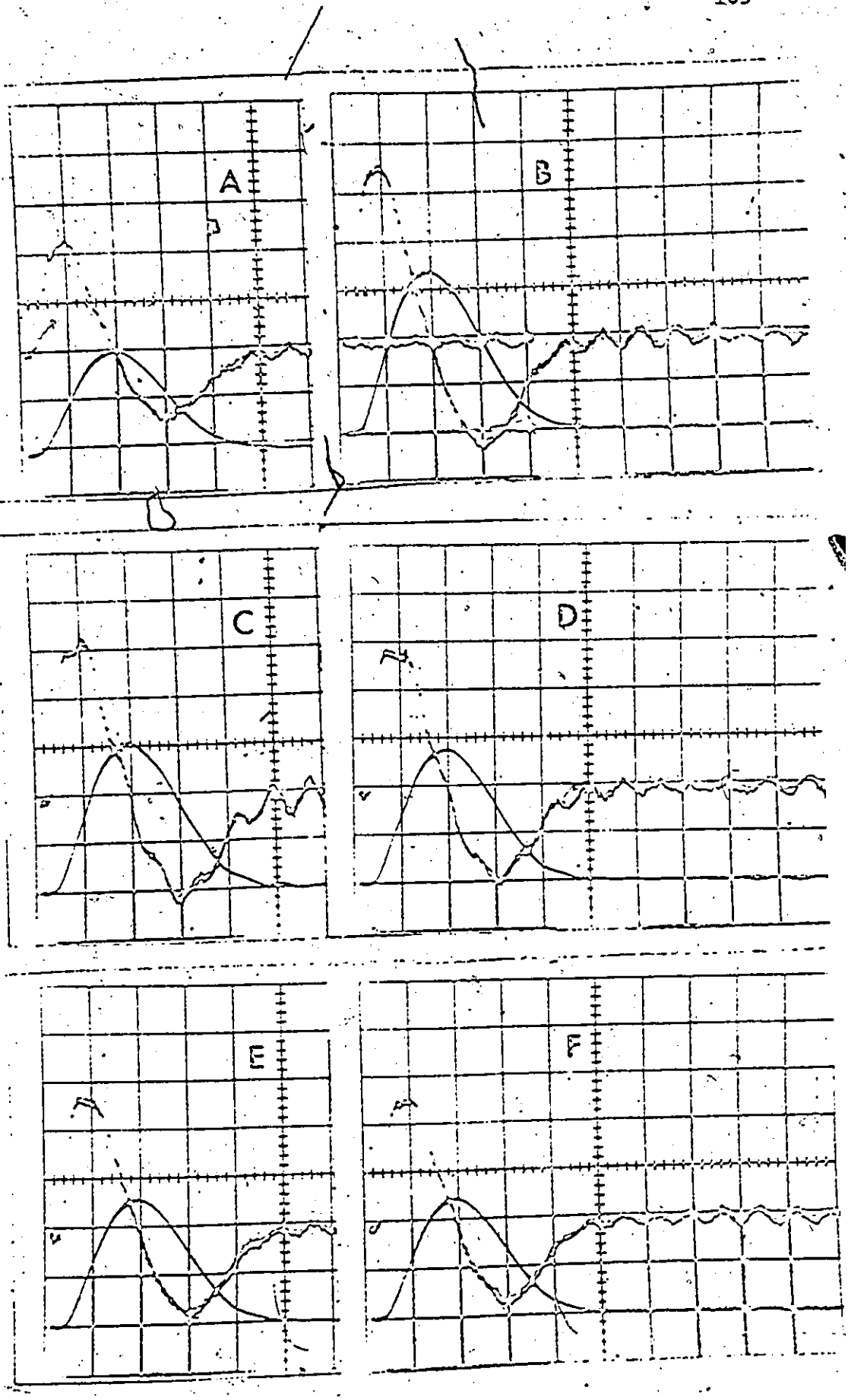

A
B
C
D
E
F

Figure IV-7. Effect of Ouabain on Isometric Tension in Isolated Papillary Muscle of the Rat.

$[Ca^{++}]_o$ = 1.0mM.

Exposure in Minutes.

A) 0; B) 7; C) 10; D) 15; E) 40.

Upper Tracing: dT/dt; 1 division = 5g./sec.

Lower Tracing: Tension (T); 1 division = 0.5g.

Time Scale: 1 division = 0.1 sec.

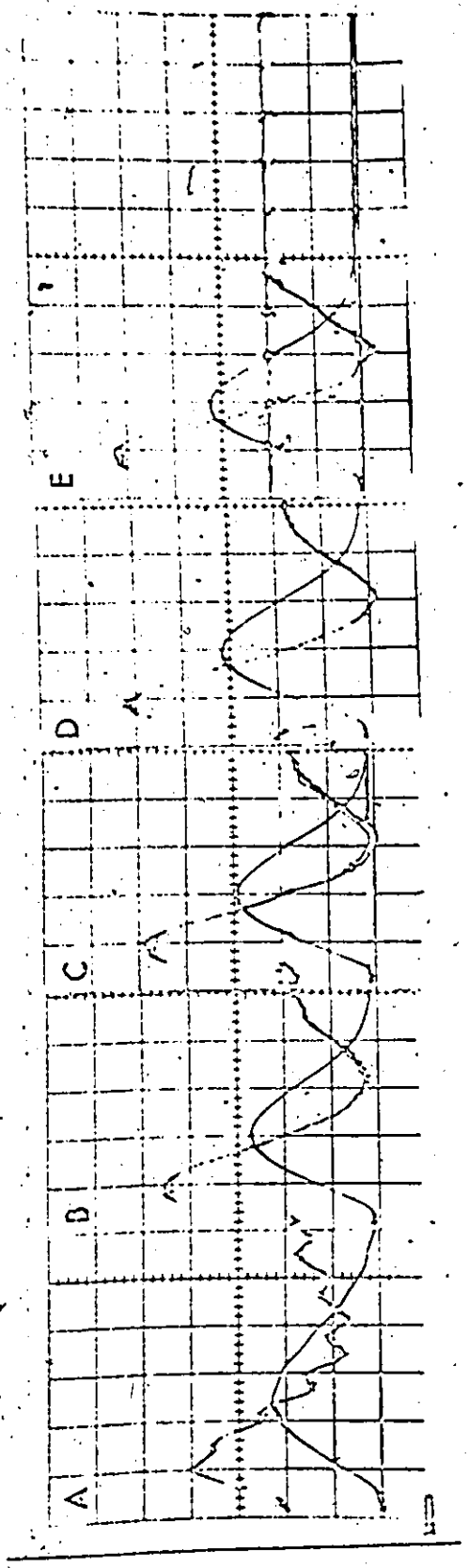

E
D
C
B
A

Figure IV-8. Effect of Ouabain (10^{-5}M.) on Post-Stimulation Potentiation in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 1.0mM. Exposure time, 20 minutes.

A) Control

B) $PSP_{30"}$

C) $PSP_{30"}$ + 30"

D) $PSP_{30"}$ + 60"

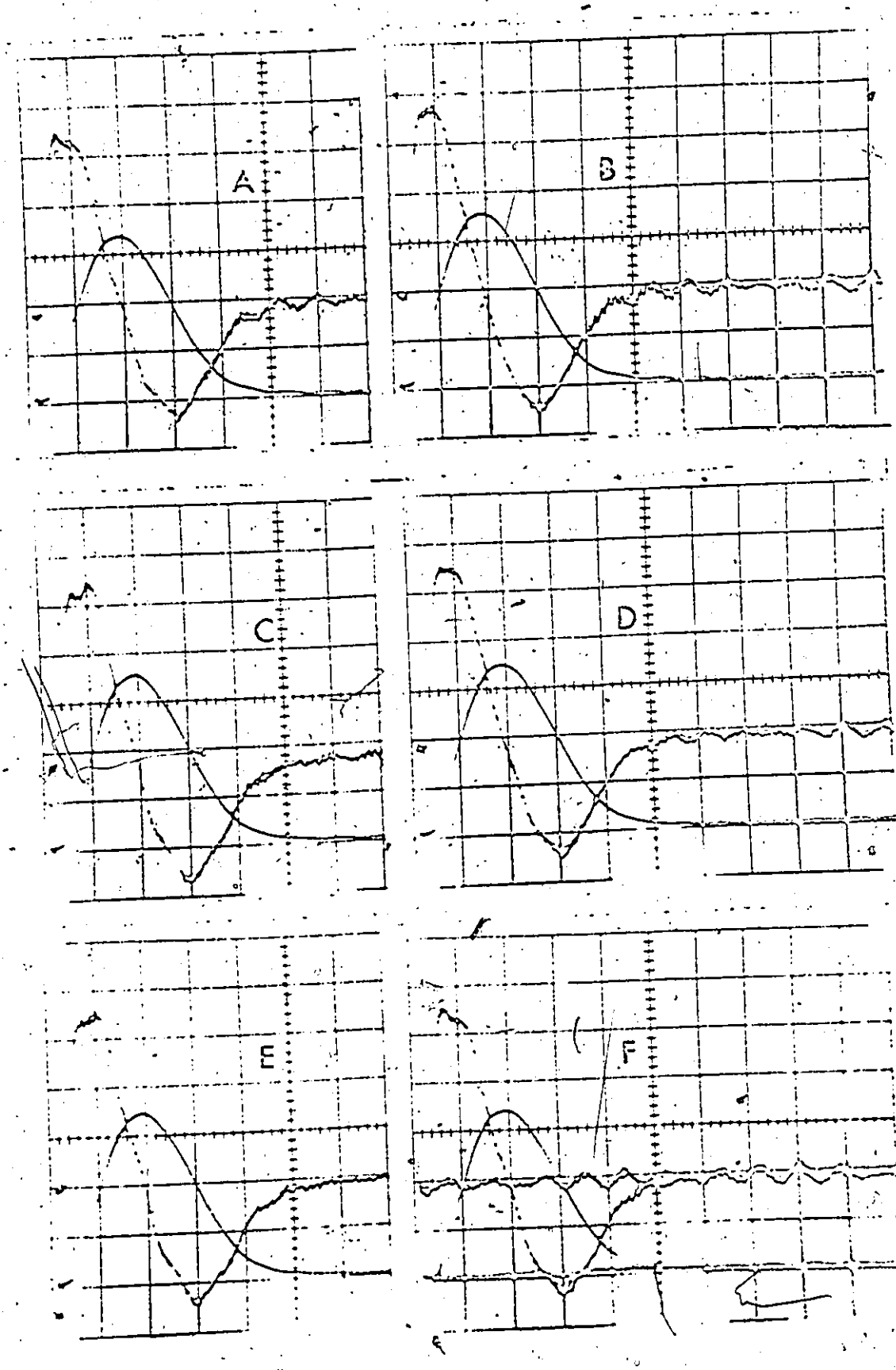
E) $PSP_{30"}$ + 90"

F) $PSP_{30"}$ +120

Upper Tracing: dT/dt; 1 division = 5.0g./sec.

Lower Tracing: Tension (T); 1 division = 0.5g.

Time Scale: 1 division = 0.1 sec.


A
B
C
D
E
F

At 42 minutes exposure to ouabain (Figure IV-9-A) rested state contraction tension was greater than without ouabain, and the same as that recorded at 20 minutes (Figure IV-8-A); $PSP_{30"}$ (Figure IV-9-B) was not significant.

At low calcium concentrations ($[Ca^{++}]_o$ = 0.25 mM.) (Table IV-II, Figure IV-10), the effect of ouabain (1.37×10^{-5} M.) is seen as a decrease in the slope of the positive component of the T-F curve. This is evident at 34 minutes exposure to ouabain (curve b) but even more pronounced after 64 minutes exposure (curve c). Rested state contraction tension increased from 0.44 g. (without ouabain) to 0.85 g. at 34 minutes and 1.3 g. at 64 minutes exposure, an overall increase of 53%. The inotropism is illustrated further in Figure IV-11 which traces the phase plane plot of the contractions following the addition of ouabain (1.37×10^{-5} M.) with 0.25 mM calcium in the muscle bath; oscilloscope tracings are shown in Figure IV-12. The inotropic effect is seen as an increase in rested state contraction tension over the 60 minute exposure period. Washout of the bath for 31 minutes with ouabain-free low calcium (0.25 mM) Tyrode resulted in a fall in the rested state contraction tension (Figure IV-13-A) and the reestablishment of a significant $PSP_{30"}$ (Figure IV-13-B).

Effect of Reduced Sodium Ion Concentration

Figure IV-14 shows the T-F response of rat papillary muscle at 2.5 mM calcium at normal (100%) and 50% sodium concentration. Sodium

Figure IV-9. Effect of Ouabain (10^{-5}M.) on Post-Stimulation Potentiation in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 1.0mM. Exposure time to Ouabain, 42 minutes.

A) Control

B) $PSP_{30"}$

C) $PSP_{30"}$+30"

D) $PSP_{30"}$ + 60"

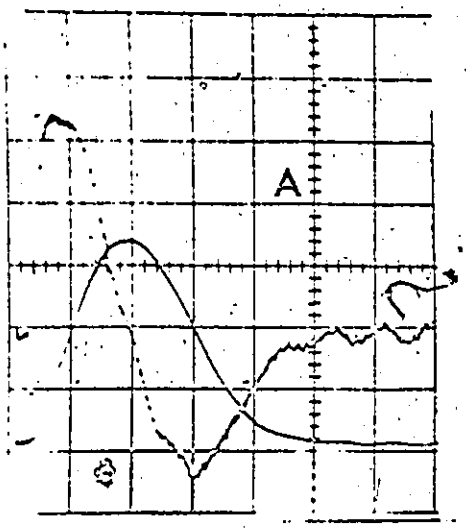
E) $PSP_{30"}$ + 90"

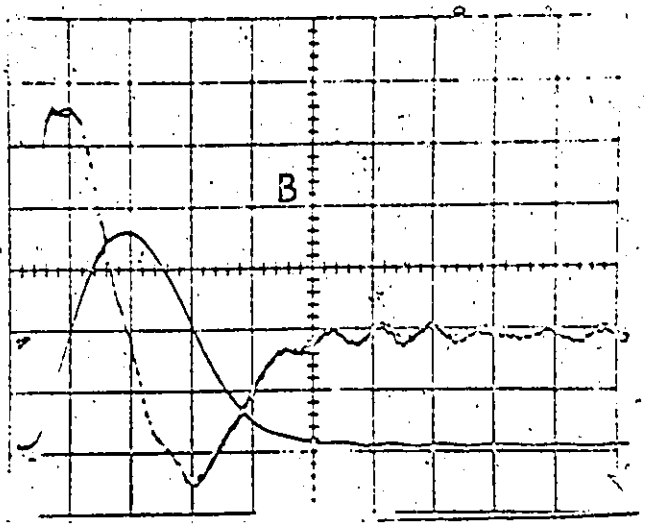
F) $PSP_{30"}$+120"

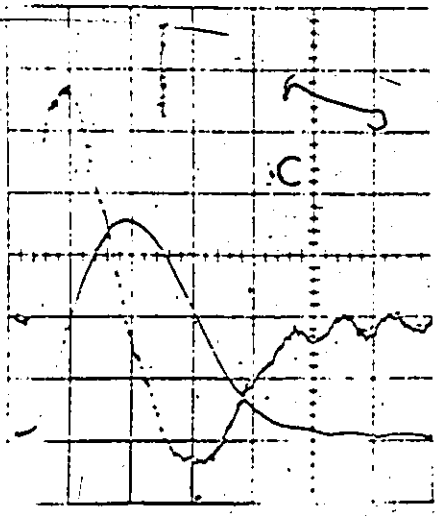
Upper Tracing: dT/dt; 1 division = 5.0g./sec.

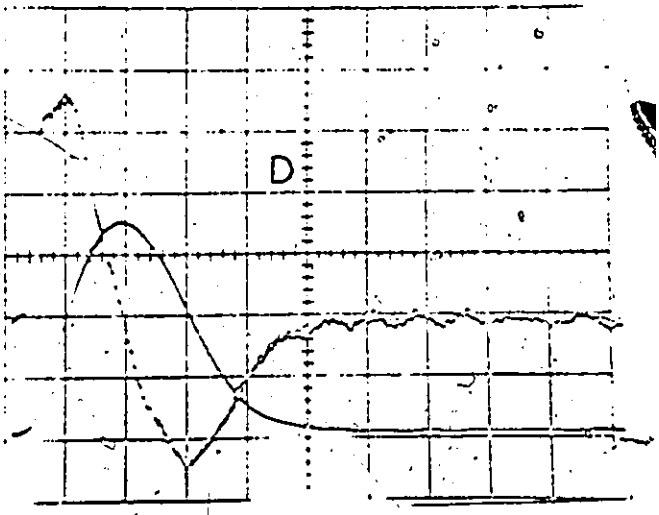
Lower Tracing: Tension (T); 1 division = 0.5g.

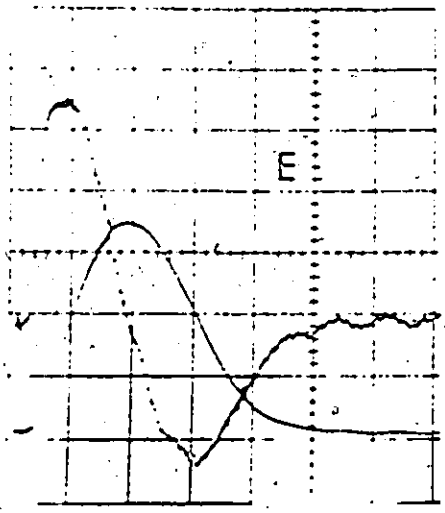
Time Scale: 1 division = 0.1 sec.


A


B


C


D


E

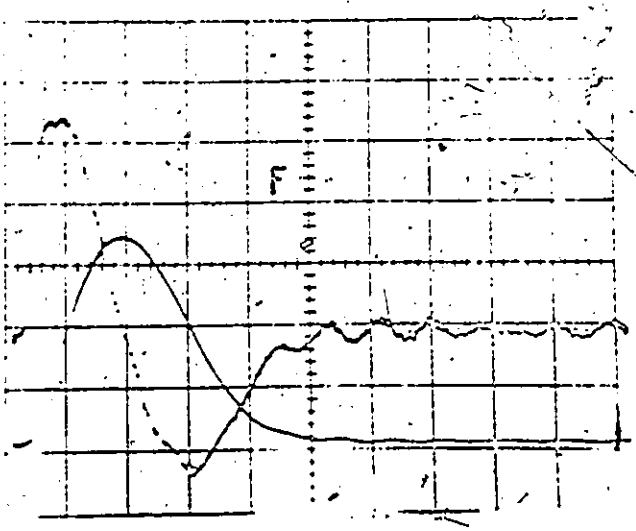

F

Table IV-II

Effect of Ouabain on the Tension-Frequency Response of Isolated Papillary Muscle of the Rat.

Treatment	Freq./Stim. Imp./Min.	T (g.)	%Tmax	Treatment	Freq./Stim. Imp./Min.	T (g.)	%Tmax
	2	.44	95.6		2	.85	100
	6	.285	61.9		6	.47	55.3
$[Ca^{++}]_o$=0.25mM	12	.235	51.1		12	.33	38.8
	30	.245	53.2	$[Ca^{++}]_o$=0.25mM	30	.30	35.3
	60	.330	71.7	1.37×10^{-5}M.	60	.40	47.1
	95	.435	74.6	Ouabain	95	.55	64.7
	120	.460	100		120	.60	70.6
	150	.450	97.8	(34 min.)	150	.635	74.7
	180	.400	87		180	.55	64.7
	2	1.3	100				
	6	.80	53.8				
	12	.45	34.6				
$[Ca^{++}]_o$=0.25mM	30	.40	30.8				
	60	.46	35.4				
1.37×10^{-5}M.	95	.59	45.4				
Ouabain							
	120	.66	50.8				
(64 min.)	150	.685	57.7				
	180	.59	45.4				

Figure IV-10. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. Effect of Ouabain (10^{-5} M.).

a) $[Ca^{++}]_o$ = 0.25mM.

b) $[Ca^{++}]_o$ = 0.25 mM Ouabain (10^{-5}M.) - 34 min. Exposure.

c) $[Ca^{++}]_o$ = 0.25mM Ouabain (10^{-5}M.) - 64 min. Exposure.

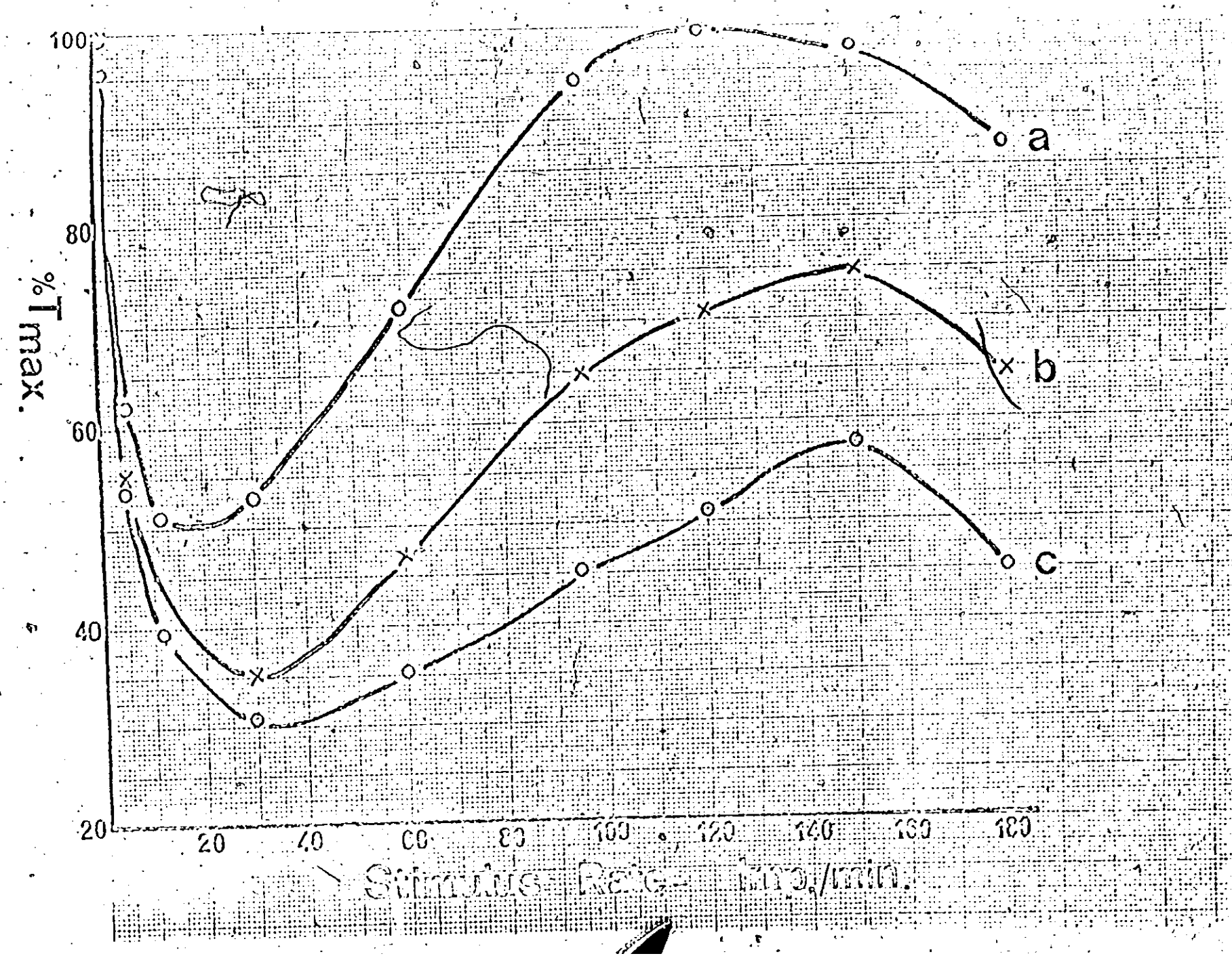

100
80
60
40
20
$T_{max.}$ %
20
40
60
80
100
120
140
160
180
a
b
c

Figure IV-11. Phase-Plane Plot of Isometric Contractions of Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 0.25 mM. Effect of Ouabain (10^{-5} M.) after 0, 5, 10, 15, 20, 60, 75 minutes exposure.

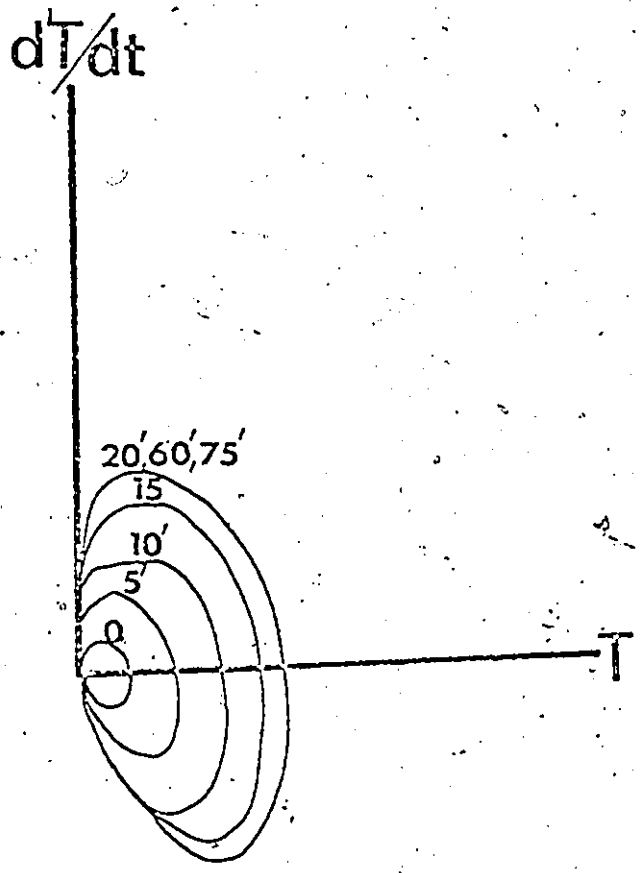

dT/dt
20',60',75'
15'
10'
5'
0
T

Figure IV-12. Isometric Contractions in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 0.25mM: Effect of Ouabain (1.37×10^{-5} M.) Exposure (min.).

A) 0 B) 5

C) 10 D) 15

E) 20 F) 60

Upper Tracing: Tension (T); 1 division = 0.5g.

Lower Tracing: dT/dt; 1 division = 10g./sec.

Time Scale: 1 division = 0.1 sec.

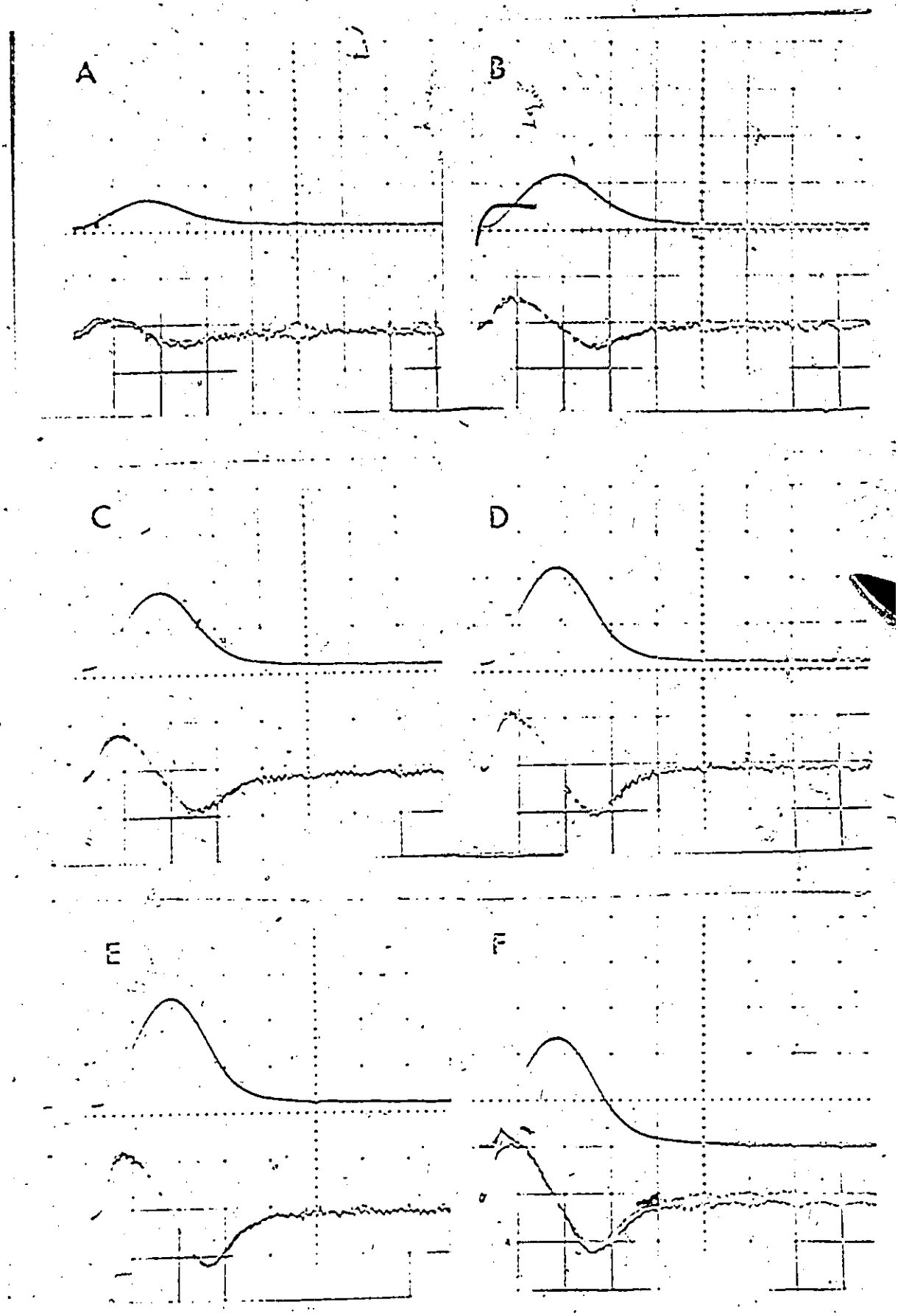

A
B
C
D
E
F

Figure IV-13. Isometric Contractions in Isolated Papillary Muscle of the Rat. Post-Stimulation Potentiation after 31 min. washout from 1.37×10^{-3} M. Ouabain. $[Ca^{++}]_o$ - 0.25mM.

A) Control B) $PSP_{30"}$ C) $PSP_{30"}$ + 30"

Upper Tracing: dT/dt; 1 division = 10g./sec.

Lower Tracing: Tension (T); 1 division = 0.5g.

Time Scale: 1 division = 0.1 sec.

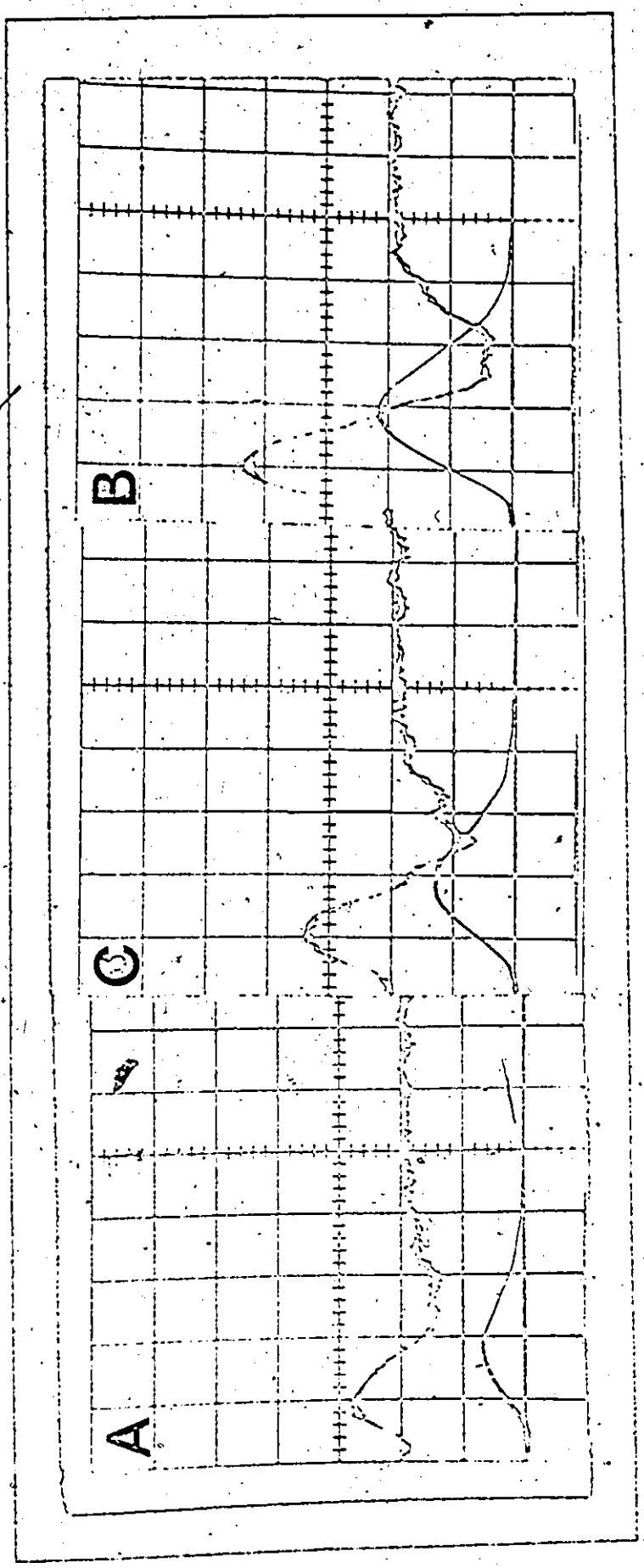
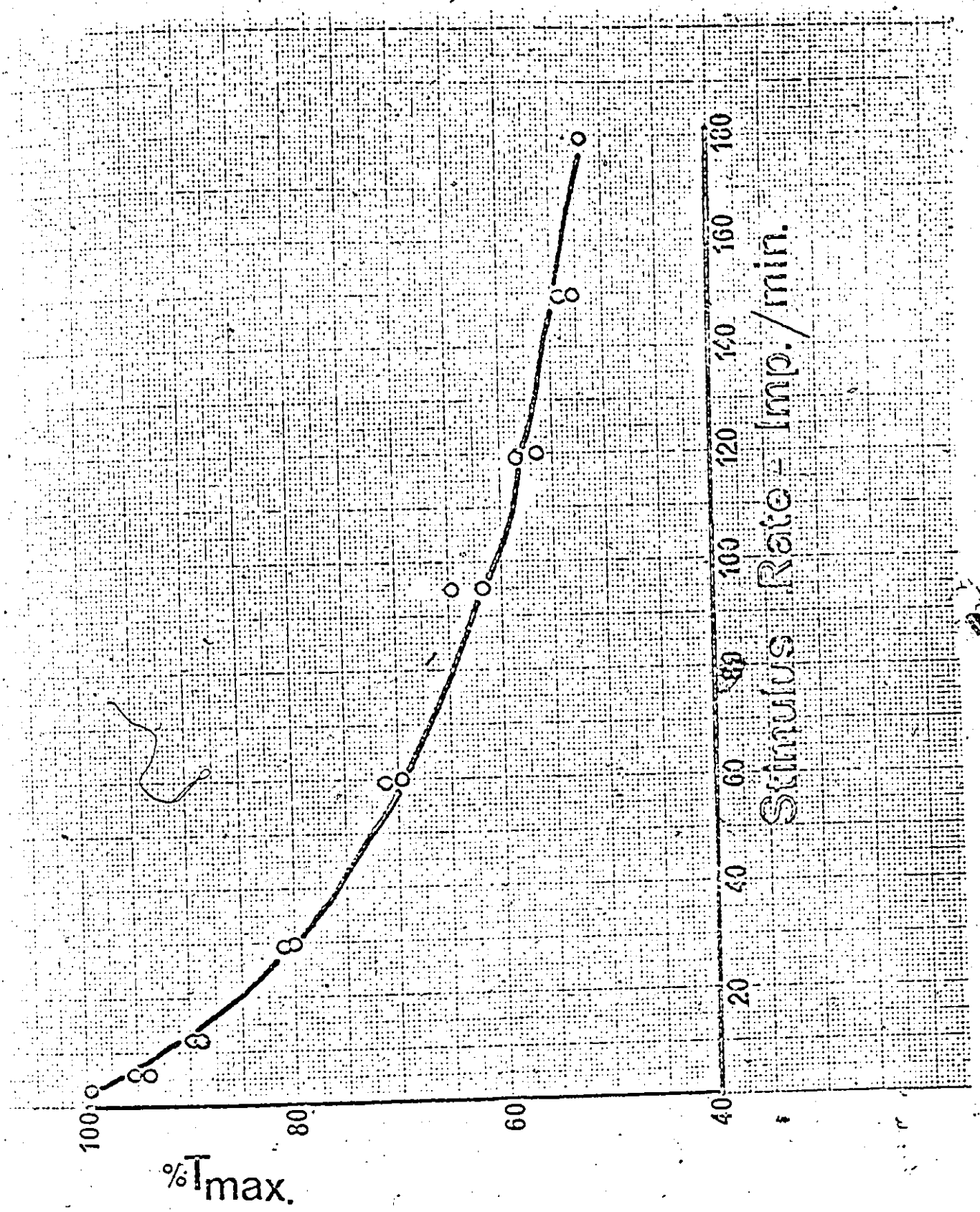

B
C
A

Figure IV-14. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 2.5mM.

(●) $[Na^+]_o$ = 100%; (○) $[Na^+]_o$ = 50%.


100
80
60
40
%Tmax.
20
40
60
80
100
120
140
160
180
Stimulus Rate - Imp./min.

was replaced by sucrose to maintain osmolarity in the Tyrode solution. Little change in the T-F response curve is seen, with no significant change in rested state tension. $PSP_{30''}$ also showed no evidence of potentiation at either sodium concentration. When $[Ca^{++}]_o$ is reduced to 1.0 mM, the T-F response at normal sodium concentration (100%) produces the expected positive inotropic component (Figure IV-15). However, in 50% sodium solution, the positive component of the T-F relationship is not as prominent (curve b) with no significant change in tension occurring after reduction of the sodium concentration. $PSP_{30''}$ also was not affected by this change in sodium concentration, (Table IV-III).

At low calcium concentration, $[Ca^{++}]_o$ = 0.25 mM, the T-F response showed the complete positive inotropism (Figure IV-16) (curve a). When sodium ion concentration was then reduced to 10% of normal value, the T-F response appeared similar to that seen at higher calcium concentrations although rested state tension was only 71% of the tension at 2.5 mM calcium. The addition of ouabain (1.37×10^{-5} M.) at 10% $[Na^+]_o$ and 0.25 mM $[Ca^{++}]_o$ actually caused a decrease in rested state contraction tension and no significant change in the shape of the T-F response; the negative inotropism still being apparent.

Effect of pH Decrease on the T-F and $PSP_{30''}$ Response

The pH of the Tyrode solution was modified by changing from a 5%/95% CO_2/oxygen mixture bubbled through the bath to a mixture

Figure IV-15. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 1.0mM.

A (●) $[Na^+]_o$ = 100%; B (○) $[Na^+]_o$ = 50%.

%Tmax
100
80
60
40
20
40
60
80
100
120
140
160
180
Time / min
A
B

Table IV-III

Effect of 50% Na^+ on T-F Response in Isolated Papillary Muscle of the Rat.

Treatment	Freq:/Stim. Imp./Min.	T (g.)	%Tmax.	dT/dt (g./sec.)	%dT/max dt
1.0mM $[Ca^{++}]_o$ Normal $[Na^+]_o$	2	2.95*	100	27.0	90.
	6	2.70	91.5	25.0	83.3
	12	2.60	88.1	23.5	78.3
	30	2.65	89.8	25.5	85.0
	60	2.70	91.5	29.0	97.0
	95	2.50	84.7	30.0	100
	120	1.70	57.6	25.0	83.3
	150	1.55	52.5	24.5	81.7
	180	1.30	44.1	22.0	73.3
1.0mM $[Ca^{++}]_o$ 50% $[Na^+]_o$	2	2.87*	100	26.0	96.3
	6	2.35	81.9	22.0	81.5
	12	2.0	70.0	19.5	72.2
	30	2.05	71.4	21.5	79.6
	60	2.23	77.7	26.0	96.3
	95	2.20	76.7	27.0	100
	120	1.85	64.4	25.0	92.6
	150	1.75	60.9	23.5	87.0
	180	1.40	48.8	21.0	77.8

Post Stimulation Potentiation

Treatment		T(g.)	PSP(g.)	%PSP	dT/dt g./sec.	dT/dtPSP g./sec.	%PSP $(\frac{dT}{dt})$
1.0mM $[Ca^{++}]_o$	100%Na	2.95	3.8	129	27	31.5	117
1.0mM $[Ca^{++}]_o$	50%Na	2.87	3.35	117	26	30.5	117

* Tmax

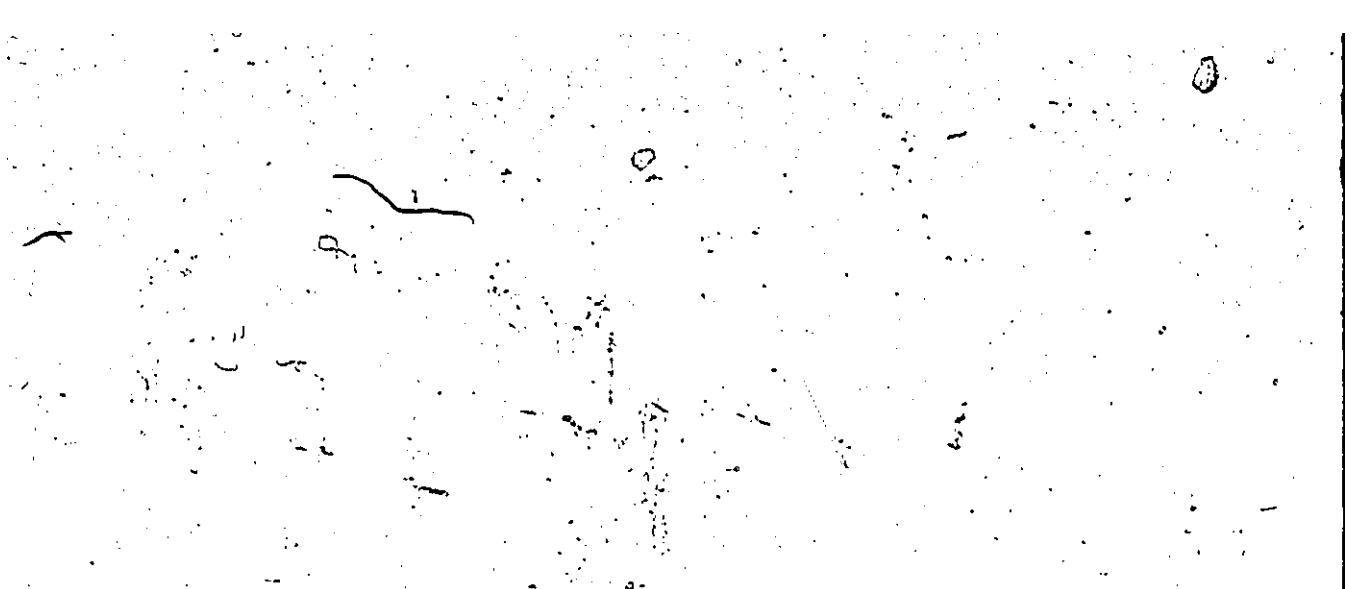


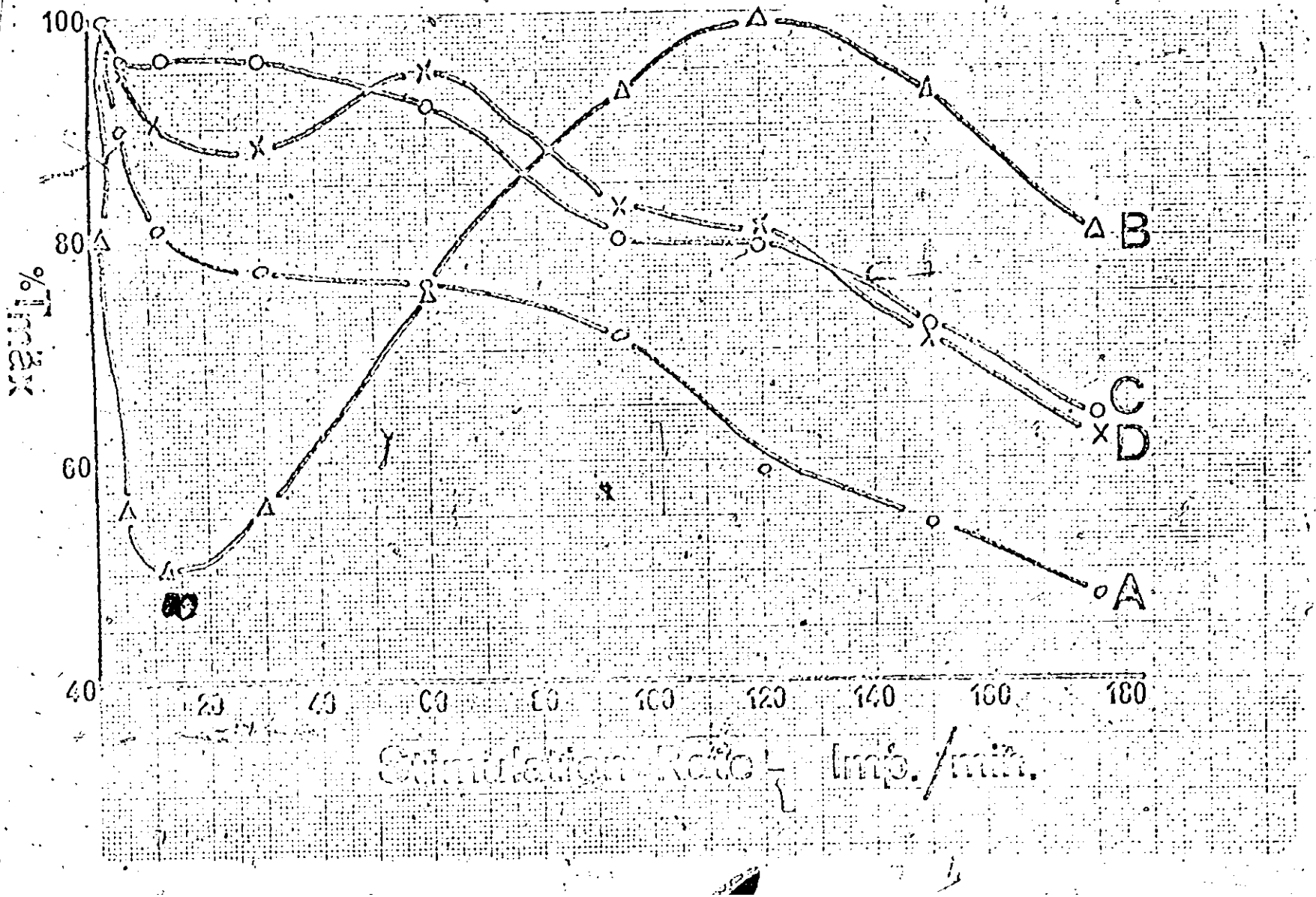
Figure IV-16. Tension-Frequency Response in Isolated Papillary Muscle of the Rat.

A (●) $[Ca^{++}]_o$ = 2.5mM, $[Na^+]_o$ = 100%, Tmax = 1.76g.;

B (△) $[Ca^{++}]_o$ = 0.25mM, $[Na^+]_o$ = 100%, Tmax = 1.25g.;

C (○) $[Ca^{++}]_o$ = 0.25mM, $[Na^+]_o$ = 10%, Tmax = 1.25g.;

D (X) $[Ca^{++}]_o$ = 0.25mM, $[Na^+]_o$ = 10%, Tmax = 1.05g., Ouabain - 1 x 10^{-5}M.


100
80
60
40
20
40
60
80
100
120
140
160
180
Stimulation Rate
Imp./min.
B
C
D
A

containing 20%/80% CO_2/O_2. This resulted in a pH drop from 7.40 to 6.73. Figure IV-17 shows the results of an experiment conducted at $[Ca^{++}]_o$ = 2.5 mM. After changing to the 20% CO_2, tension fell from 2.5 to 1.94 g. (-22%) after 20 minutes. While no $PSP_{30"}$ was obtainable at 5% Co_2, 20 minute exposure resulted in a $PSP_{30"}$ of 108% (Figure IV-17-B). After 60 minute exposure to 20% CO_2 RSC tension had fallen to 1.3 g., about 50% of the tension at 5% CO_2, while $PSP_{30"}$ now increased to 134%. At this point ouabain (1.37×10^{-5} M.) was added to the bath. Within 90 minutes RSC tension rose to 2 g. (Figure IV-17-D). $PSP_{30"}$ at this point was not obtainable.

The T-F response of the rat papillary muscle preparation at 20% CO_2 is illustrated in Figure IV-18. At 20% CO_2, the negative inotropism normally seen at 2.5 mM calcium and 5% CO_2 is changed so that a positive component is produced to a peak at about 100 stimuli/minute. The addition of ouabain at 20% CO_2 resulted in an increase in the rested state contraction tension (Figure IV-17) and the T-F relationship shows a reduced slope of the positive component in the inotropic response of the papillary muscle (Figure IV-18-B).

Figure IV-19 shows oscilloscope photographs of the rested-state contractions and $PSP_{30"}$ at 5% and 20% CO_2 in Tyrode containing 2.5 mM $[Ca^{++}]_o$. No $PSP_{30"}$ is present at 5% CO_2; 20% CO_2 reduced rested state contraction tension and $PSP_{30"}$ indicates a definite potentiation. After continued bubbling with 20% CO_2, rested state tension falls further (Figure IV-20-A). The addition of ouabain (1.37×10^{-5} M.) caused

Figure IV-17. Effect of 20% CO_2 on RSC tension and $PSP_{30''}$.

A) 5% CO_2 RSC tension, changed to 20% CO_2.

B) 20 min. exposure,

C) Ouabain, 10^{-5}M. added,

D) 90 min. exposure to Ouabain. $[Ca^{++}]_o$ = 2.5 mM.

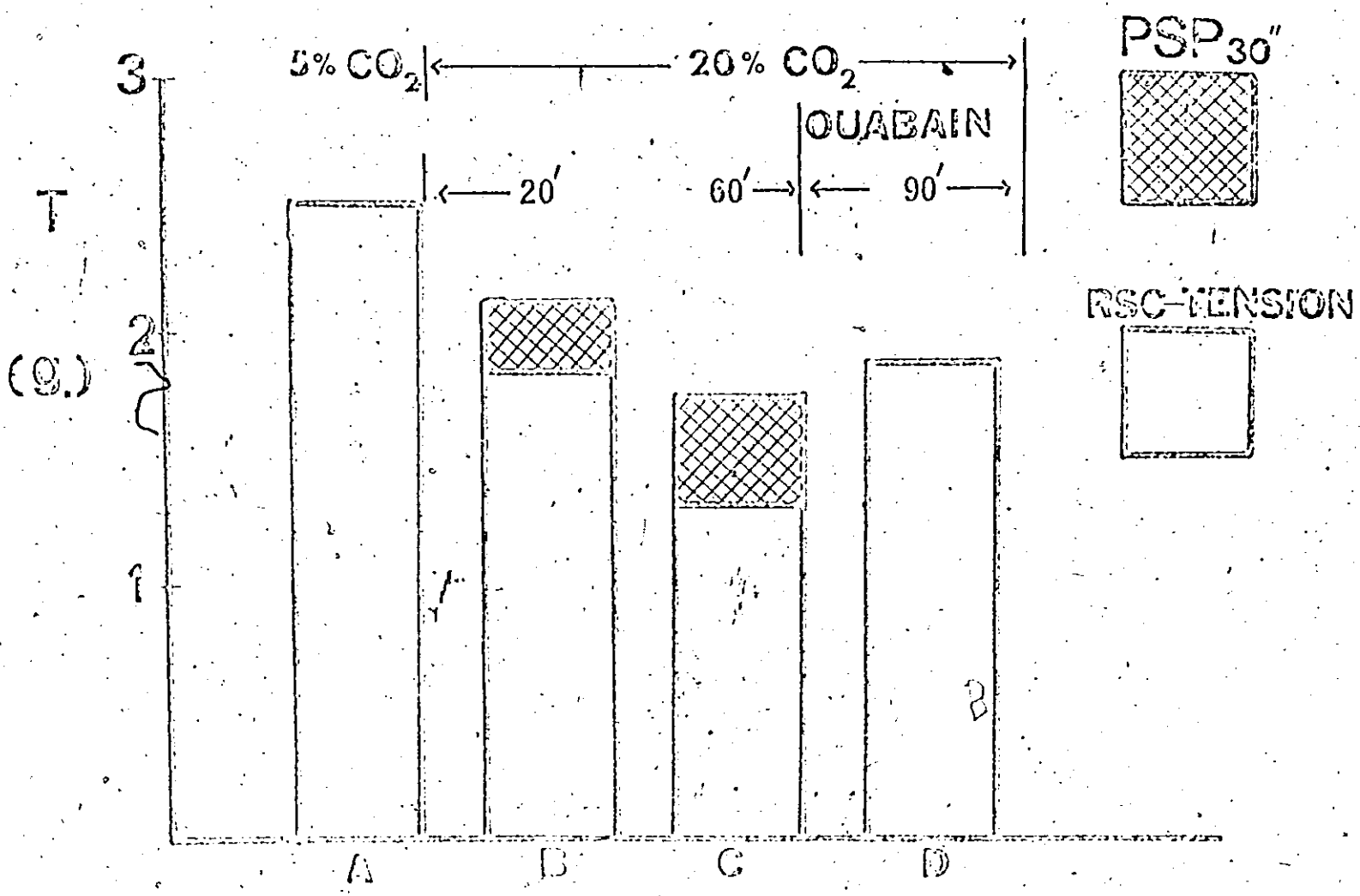

5% CO_2
20% CO_2
OUABAIN
20′
60′
90′
$PSP_{30''}$
RSC-TENSION
T
(g.)
3
2
1
A
B
C
D

Figure IV-18. Tension-Frequency Response in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 2.5mM. Aeration: 20% O_2.

A (⊖), Control; B (0) Effect of Ouabain (10^{-5}M.).

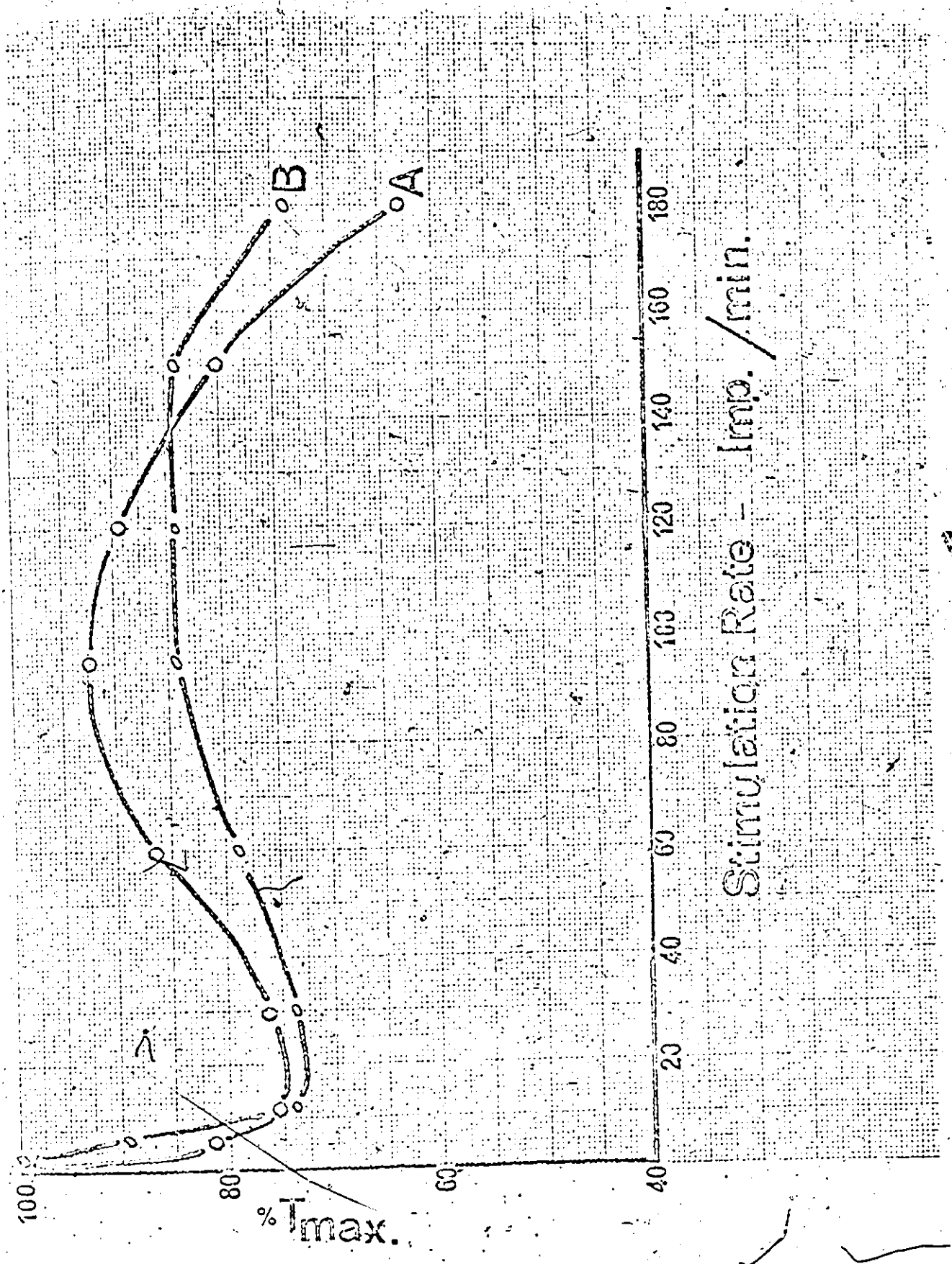

B
A
100
80
60
40
% Tmax.
20
40
60
80
100
120
140
160
180
Stimulation Rate - Imp./min.

Figure IV-19. Effect of CO_2 on Post-Stimulation Potentiation in the Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 2.5mM.

A) Control - 95% O_2/5% CO_2

B) $PSP_{30"}$ - 95% O_2/5% CO_2

C) Control - 80% O_2/20% CO_2

D) $PSP_{30"}$ - 80% O_2/20% CO_2.

Upper Tracing: dT/dt; 1 division = 5.0g./sec.

Lower Tracing: Tension (T); 1 division = 0.5g.

Time Scale: 1 division = 0.1 sec.

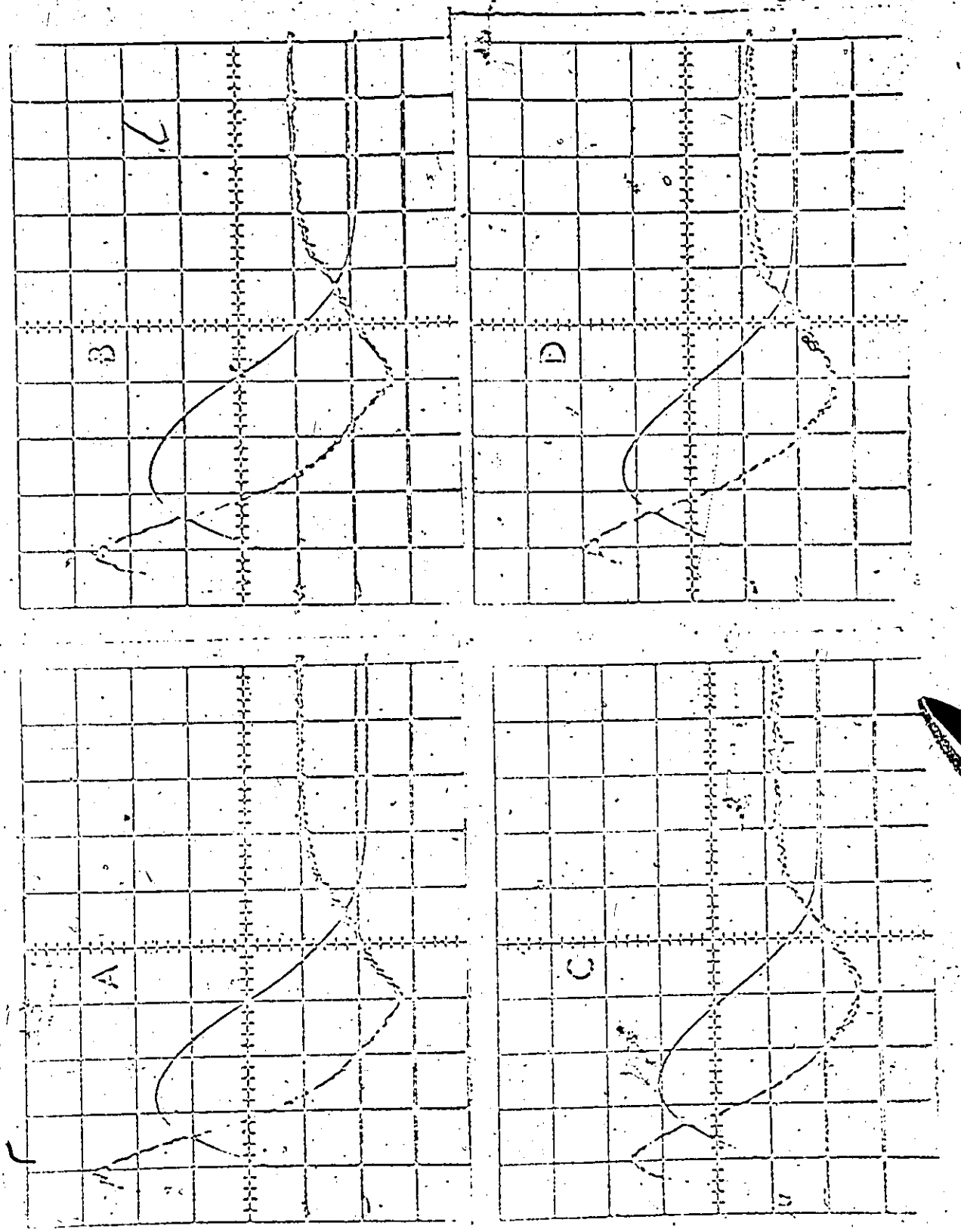

B
D
A
C

Figure IV-20. Effect of Ouabain on Isometric Tension in Isolated Papillary Muscle of the Rat. $[Ca^{++}]_o$ = 2.5mM. 20% CO_2/80% O_2.

A) Control B) 5 min. exposure to Ouabain (10^{-}

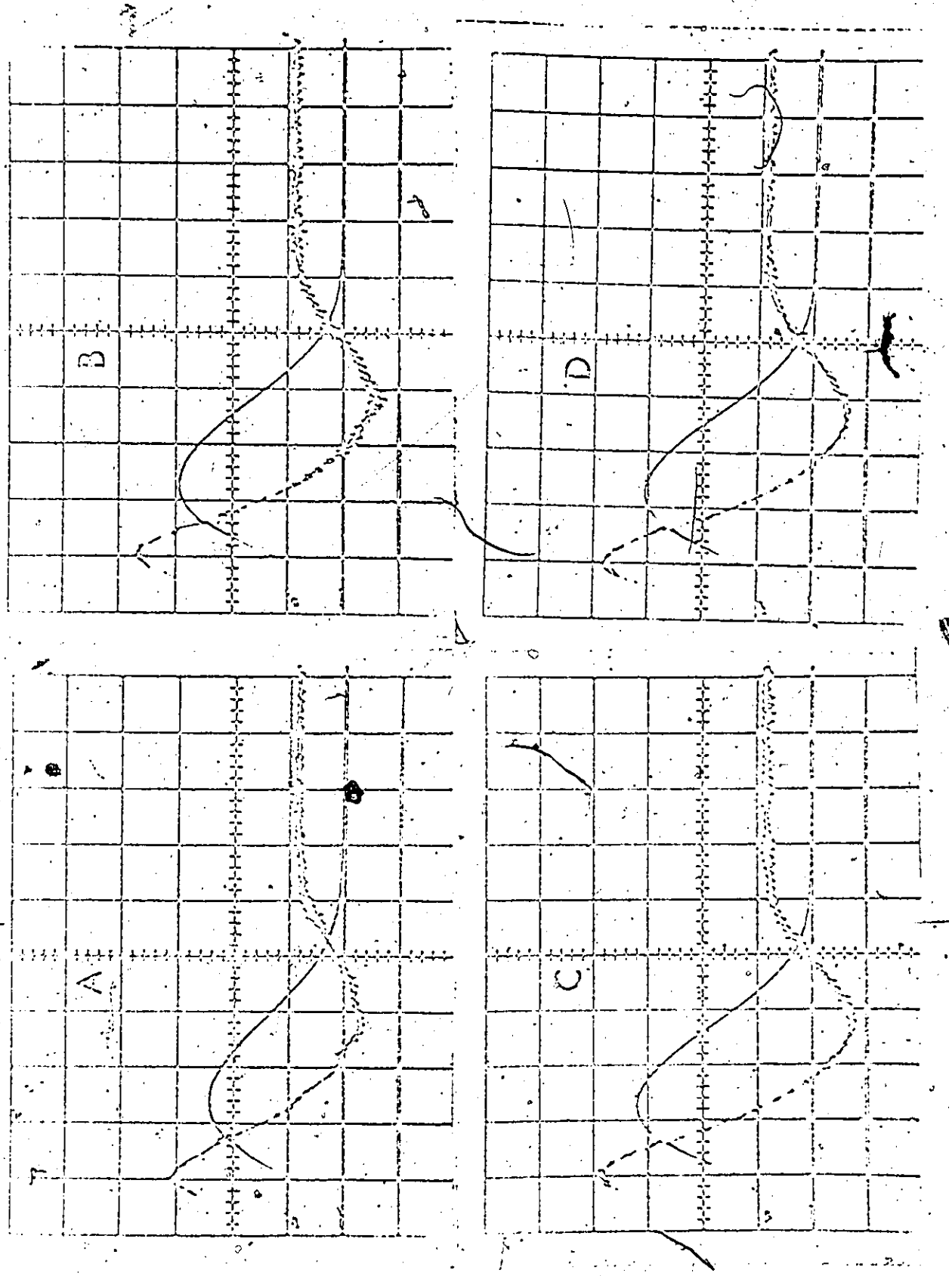
C) $PSP_{30"}$ @ 7 min. Ouabain exposure.

D) 20 min. Ouabain exposure.

Upper Tracing: dT/dt; 1 division = 5.0g./sec.

Lower Tracing: Tension (T); 1 division = 0.5g.

Time Scale: 1 division = 0.1 sec.


B
D
A
C

an increase in rested state contraction tension Figures IV-20-B and IV-20-D (5, and 20 minutes respectively). Figure IV-20-C shows $PSP_{30''}$ after 7 minutes exposure to ouabain.

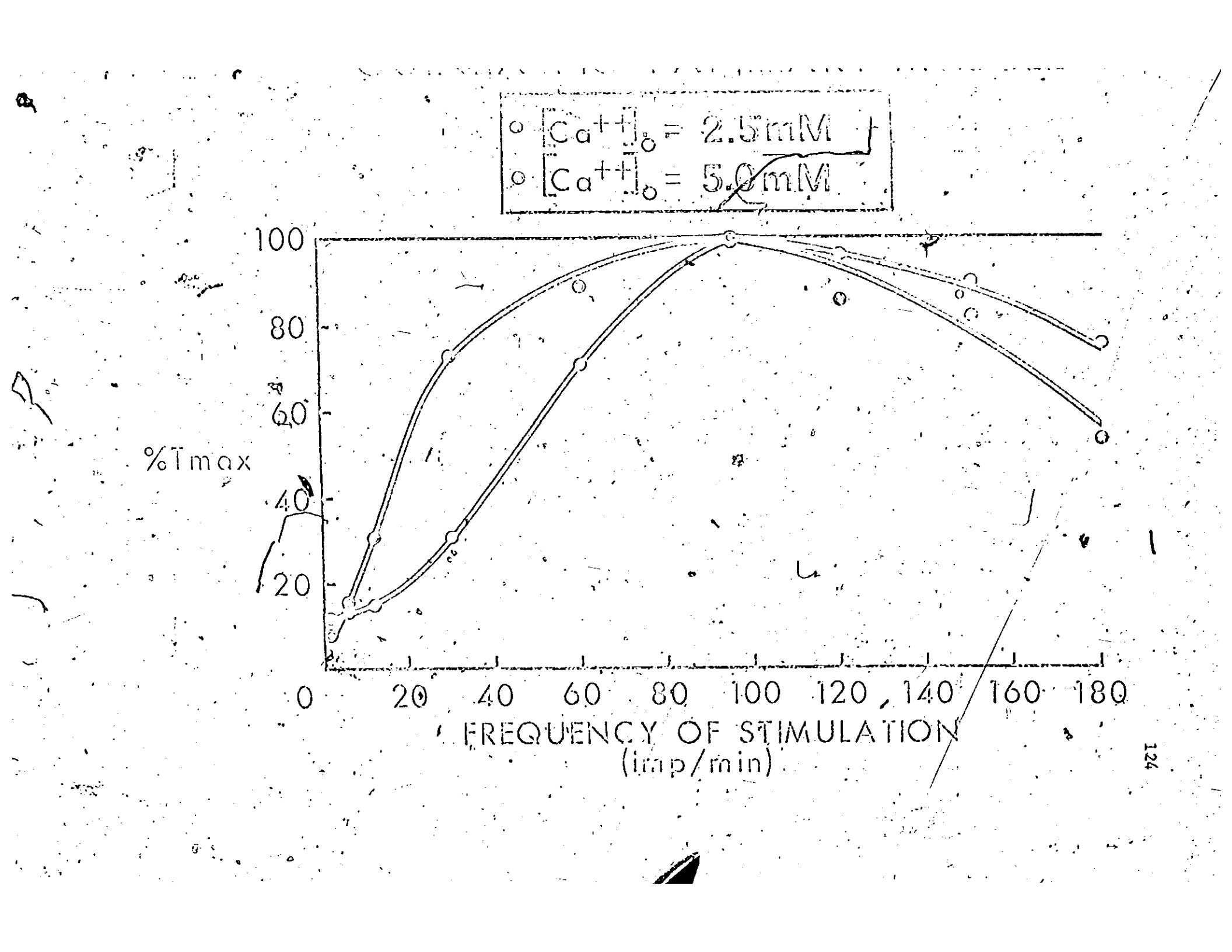
Effect of Ouabain in Guinea Pig Papillary Muscle

Figure IV-21 shows the T-F response of guinea-pig papillary muscle at 2.5 and 5.0 mM calcium. At 2.5 mM $[Ca^{++}]_o$ Tmax = 065 g. Changing the calcium concentration from 2.5 to 5.0 mM resulted in an increase in the rested-state contraction tension to 0.68g. and the T max (.85 g.) but did not change the slope of the positive component of the T-F response as drastically as a doubling in $[Ca^{++}]_o$ had caused in the rat (eg. from 1.0 mM to 2.0 mM). However, the addition of Ouabain (1.37×10^{-5}M.) to the bath containing Tyrode solution with $[Ca^{++}]_o$ = 2.5 mM changed the T-F response of the guinea-pig from a positive to a negative inotropic relationship within 9 minutes. (Figure IV-22). Washout after this run with ouabain-free Tyrode resulted in a reversal of the phenomenon. $PSP_{30''}$ tested before Ouabain exposure was 153% at 2.5 mM $[Ca^{++}]_o$. While $PSP_{30''}$ was reduced to 93% when ouabain had been introduced to the bath for 5 to 7 minutes (Table IV-IV-C). Washout with Ouabain-free Tyrode reestablished $PSP_{30''}$, provided the ouabain was not in contact with the muscle for longer than 20 minutes (Table IV-IV-C).

Discussion

These experiments clearly demonstrate the ability of ouabain to exert a positive inotropism upon rat heart muscle. This was also

Figure IV-21. Tension-Frequency Response in Isolated Papillary Muscle of Guinea-Pig. Effect of $[Ca^{++}]_o$.


○ [Ca++]o = 2.5mM
○ [Ca++]o = 5.0mM
100
80
60
40
20
%Tmax
0
20
40
60
80
100
120
140
160
180
FREQUENCY OF STIMULATION
(imp/min)

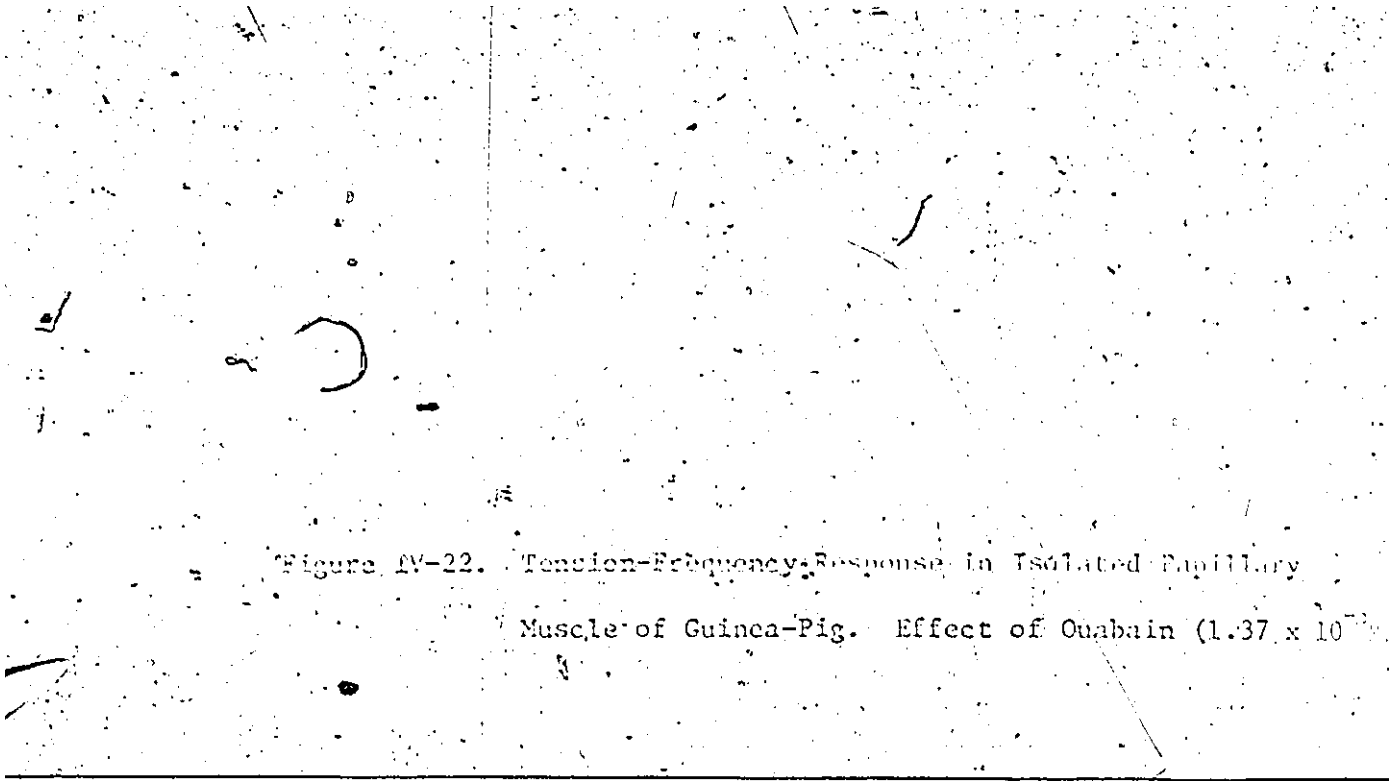


Figure IV-22. Tension-Frequency Response in Isolated Papillary Muscle of Guinea-Pig. Effect of Ouabain ($1.37 \times 10^{-}$

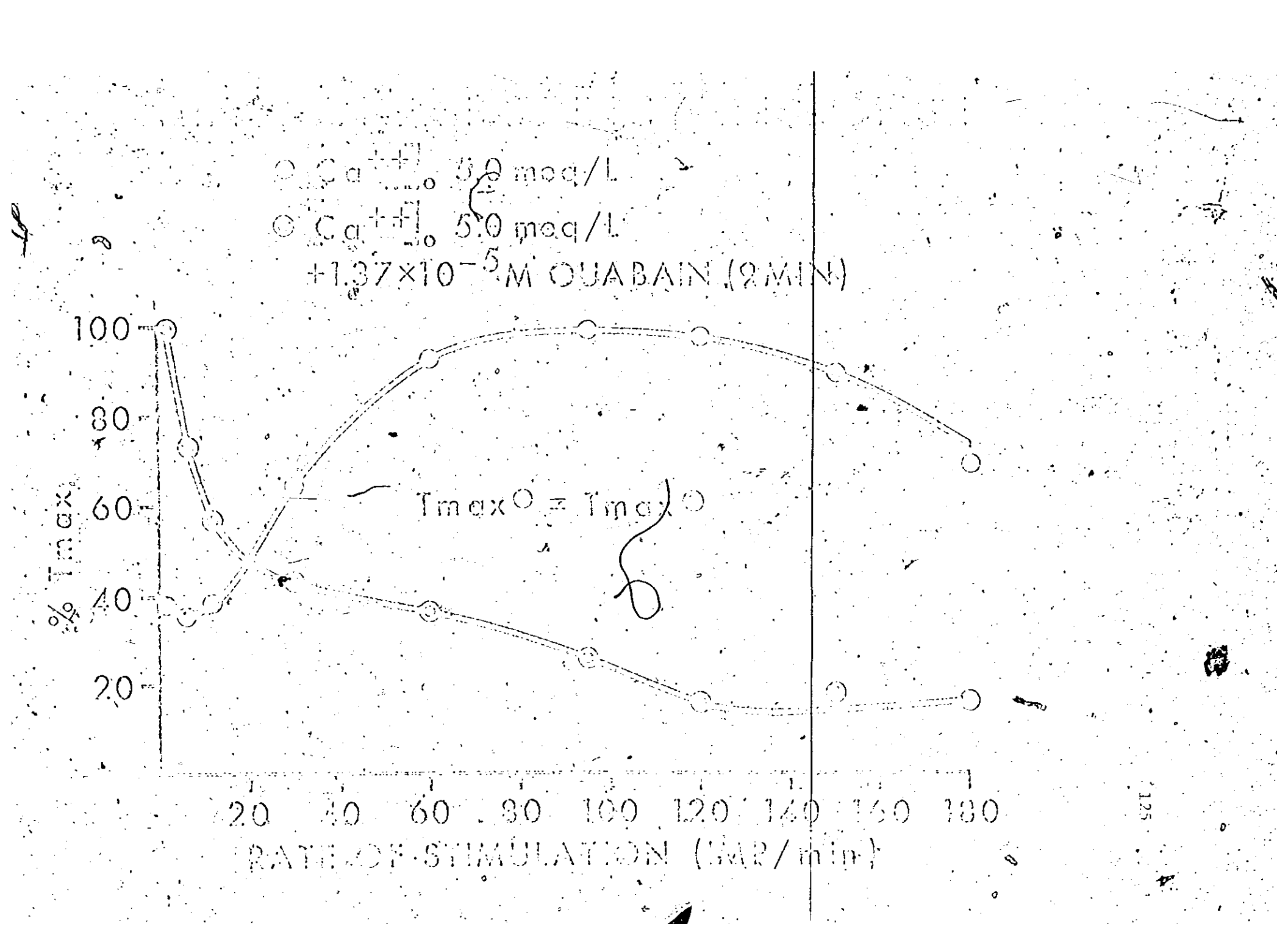

○ $Ca^{++}]_o$ 5.0 meq/L
◎ $Ca^{++}]_o$ 5.0 meq/L
+1.37×10⁻⁵M OUABAIN (2MIN)
100
80
60
40
20
% Tmax
Tmax◎ = Tmax○
20 40 60 80 100 120 140 160 180
RATE OF STIMULATION (IMP/min)

Table IV-IV

(A)

Guinea Pig Papillary Muscle - Tension Frequency Relationship

Treatment	Frequency of Stimulation Impulses/min.	Tension g.	%Tmax
$[Ca^{++}]_o$=2.5mM	2	.26	38.2
	6	.25	36.7
	12	.26	38.2
	30	.44	64.7
	60	.64	94.1
	95	.68*	100
	120	.67	98.5
	150	.62	91.1
	180	.48	70.5

(B)

$[Ca^{++}]_o$=2.5mM	2	.68*	100
	6	.50	73.5
	12	.39	57.3
1.37×10^{-5}M.	30	.30	44
	60	.26	38.2
Ouabain	95	.18	26.4
	120	.11	16.1
(9 min.)	150	.13	19.1
	180	.12	17.6

*Tmax.

(C)

Post Stimulation Potentiation (PSP)-

Guinea Pig Papillary Muscle

Treatment	Control Tension (g.)	$PSP_{30''}$ tension (g.)	$\%PSP_{30''}$
$[Ca^{++}]_o$ = 2.5mM.	0.3	0.46	153
$[Ca^{++}]_o$ = 2.5mM. + 1.37x 10^{-5}M. ouabain (12 minutes exposure)	0.8	0.75	93
$[Ca^{++}]_o$ = 2.5mM - Ouabain washout	0.65	0.81	125

suggested in 1950 by Masuoka and Saunders who demonstrated a positive inotropic effect on rat ventricular strips in low calcium (.3 mM) phosphate buffered solution containing ouabain. Our studies suggest that the effectiveness of ouabain in rat muscle is related to an intrinsic ceiling of contractility (COC). This term has been used to denote the maximum attainable tension (Koch-Weser and Blinks, 1963). The ceiling which determines the maximum contractile force would be controlled mainly by the level of releaseable calcium within the myocardium. This view is corroborated by many studies (review by Lee and Klaus, 1971; Shina and Miuhira, 1971) which indicate that the inotropic effect of ouabain *in vitro* is caused by an increase in free calcium available for contraction within the myocardial cell. In fact there are numerous reports which indicate the synergism between $[Ca^{++}]_o$ and exogenously administered ouabain. The review by Klaus and Lee (1971) also points out the instances where both cardiac glycosides and an increase in $[Ca^{++}]_o$ abolish the staircase phenomenon (T-F relationship).

It is clear from our data that an increase in the inotropic state after administration of ouabain (1.37×10^{-5} M.) to rat papillary muscle preparations causes 1) a decrease in the slope of the positive component of the T-F curve, and 2) a decrease in the $\% PSP_{30}$. As we have shown in the previous section, both of these phenomena can be elicited by increasing the calcium concentration in the bath. Furthermore, we demonstrate that the maximum attainable tension for rat cardiac muscle normally obtained around 2.5 mM $[Ca^{++}]_o$ appears to be the ceiling

for the inotropic effects of ouabain in this species. We thus believe that the effectiveness of the cardiac glycoside ouabain is intimately tied to the same mechanism which controls contractility (and the phenomena of post-stimulation potentiation and the T-F relationship) in the myocardial cell of the rat.

The phase-plane plot showing the effect of ouabain at 0.25 mM $[Ca^{++}]_o$ indicates that the temporal relationship between dT/dt and T is maintained as the inotropism develops. This is similar to the effect of dSP_{-30} and a change in the $[Ca^{++}]_o$ which was shown in Section III. This is important since it provides another proof that the inotropic interaction of ouabain produces an effect comparable to the inotropic states produced by changing $[Ca^{++}]_o$ in the rat papillary muscle.

By increasing $[Ca^{++}]_o$ Teiger and Farah (1968) were able to abolish the T-F (Bowditch) response in rabbit heart muscle; by decreasing $[Ca^{++}]_o$ below 2.5 mM we were able to produce the Bowditch response in rat heart muscle, and by addition of ouabain, Tuttle and Farah (1961) were able to abolish the Bowditch response in guinea-pig heart preparations.

We have also demonstrated that addition of ouabain (1.37×10^{-5} M.) to the bath at 2.5 mM calcium caused the conversion of the positive inotropic T-F response to a negative T-F response in the guinea-pig muscle, and that this phenomenon was reversible. The short exposure (less than 9 minutes) minimized any toxicity due to the presence of the foreign base of the glycoside. Tuttle and Farah (1961) were able to show a gradual decrease in the slope of the positive component of the T-F

response by addition of ouabain in both guinea-pig, cat and rabbit myocardium. In normal heart (non-depressed) the cardiac glycoside also abolished post-stimulation potentiation.

Our experiments with changes in calcium and the effects of ouabain on isolated rat heart muscle suggest that the cardiac glycoside ouabain and calcium act upon the same mechanism. Furthermore, these results indicate that the rat heart muscle is capable of demonstrating similar properties to the myocardium of cat, rabbit and guinea-pig. The critical and common factor to the changes appears to be the external concentration of the calcium ion. The conclusions reached by Lee and Klaus in their review in 1971 on the mechanism of action of cardiac glycosides implies that one of the effects is an increase in releasable calcium within the myocardial cell. An interesting study by Shina and Mizuhira (1971) showed an increase in precipitable calcium in electron micrographs of mouse heart treated with therapeutic doses of ouabain. The increase in precipitate was found specifically in the terminal cisternae and to a lesser extent inside and around the longitudinal tubules of the sarcoplasmic reticulum. Since the sarcoplasmic reticulum is considered a source of calcium in contraction coupling (Lee, 1967), this study provides most direct evidence that ouabain is involved in an increase in releaseable calcium. Less direct evidence is provided in numerous other studies (see review by Lee and Klaus, 1971; and Bailey and Sures, 1971) which simply suggest that a redistribution of calcium contained within the myocardial cell occurs with ouabain administration.

While no definitive proof exists for the mechanism involving the increase in releaseable calcium, evidence has accumulated from many sources (Glynn 1964; Skou, 1957; review by Lee and Klaus, 1971, Schwartz et al., 1969) that ouabain inhibits the Na^+, K^+, ATPase, the enzyme primarily concerned with Na^+ transport. Experimental evidence has also accumulated indicating that a probable sequence for the inotropic action of ouabain would be an inhibition of the Na^+, K^+ ATPase, blocking sodium transport, and resulting in an intracellular accumulation of sodium ion. (Langer, 1971). According to a proposal by Langer (1970), Na^+ ions compete for membrane sites with Ca^{++} ions. An increase in intracellular Na^+, caused by the Na^+, K^+ ATPase inhibition, would then displace Ca^{++} resulting in an increase in the releaseable Ca^{++} fraction. Our data on the effects of low sodium concentration (10%) on the T-F relationship shows a similarity to the effect of raising the calcium concentration i.e., abolishing the positive inotropism. These results are in agreement with similar experiments performed by Teiger and Farah (1968) on rabbit myocardium and Niedergerke and Lüttgau (1957) on frog heart. The suggestion is that calcium and sodium ions compete at the same locus for a common carrier; thus a decrease in sodium concentration would be analogous to an increase in the myocardial releaseable calcium (that calcium fraction utilized in tension development). Langer (1970) also suggests that H^+ ions compete for calcium sites in the myocardial membrane. Our data support this view as well. An increase in $[H^+]$ caused by an increase in pCO_2 with our bicarbonate buffer results in 1) a

increase in rested state tension and 2) the development of a positive component to the T-F response even at 2.5 mM $[Ca^{++}]_o$. The ability of ouabain to abolish this inotropism and remove $PSP_{30"}$ indicates that the H^+ ion mechanism may be working as Langer suggests. The ability of ouabain to restore the rested state tension at 20% CO_2 and to remove potentiation at 2.5 mM $[Ca^{++}]_o$ may test the state of normalcy in rat myocardium. Tuttle and Farah (1961) suggested that rabbit heart preparations from "poor" hearts were restored to normal T-F-responses easily by low doses of ouabain; anoxic and hypercapnic regions may very easily be present in damaged or infarcted myocardium and cardiac glycosides supposedly have the most pronounced effect in depressed myocardial tissue (Coko et al., 1969). By depressing the myocardial tissue of the rat heart with 20% CO_2, we have been able to demonstrate the ability of ouabain to become effective. This suggests that 1) an increase in $[H^+]$ may displace releaseable calcium and 2) that ouabain can counteract this phenomenon. One may question this by saying that if an increase in $[H^+]$ caused the displacement of calcium then how is it that developed tension does not increase instead of falling? It may be that this calcium is shifted to a less accessible region by the H^+ ion and that an increase in $[Na^+]_i$ caused by ouabain releases this calcium to more accessible sites so that tension is restored. While this explanation remains speculative, it is interesting in that it relates well to the ceiling of contractility noted in rat heart. Furthermore, these data suggest that the internal

calcium stores play a dominant role in determining rested state contraction tension since even with $[Ca^{++}]_o$ = 2.5 mM, a tension reserve capacity can be demonstrated in the presence of 20% CO_2. It is unlikely that the increase in pCO_2 reduced $[Ca^{++}]_o$, but more likely resulted in an apparent decrease in $[Ca^{++}]_i$; thus the fall in the rested state contraction tension. The fact that the shape of the T-F response at 20% CO_2 follows the curve expected for a decrease in the RSC tension below Tmax at 2.5 mM $[Ca^{++}]_o$ indicates that there has been no significant change in excitation contraction coupling over the range of frequencies tested. In other words, a pH decrease caused a decrease in RSC tension and the T-F curve followed the inotropic response expected for the tension state rather than the $[Ca^{++}]_o$ level.

The negative inotropic effect of acidosis in heart has been shown to be due to the effect of hydrogen ion itself rather than the increase in carbon dioxide concentration (Jordovic, 1966, McElroy et al., 1968; Katz, 1970). Bass and Moore indicated in 1966 that hydrogen ions and protons generally can displace Ca^{++} from a number of biological structures while Caryalho (1966) found that hydrogen ions had a much greater affinity than Ca^{++} for muscle microsomal fragments. Similarly, Taylor and Morrillees (1971) have shown that the amount of calcium bound to extracellular, membrane located sites is reduced by hypoxia, and that prolonged hypoxia impairs the calcium accumulating ability of microsomal fractions (S.R.). It is evident that the depression of the rested state

contraction by the reduction in pH can be reversed by ouabain and that the apparent ceiling of contractility at low pH has not changed appreciably in contrast to the results obtained at low sodium concentrations. At low $[Na^+]_o$, the apparent ceiling of contractility is reduced so that even at low calcium, $[Ca^{++}]_o = 0.25$ mM, potentiation and the positive inotropic response to an increase in stimulation frequency is abolished without a change in the rested state contraction tension. The inability of ouabain to increase tension also provides evidence that the apparent ceiling of contractility is lowered by the reduced sodium. The lowered ceiling of contractility (COC) could be caused by a reduction in the number of displaceable loci available for Na^+/Ca^{++} interaction; a reduced $[Na^+]_o$, may also result in less Na^+ entry per beat during a frequency change and also displace less Ca^{++}. But if the latter were true, then during ouabain administration one might observe a positive inotropic effect on rested state tension. It is therefore more likely that the COC is reduced because of a diminution in the number of reactable sites, probably at the sarcolemma or sodium exchanging surface.

We do know that the reduced ceiling can be moved to a higher level by increasing $[Ca^{++}]_o$ even at low $[Na^+]_o$. However, the ineffectiveness of ouabain at low $[Ca^{++}]_o$, low $[Na^+]_o$, suggests that an increase in releaseable calcium is not possible under these conditions and implies that Ca^{++} has been lost and cannot be mobilized during the

low Na^+, low Ca^{++} equilibration period.

The concepts of the ceiling of contractility and reserve capacity while not unique are useful in dealing with the model system we envisage. Although we imagine that the level of $[Ca^{++}]_o$ determines the level of intracellular releaseable calcium which in turn determines the number of Actin-Myosin cross bridges activated, we must also make some assumptions about other events in the excitation-contraction and relaxation cycle. For instance we must consider that the interventions we have utilized in an attempt to alter the contractile state do not alter depolarization, transmission, calcium release, Ca^{++}-troponin interaction, the Myosin-ATPase activity and calcium removal (relaxation). Or if the interventions do interfere with any stage of E-C coupling that the changes are parallel to the effects of $[Ca^{++}]_o$ on tension development.

Interpretation of the Model

Based upon the results in Section IV our model of calcium movement in rat cardiac muscle may have to be modified slightly. Figure IV-23 shows this revised model.

Process 1 is the passive flux of calcium into and out of the myocardial cell across the sarcolemma. Thus after a sufficiently long interval Ca_s will be in equilibrium with $[Ca^{++}]_o$; the quantity of calcium in Ca_o is considered as being infinite compared to Ca_s. Calcium can also enter Ca_s through process 2 associated with the action potential and depolarization of the cell (gated calcium). This process also is

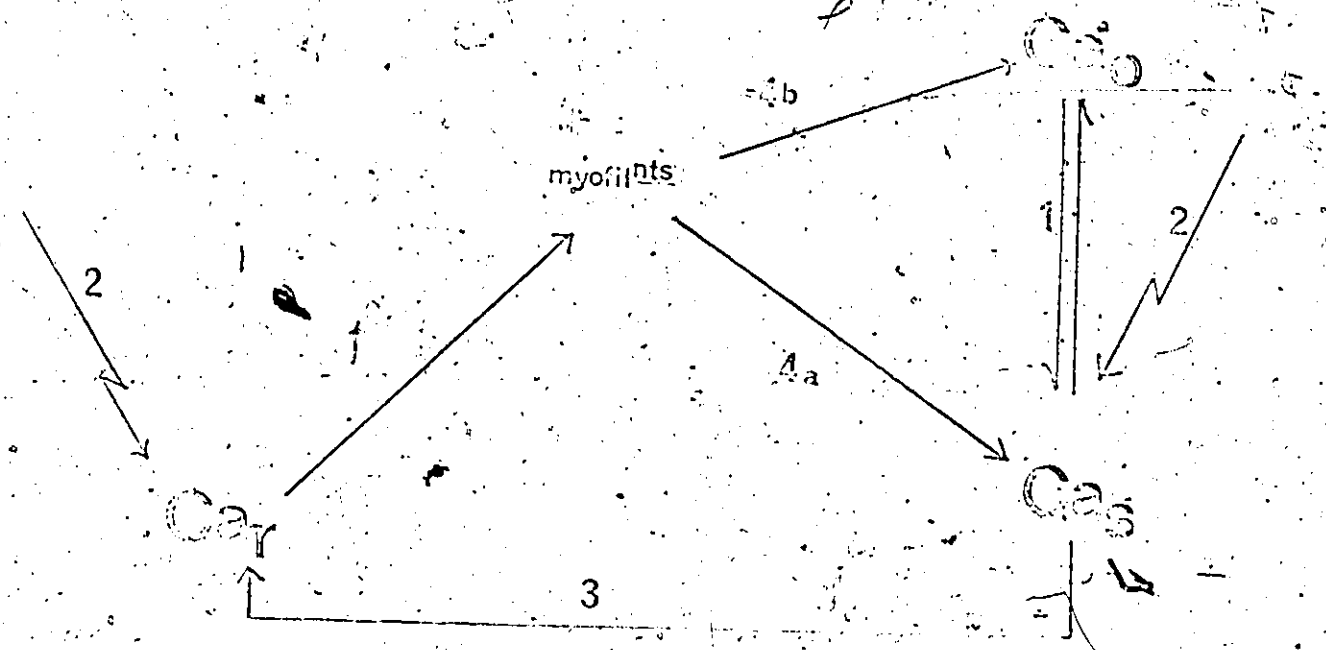


Figure IV-23. Hypothetical Model of Calcium Movement in Rat Myocardium.

responsible for completely releasing Ca_p to the myofilaments and causing contraction. Peak tension would be dependent upon the level of calcium in Ca_r just before it is released. The calcium can then be sequestered by process 4 (a) or expelled from the cell by 4 (b) (relaxation). Process 3 is a rate limiting process, undirectional with the rate dependent upon the level of Ca_s.

In Section III we suggested that the appearance of the positive component in the T-F relationship and also of post-stimulation potentiation (PSP) was related to the level of $[Ca^{++}]_o$. At high $[Ca^{++}]_o$, the COC is reached while low $[Ca^{++}]_o$ results in an apparent reserve capacity. It now appears that we can modify the size of the reserve in at least two ways:

1) by decreasing $[Ca^{++}]_o$ we increase the apparent reserve capacity, or alternately by decreasing pH we would achieve a similar result

2) by addition of ouabain we decrease the apparent reserve capacity.

We have shown that ouabain and $[Ca^{++}]_o$ are complementary in their effects on PSP and the T-F relationship. Similarly, an alteration of the pH in the bathing medium by 20% CO_2 created a reserve capacity abolished by ouabain administration.

However, the effect of low sodium concentration (10% of normal) apparently caused a different change in the system. Low sodium seems to lower the apparent ceiling of contractility; at low Na^+ (10%) and low

$[Ca^{++}]_o$ (0.25 mM), the RSC tension is not significantly different than at 100% $[Na^+]_o$; however $PSP_{30'}$ and the positive component of the T-F beats disappear. Furthermore, at low $[Na^+]_o$ ouabain has no apparent effect on the T-F response. Thus sodium concentration seems to control the ceiling of contractility in the rat heart preparation; the apparent capacity of Ca_s may be reduced in lowered $[Na^+]_o$.

Rat heart apparently has a ceiling of contractility (COC) around 2.5 mM $[Ca^{++}]_o$, however, it is apparent that even an increase to $[Ca^{++}]_o$ = 5.0 mM does not produce the COC in guinea-pig. Similarly, rabbit heart has a COC around 7.2 mM $[Ca^{++}]_o$. (Teiger and Farah, 1968). The shift in the shape of the T-F response by ouabain in guinea-pig papillary muscle suggests that a system analogous to that of the rat may be present; the COC of rat, guinea-pig, and rabbit are at different levels, and hence the saturability of calcium stores similar to Ca_s may also be different in these species at the same $[Ca^{++}]_o$. This may also be related to the passive flux (Process 1) in our newest model (Figure IV-23). Hence the loading of a Ca_s (% saturation) will be variably changed from species to species at say 2.0 mM $[Ca^{++}]_o$. It is however obvious that the rat heart can be demonstrated to have characteristics similar to the guinea-pig and rabbit using appropriate experimental conditions.

From our data we may suggest where interactions of pH, Na and ouabain may possibly occur in our model (Figure IV-23). Ouabain - since ouabain has been implicated in Na^+, K^+ ATPase inhibition at

the sarcolemmal membrane (Lee and Klaus, 1971), process 1 (passive Ca^{++} influx) may be affected. Although K^+ is exchanged for Na^+ by this "pump", $[Ca^{++}]_o$ also has a lesser affinity for the outer cation exchange site (Skou, 1969). The change in $[Na^+]_i$ caused by Na^+ accumulation may also affect bound Ca^{++} distribution and so Ca_s may increase; the driving force to Ca_r through process 3 would therefore be elevated.

$[Na^+]_o$ - a decrease in $[Na^+]_o$ would have little effect on $[Na^+]_i$ at low rates of stimulation, since the Na^+, K^+ ATPase tends to keep $[Na^+]_i$ low (around 10-20 mM). However, an increase in the frequency of stimulation could displace calcium from Ca_s - resulting in a positive inotropic at certain frequencies.

pH - the primary effect of a decrease in pH would be on Ca_s - bound calcium stores - resulting in a decrease in available Ca^{++} to Ca_r.

Unlike the possible $[Na^+]_i$ effect which may increase the releasable calcium - a pH fall appears to displace this calcium away from Ca_r. However, this effect is complicated by the fact that an alteration in pH has been shown to have profound effects on the myosin ATPase; an increased pH causes activation while acid medium depresses ATPase activity (Katz, 1970). Hence the fall in RSC tension in lowered pH may be the result of more than the interaction of H^+ with Ca^{++} binding at the sarcolemmal surface or with internal Ca^{++} storage.

While the reduction in the $[Ca^{++}]_o$ is shown to allow the positive T-F relationship and potentiation to appear in the rat, the model has not yet explained fully how a decrease in Ca_s will allow this

to occur.

We propose that an increase in the calcium released/beat from Ca_r may occur if a) process 3 is a time limited event b) Ca_s increases in calcium content due to gating (process 2). To comprehend these concepts more fully it is appropriate to define the recharge process that may occur between rested state contractions.

The recharge of Ca_r in our model can be represented by the amount of calcium that can be released from Ca_r at any time during the rested state contraction interval, and is proportional to these peak tensions. We assume a linear relationship between calcium released and peak developed tension (Katz, 1970), as long as $[Ca^{++}]_o$ (and hence Ca_s and/or Ca_r) is equal to or below a value producing maximum attainable tension (the COC).

SECTION V

A. $[Ca^{++}]_o$ and Rested State Contraction Tension

B. Analysis of Tension Recovery Between Rested State Contractions.

Introduction

In this section we present the results of two critical types of experiments necessary to describe the model we have proposed for the movement of calcium ion in rat heart muscle.

A) In the preceding sections we suggested that the relationship between $[Ca^{++}]_o$, pH, $[Na^+]_o$ and ouabain were inter-related to a ceiling of contractility, (COC) which was primarily determined by $[Ca^{++}]_o$. Moreover, we suggested that the COC in rat heart was around 2.5 mM $[Ca^{++}]_o$. In this section we present results on the precise relationship between $[Ca^{++}]_o$ and RSC tension. We also conducted experiments to determine the relation between $[Ca^{++}]_o$ and the calcium content of the preparation.

B) In the previous sections we suggest that the tension-determining Ca^{++} is derived from a pool of calcium that is refilled or recharged betweem contractions. In a series of experiments we investigate the peak developed tension at intervals less than the rested state contraction interval. This would correspond to the recharge of the Ca_r pool from Ca_s in our model (Figure IV-23) through process 3.

From these data, we then attempt to build a complete model of the calcium movement in rat myocardium that will account for the experimentally determined interval-strength relationship (T-F response) and related phenomena.

A. The Relation Between $[Ca^{++}]_o$ and Rested State Contraction Tension.

To determine the relationship between external calcium concentration and rested state contraction tension, the following experiments were performed:

The isolated papillary muscle preparation was set up as outlined in Section II, with the initial $[Ca^{++}]_o$ at 2.5 mM. After equilibration, the bath $[Ca^{++}]_o$ was decreased in 0.1 mM steps. At each step, tension was monitored until a true rested state contraction tension was obtained i.e., a steady peak tension was recorded. When $[Ca^{++}]_o = 0$ was reached, the bath calcium concentration was increased in 0.1 mM steps with the RSC tension recorded at each step. In a second series of experiments, the $[Ca^{++}]_o$ was changed to 0 immediately after equilibration and the tension monitored until peak tension was less than 10 mg. (about the level of noise in the system). At this point $[Ca^{++}]_o$ was increased in 0.1 mM steps and the rested state contraction tension was followed after each increase. The calcium concentration in the 100 ml. muscle bath was maintained at each stage by replacing the Tyrode solution several times. This was especially critical when we maintained $[Ca^{++}]_o$ at 0. The Tyrode solution was always made up with deionized double distilled water (prepared daily), and the only calcium contamination was believed to be derived from chemicals used in preparing the balanced salt solution (eg. NaCl, $NaHCO_3$, etc.).

Results

Figure V-I shows the rested state contraction tension as a

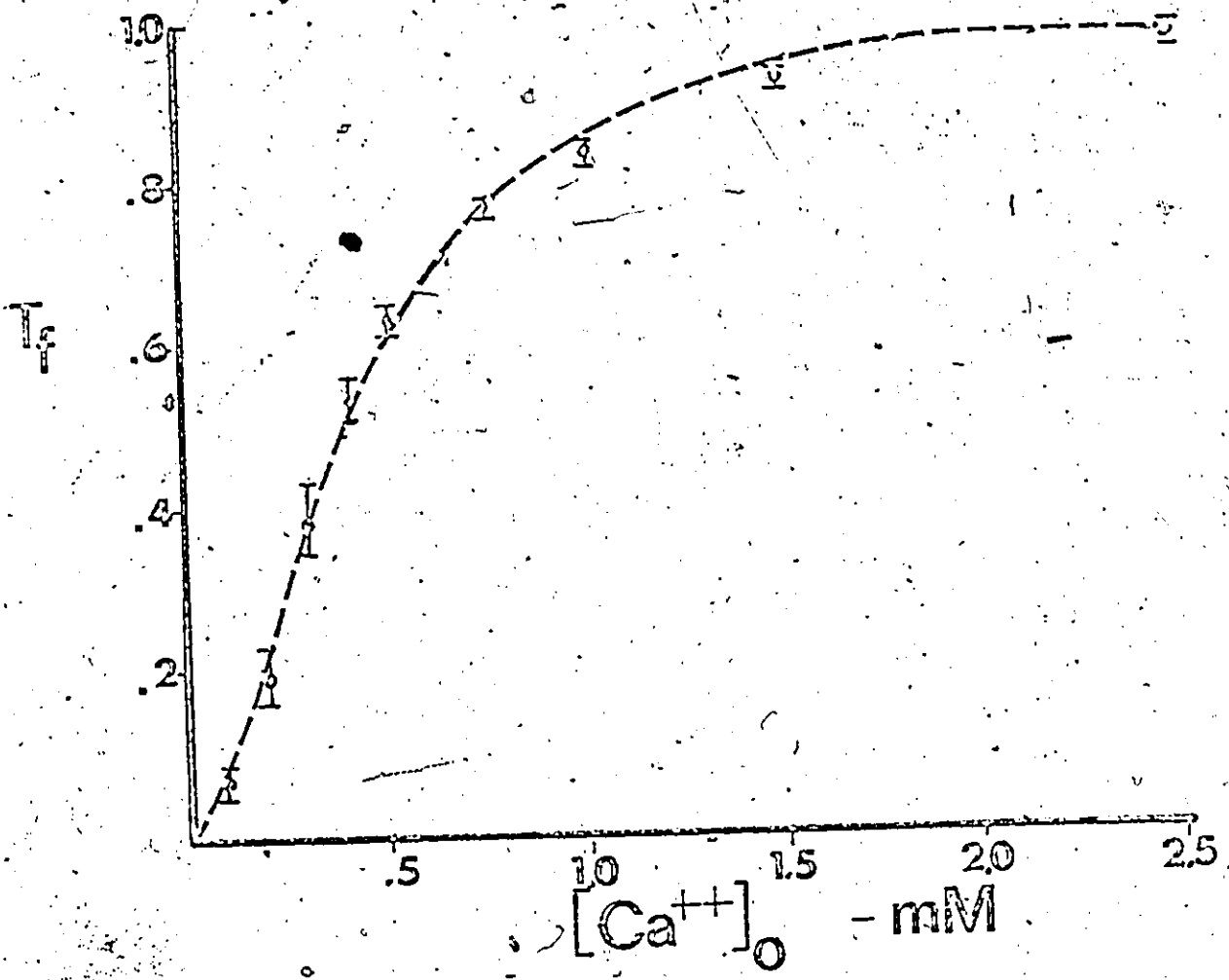


Figure V-1: Steady State Tension in Isolated Papillary Muscle of the Rat. Effect of $[Ca^{++}]_o$. (o) Experimentally determined values ± S.E.M. Solid line indicates values calculated from mathematical expression for T_f (Formula 1).

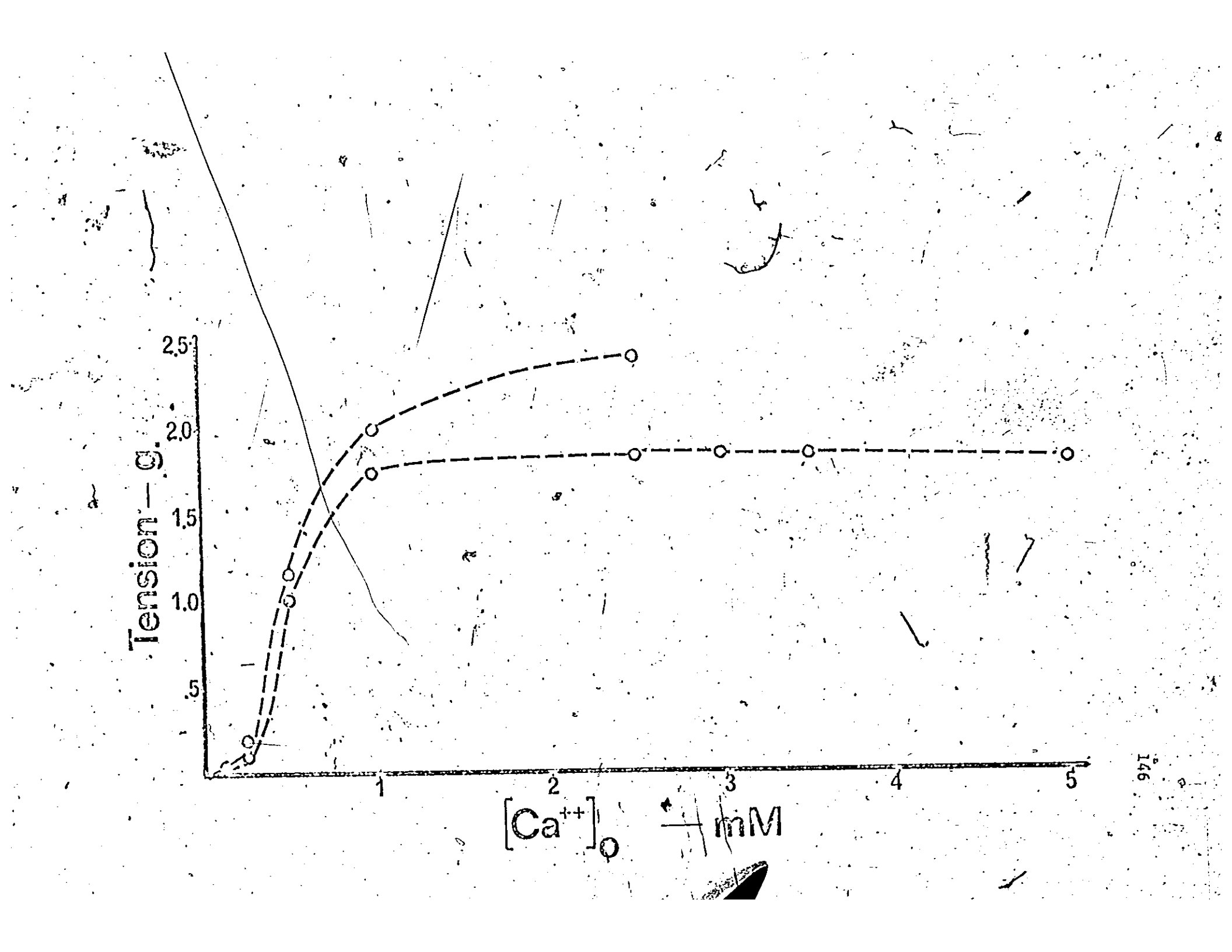
function of $[Ca^{++}]_o$ (solid points ± S.E.M.) The curve has a characteristic S-shape with the steepest slope occuring between 0.25 and 0.5 mM $[Ca^{++}]_o$. This curve was obtained at each $[Ca^{++}]_o$ when the external calcium concentration was increased from 0 to 2.5 mM (ascending RSC tension).

A typical experiment in which both the ascending RSC tension and descending RSC tension was followed at each $[Ca^{++}]_o$ is illustrated in Figure V-2. Both the ascending tension curve (open circles) and the descending tension curve (closed circles) have a sigmoid shape. But the tension at the start of the experiment was greater than the ascending RSC tension at 2.5 mM $[Ca^{++}]_o$; at least 2 hours had elapsed between these two readings. As $[Ca^{++}]_o$ was reduced, both the descending and ascending tension approach each other; descending tension was always greater.

There may be several factors contributing to the tension difference at 2.5 mM $[Ca^{++}]_o$. Since we have chosen to utilize the ascending tension curve as an indication of the RSC tension/ $[Ca^{++}]_o$ relationship, it is appropriate that the criteria for using these data are discussed at this point.

We have observed that after equilibration at 2.5 mM $[Ca^{++}]_o$, the RSC tension remains stable for up to 5-6 hours. However, after this period of time, RSC tension falls by about 10% for every hour the isolated preparation is maintained. This occurs irrespective of the addition of freshly prepared Tyrode to the bath. This change can there-

Figure V-2. Steady State Tension versus $[Ca^{++}]_o$. (●) Values determined in descending $[Ca^{++}]_o$. (○) Values determined in ascending $[Ca^{++}]_o$.


Tension — g.
2.5
2.0
1.5
1.0
.5
1
2
3
4
5
$[Ca^{++}]_o$ — mM

fore be attributed to a general failure of the heart muscle preparation. However, with sheep heart trabeculae, the decline in tension after hours of perfusion has been attributed to a gradual loss of Ca^{++} by the tissue (S. Weidmann, personal communication). It is therefore possible that this mechanism may have contributed to the difference in the ascending and descending tension maxima. The E.C. coupling mechanism appears to be intact in both the ascending and descending phases at 2.5 mM $[Ca^{++}]_o$ since the T-F relationship and PSP appeared similar. It may also be suggested that prolonged soaking in $[Ca^{++}]_o = 0$ Tyrode may have damaged some fibers particularly the most peripheral fibers in the papillary muscle bundle. Low $[Ca^{++}]_o$ has been reported to be disruptive upon membranes generally (Rothstein, 1963).

And finally there may have been a shift in internal Ca^{++} stores within the myocardial cells. Since we waited until RSC tension was achieved at each $[Ca^{++}]_o$ in the descending phase, we may have removed Ca^{++} from very slow exchanging sites and transferred them to faster tension determining sites. Indeed we found that it took longer to descend to RSC tension than to increase to an RSC tension stage.

In summary, there are at least 3 possible reasons for the difference in the ascending-descending RSC tension. 1) an irreversible loss of Ca^{++} from the tissue 2) damage to a portion of the muscle cells in 0 $[Ca^{++}]_o$ Tyrode and 3) a shift in internal Ca^{++} stores.

We believe that it may be the third possibility that is

occuring since a) we have minimized the time between equilibration and RSC tension measurement and b) the E.C. coupling response appears to be intact. Hence, ascending RSC tension has been utilized in determining the RSC tension/$[Ca^{++}]_o$ relationship.

A Mathematical Expression for Rested State Contraction Tension (RSC-tension) as a Function of External Calcium Concentration.

A mathematical expression to describe the RSC-tension/$[Ca^{++}]_o$ relationship has been found. RSC tension is expressed as the fraction of maximum obtainable tension i.e., the tension at the ceiling of contractility (COC).

Thus $$T_f = \frac{K[Ca]^n}{K[Ca]^n + 1} \quad \ldots\ldots 1$$

where T_f = fractional tension (RSC tension)

$[Ca]$ is the calcium concentration (mEq/L.)

$K = 1.8$

$n = 2$.

This expression provides the best fit to the experimental data (Figure V-1) by a simple arbitrary mathematical expression. Equation (1) is a modification of the Hill (1910) equation for the saturation of hemoglobin by oxygen and describes a sigmoid relationship. In our system, the values of n = 2 and K = 1.8 were obtained by assessing the fit of the theoretical curve to fit over the experimental values when n was varied from 2-4 and K was estimated at 1.8 by putting $[Ca]^n \times K = 1$. This gives the value of K at the point where T = ½ RSC tension, i.e.,

$T_f = 0.5$. Formula (1) gives a curve which fits directly over the broken line in Figure V-1 and hence can be considered as describing the experimental data accurately.

The Relationship between RSC tension and Potentiation

In an experiment described in section III, we showed maximum $PSP_{30''}$ as a function of $[Ca^{++}]_o$. Figure V-3, which is the same as Figure III-5 traces $PSP_{30''}$ potentiation maxima at each $[Ca^{++}]_o$. The RSC tension curve (solid circles) is similar to the curve in Figure V-1. However, the $PSP_{30''}$ curve (X's) indicates that the ceiling of contractility is reached at a lower $[Ca^{++}]_o$; at around 0.75 mM the $PSP_{30''}$ tension peaks and plateaus at a level with the tension ceiling for RSC tension at 2.5 mM $[Ca^{++}]_o$. (Table III-III pp. 66-67).

Twin-Pulse Stimulation and RSC tension

As a sequel to the above experiment, the RSC tension at each $[Ca^{++}]_o$ was determined and compared to the tension produced with twin-pulse stimulation; the second stimulus was triggered 200 msec. after the first. Figure V-4 indicates that the slope of the twin-pulse curve (open circles) is greater than the slope of the RSC tension curve (closed circles).

The paired pulse stimulation was only effective between 0.5 mEq/L. (0.25 mM.) and 5 mEq/L. (2.5 mM.) $[Ca^{++}]_o$ and resulted in an increase in tension in response to the second pulse compared to RSC tension.

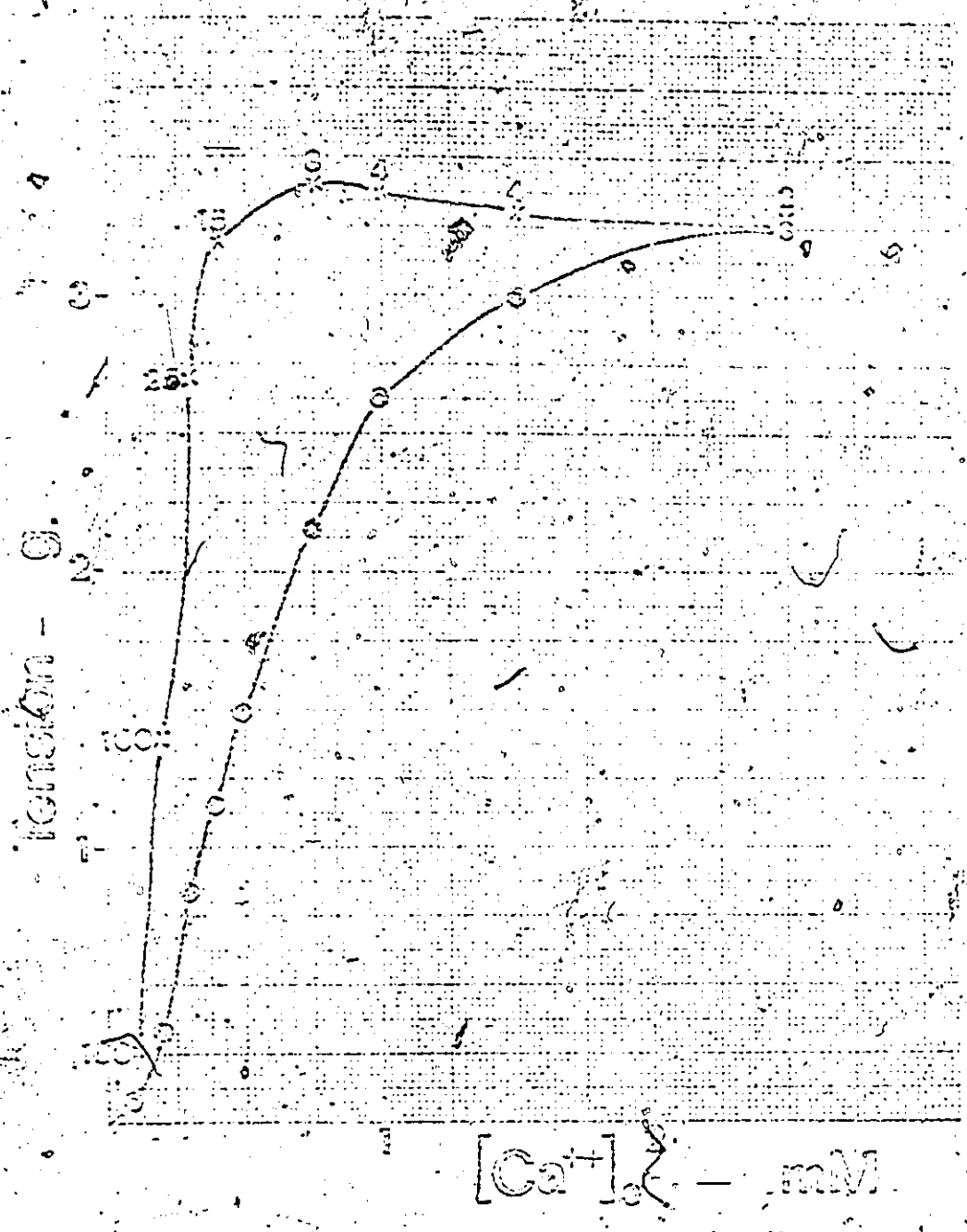
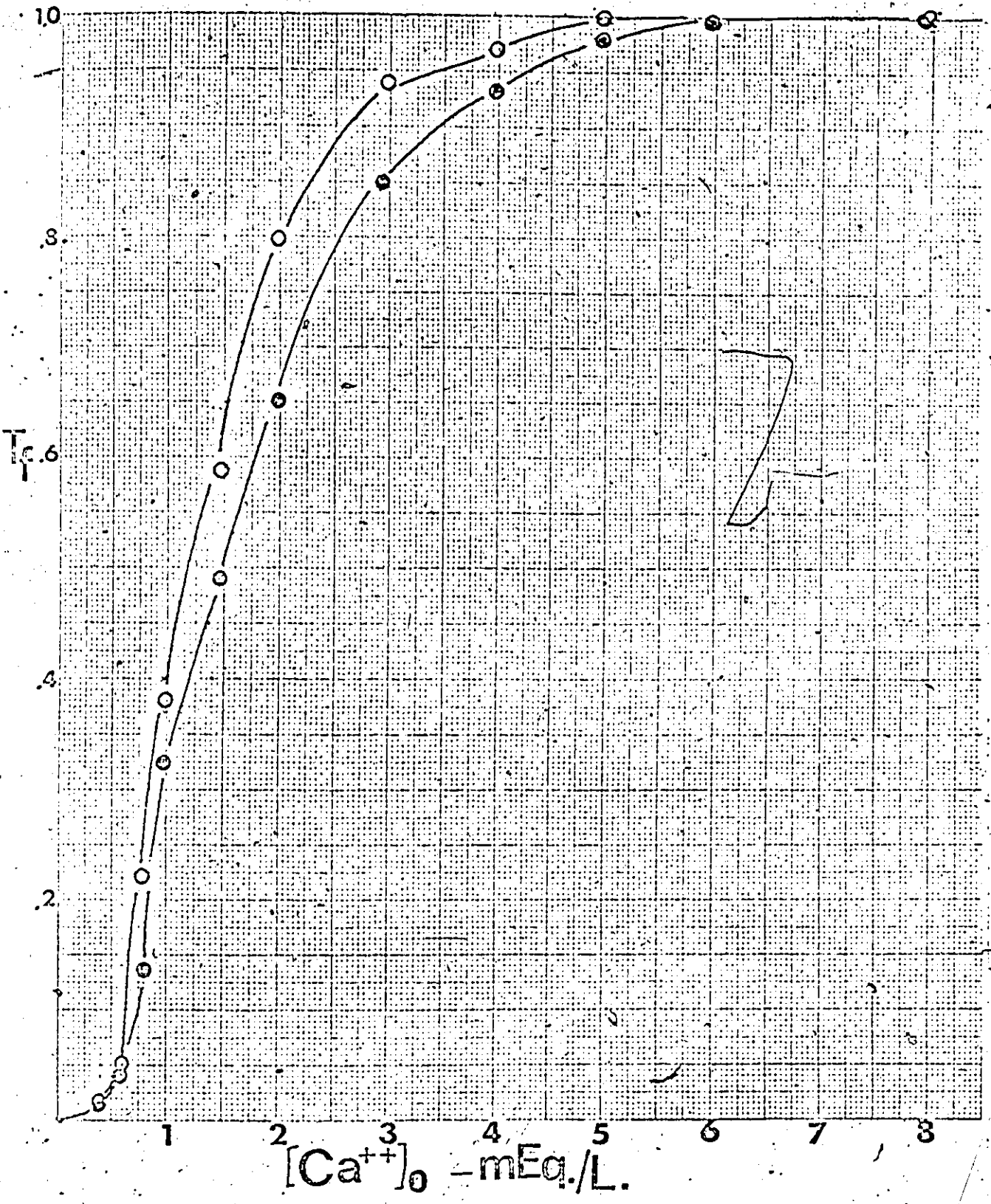


Figure V-3. Effect of $[Ca^{++}]_o$ on Maximum $PSP_{30''}$. (X) ($PSP_{30''}$) - Number of Conditioning Pulses @ 200 mSec. pulse interval. (○) - RSC tension.

Figure V-4. Steady State Tension. Effect of $[Ca^{++}]$ and Paired-Pulse Stimulation. (⊙) - Single Pulse Stimulation (2/minute). (0) - Paired - Pulse Stimulation (200msec.)


1.0
.8
.6
.4
.2
T_c
1
2
3
4
5
6
7
8
$[Ca^{++}]_0$ – mEq./L.

The results of these experiments may be summarized as follows:

a) few pulses are required at high $[Ca^{++}]_o$ to obtain maximum potentiation of tension.

b) for each $[Ca^{++}]_o$ level, there is an optimum number of conditioning pulses in order to achieve maximum potentiation. At higher $[Ca^{++}]_o$ a large number of conditioning pulses may result in no potentiation or a fall below RSC tension (Table III-III pp. 66-67).

c) above 0.4 mM $[Ca^{++}]_o$ the maximum obtainable tension (the so-called ceiling of contractility) may be approached by PSP; below 0.4 mM $[Ca^{++}]_o$, $PSP_{30"}$ does not reach this ceiling although conditioning pulses up to 100 stimuli (over 20 second period) were applied.

d) at the lowest $[Ca^{++}]_o$ used, 0.2 mEq./L (0.1 mM), the response of $PSP_{30"}$ tension to the number of applied conditioning stimuli was almost perfectly linear up to the maximum number of stimuli we applied (100 stimuli). When $[Ca^{++}]_o$ is increased to 0.4 mEq./L. (0.2 mM), the linear response in PSP was obtained only to 25 stimuli; an increase in the number of pulses produced no further potentiation, but did not reduce the potentiation. However, at $[Ca^{++}]_o$ greater than 0.4 mEq./L. (0.2mM), $PSP_{30"}$ was reduced with an increase in conditioning pulses. By 0.6 mEq./L. (0.3mM) $[Ca^{++}]_o$, $PSP_{30"}$ tension plateaued at 15-25 pulses, with an increase in conditioning pulses resulting in a fall of $PSP_{30"}$ tension.

Decay of RSC tension at $[Ca^{++}]_o = 0$

Washing out muscle calcium by maintaining bath $[Ca^{++}]_o$ at 0

afforded the opportunity to follow the decay of tension during this period. The decay of tension from muscles equilibrated at 2.5 mM $[Ca^{++}]_o$ and changed to 0 $[Ca^{++}]_o$ generally took close to 1½ hours. During this period the Tyrode solution was changed several times to maintain calcium content in the bath as close to 0 as possible.

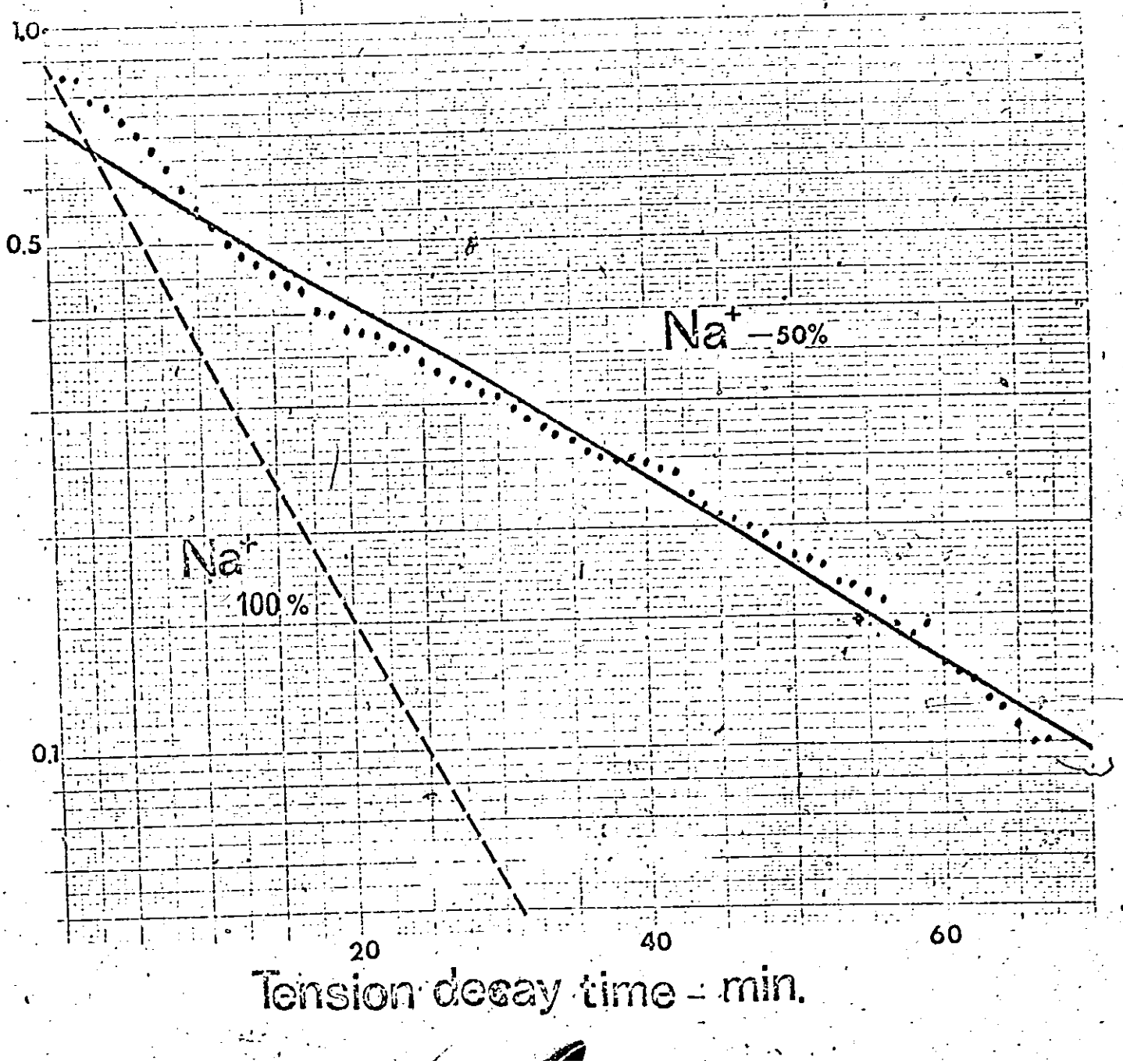
The change in tension was plotted on semilogarithmic graph paper. Initially, the curve was not linear, however after 5-6 minutes the tension decay was linear to the end of the measurement period (60 minutes - 90 minutes). The linear portion of this curve was analyzed by using a Wang 600 minicomputer program for fit of a single exponential. The results of this analysis indicated that the data fitted a curve of the format $Y = Ae^{-Bt}$; the goodness of fit was indicated with a correlation coefficient of 0.95 or greater for all of the washouts analyzed. The $t_{\frac{1}{2}}$ for 8 washouts was computed as 5.848 ± 0.803 minutes (S.E.M.). We neglected the initial decay of tension in estimating the $t_{\frac{1}{2}}$ value.

The $t_{\frac{1}{2}}$ of tension decay was also estimated in 50% $[Na^+]_o$ Tyrode. The $t_{\frac{1}{2}}$ was determined by the same procedure used at 100% $[Na^+]_o$. Figure V-5 shows the graph of an experiment in which both the experimental values (solid points) and the computer determined best fit line is indicated. The $t_{\frac{1}{2}}$ for tension decay in 50% $[Na^+]_o$ is estimated at about 24 minutes, a considerable increase in the time required to "wash-out" tension at 100% $[Na^+]_o$.

Muscle Calcium Content

The total calcium content was determined in individual

Figure V-5. Effect of $[Na^+]_o$ on Decay of Tension in 0 $[Ca^{++}]_o$ Tyr
Solution. Broken line $[Na^+]_o$ = 100%. Average $t_{\frac{1}{2}}$ =
minutes. (•) $[Na^+]_o$ = 50%; Solid line is best fit c
line. $t_{\frac{1}{2}}$ = 24 minutes. T_f is fraction of maximum d
tension when muscles were equilibrated at 2.5 mM $[Ca^+$


1.0
0.5
0.1
Na^+ — 50%
Na^+ 100 %
20
40
60
Tension decay time – min.

papillary muscles equilibrated at various $[Ca^{++}]_o$. Muscles were at first equilibrated at 2.5 mM-$[Ca^{++}]_o$, the length tension relationship adjusted to peak tension and the muscles were stimulated at 2/minute. The Tyrode solution was changed to 0 $[Ca^{++}]_o$ and maintained at that level by changing the solution frequently. When tension fell to less than 10 mg., the bath solution was changed once more to the Tyrode solution with the required $[Ca^{++}]_o$. After 45 minutes of equilibration, the muscle was removed from the isometric recording system by carefully cutting the muscle portion between the plastic clips holding the muscle. The total calcium content was determined according to the methods, outlined in Section II.

At the same time that the muscle sample was prepared, an aliquot of the bath Tyrode was taken for calcium concentration analysis. To determine a correction factor for extracellular space in our muscles a group of muscles at each $[Ca^{++}]_o$ were treated as above, but Sorbitol (H^3) (New England Nuclear) was added to the Tyrode 15 minutes after equilibration had begun. Previous experimentation had shown that 20 minutes were required to reach an equilibrium between applied H^3-Sorbitol and rat papillary muscle Sorbitol under similar conditions. The H^3 content of these muscles was determined by collecting the H^3 from the muscles with a Packard Sample Oxidizer. This recovered the H^3 in the muscle and allowed H^3 determination in a liquid Scintillation Counter (Nuclear Chicago - Mark 1); 2-channel discrimination and external standard were used to correct for quenching.

The extracellular space determined in our samples was 43 ± 3 % (± SEM) over the entire sampling. No significant difference between the samples was determined with the muscles at different $[Ca^{++}]_o$ (16 samples).

The results of the experiments to determine calcium content are shown in Table V-1, and illustrated in Figure V-6. It appears as if total calcium content does not increase above 2.0 mM $[Ca^{++}]_o$ and plateaus at 5.25 mM/Kg. muscle weight. At the lowest end of the curve, the muscle calcium content remains constant at about 1.7 mM/kg. muscle weight even when the $[Ca^{++}]_o$ is reduced close to 0 (0.0477 mM). This value of $[Ca^{++}]_o$ represents the zero $[Ca^{++}]_o$ we have used in our experiments and the calcium content from contamination of glassware, chemicals and handling. The calcium content of the muscle rises swiftly from 1.7 mM/kg. muscle weight to 5.25 mM/kg. muscle weight between $[Ca^{++}]_o$ 0.8 mM and 2 mM. We can compare muscle calcium content with RSC tension at various $[Ca^{++}]_o$ by expressing muscle calcium (Ca_m) as a fraction of the saturated value (Ca_f). Figure V-7 shows Ca_f (broken line) and T_f (solid curve) as a function of $[Ca^{++}]_o$.

Below 0.25 mM $[Ca^{++}]_o$, the RSC tension bears little relation to Ca_m. Above 0.25 mM, however, both Ca_m and RSC tension increase rapidly. RSC tension increases at a lower $[Ca^{++}]_o$ than does Ca_m; the plateau for both Ca_m and RSC tension is at 2.0 mM $[Ca^{++}]_o$.

Discussion

We have found that RSC tension is not linear with $[Ca^{++}]_o$ over

TABLE V-I

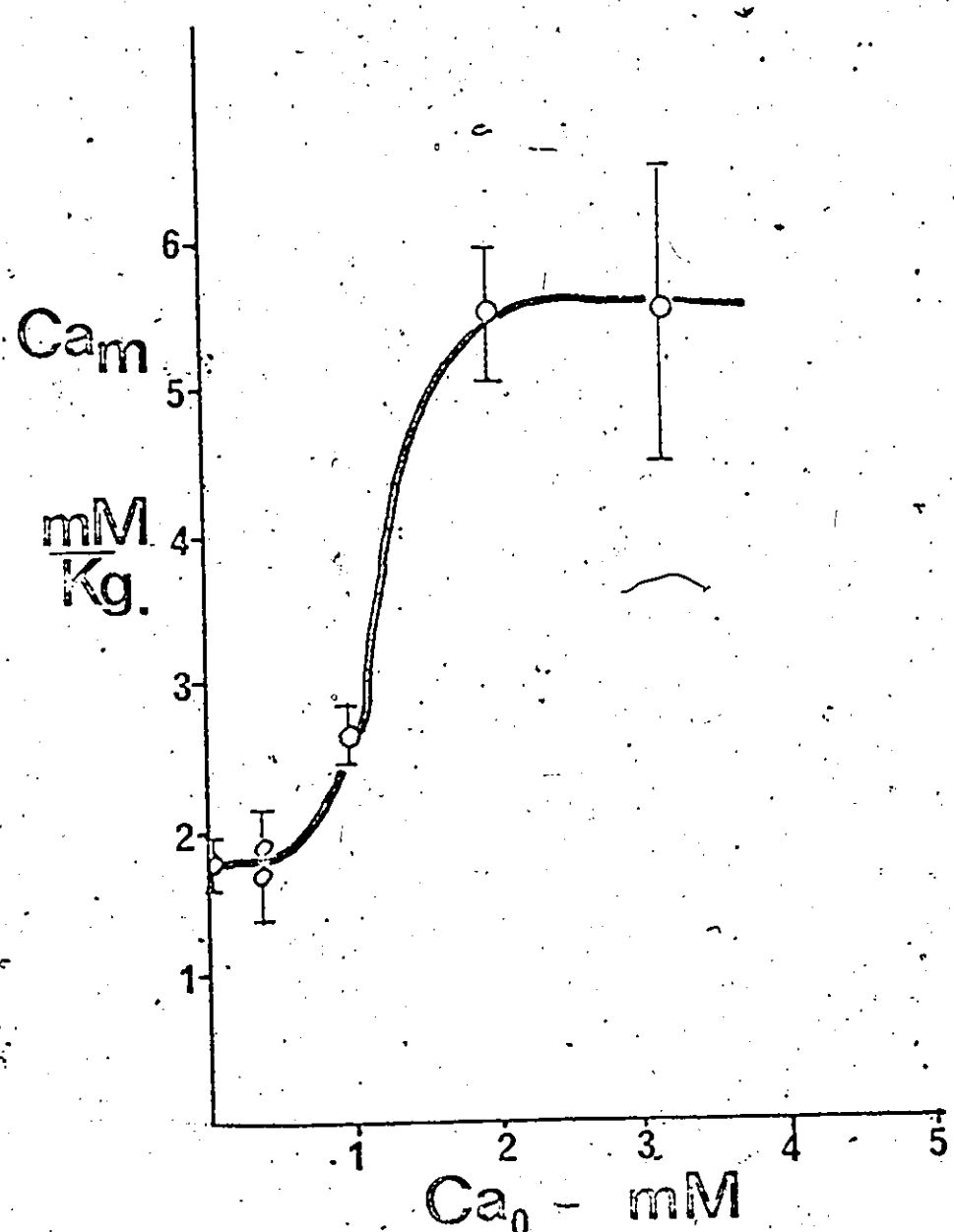
Effect of $[Ca^{++}]_o$ on Calcium Content in Papillary Muscle of the Rat.

$[Ca^{++}]_o$ mM	$[Ca]_m$* mM	Ca_f
.0477	1.773 ± 0.167 (6)	.321
.3813	1.611 ± 0.181 (6)	.292
.4013	1.877 ± .317 (6)	.340
.98	2.683 ± .205 (4)	.50
1.974	5.525** ± .504 (8)	1.0
3.19	5.524 ± 1.028 (4)	1.0

* Total Calcium in Muscle based upon Sorbitol -H^3 space of 43%. Mean ± S.E.M. Numbers in Brackets = number of muscles used in determination. Ca_F is Fractional Calcium Content based upon Maximum Calcium Content in Muscle.**

Muscle calcium is calculated on a weight basis i.e., mM/Kg. and not on tissue water. Bath calcium is determined on volume basis (mM/L.).

Figure V- 6 Total Calcium Content in the Isolated Papillary M
of the Rat. Effect of $[Ca^{++}]_o$. $[Ca]_m \pm$ S.E.M.


Cam
mM/Kg.
6
5
4
3
2
1
1
2
3
4
5
Ca0 - mM

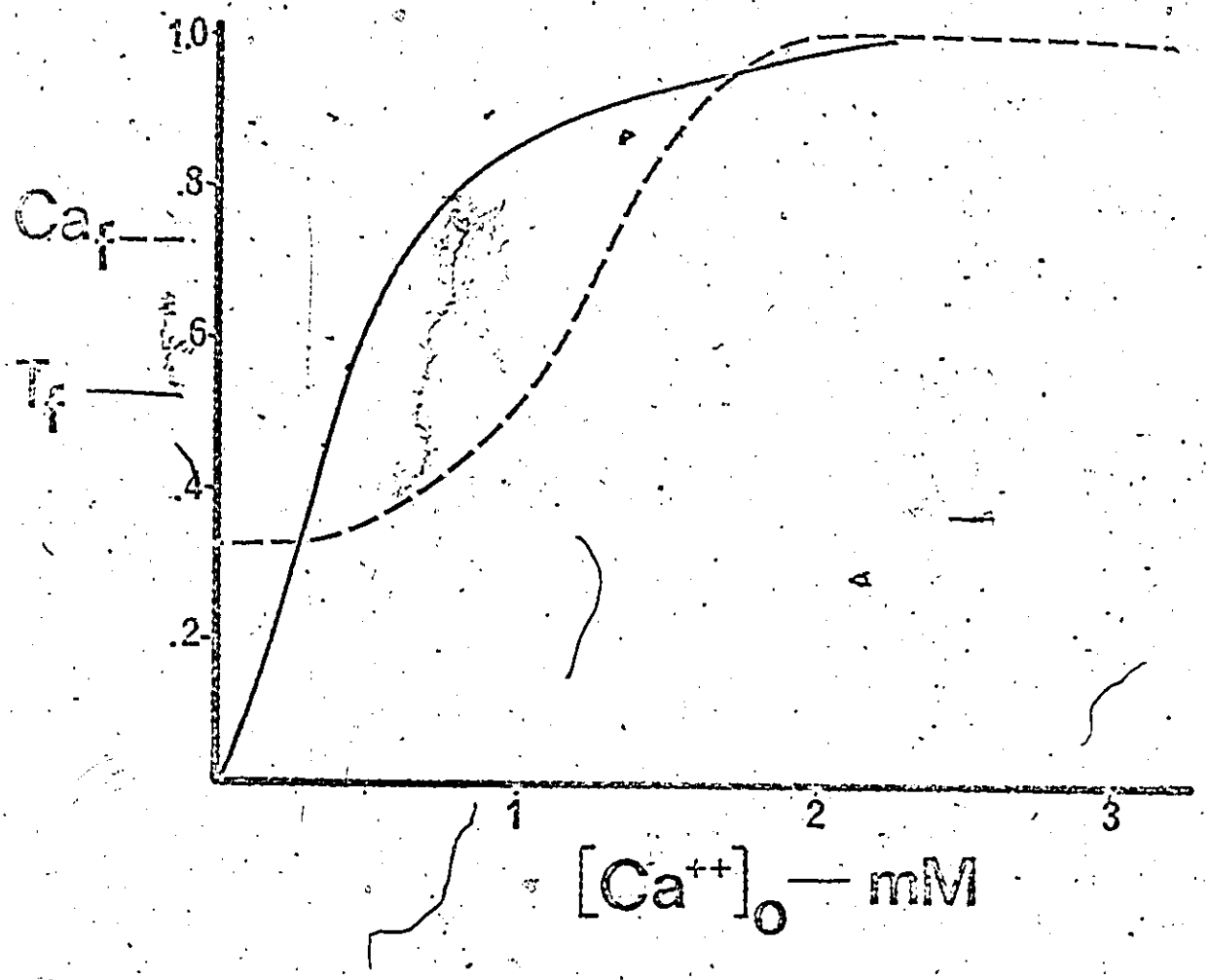


Figure V-7. Effect of $[Ca^{++}]_o$ on Papillary Muscle Tension and Calcium Content. T_f - Fractional Maximum Tension. Ca_f - fractional maximum calcium content.

the range 0 to 2.5 mM. The S-shape curve is typical of the type of response expected in a co-operative system described in biochemical textbooks where the addition of substrate facilitates and potentiates further substrate reaction. While there may be several mathematical expressions to describe this relationship, a similar sigmoid shaped curve is described by the dissociation curve of hemoglobin for oxygen. Hill (1910) describes this relationship mathematically in the form used in equation (1) as:

$$Yp = \frac{Kx^r}{Kx^r \; 1} \qquad \ldots\ldots 2$$

where Yp = fractional saturation

$K = [x]_{0.5}$ and is the value of x at Y = 0.5

While a physical interpretation for this formula is available for hemoglobin, it is speculative to apply a similar interpretation to the significance of this formula in our system beyond the fact that it can describe RSC tension at each $[Ca^{++}]_o$. For haemoglobin in equation 2, an r value of 4 was found appropriate (Hill, 1910). We now know that this value corresponds to the subunit structure of haemoglobin. Since we found a value of r = 2 in our formula fitted the experimental points we can speculate that if an analogy can be drawn between haemoglobin and our system, that a subunit structure of 2 sites is involved in the regulation of the $Ca_o - T_f$ relationship. This is interesting since a relationship of 2 Ca^{++}/troponin activation has been suggested (Ebashi and Endo, 1968; Ebashi et al., 1969) and points to the non-linearity

residing in the contractile proteins. There is as well, the evidence of Hasselbach (1966) and Hellam and Podolsky (1969) who showed that the ATPase and tension development by myofibrils at various calcium concentrations follows an S-shaped response between 10^{-5} and 10^{-8} M. Ca^{++} on a logarithmic scale.

However we must again point out that an ultrastructural significance to this equation must remain only speculative since we cannot at this point establish where the non-linearity in the relationship between $[Ca^{++}]_o$ and tension may exist.

The relationship between Ca_m and T_f indicates that RSC tension can follow an increase in muscle calcium content only in the narrow $[Ca^{++}]_o$ range of 1.25-1.5mM. At low $[Ca^{++}]_o$ particularly below 0.25 mM, RSC tension appears to be completely independent of the calcium content in the muscle. This calcium would appear to be in a very strongly bound form since it remains constant over the range of 0-0.25 mM $[Ca^{++}]_o$ and represents about 1.7 mM/kg. of muscle weight. If this amount remains constant over the $[Ca^{++}]_o$ range tested, then it would correspond to a bound fraction of 30% of the total muscle calcium when the muscle was saturated with calcium. Langer (1968) estimated that his phase 4 calcium thought to be tightly bound intracellular calcium with a $t_{\frac{1}{2}}$ of exchange of 170 minutes, represented about 20% of the tissue calcium. At the highest $[Ca^{++}]_o$'s, 2.5-5.0 mM, both Ca_m and T_f reached a plateau suggesting that internal calcium stores control maximum attainable

tension (the ceiling of contractility). In rabbit myocardium, cellular calcium was related to $[Ca^{++}]_o$ only up to 3 - 4 mM, although interstitial calcium was linear with $[Ca^{++}]_o$ from 0.5 to 12 mM (Shine, Serena and Langer, 1971). This study also indicated a limitation in the calcium influx above 4 - 5 mM $[Ca^{++}]_o$ which suggests a saturation of calcium receptor or transport sites in or on the cell membrane (Langer, 1973). If such a mechanism were present in rat papillary muscle it would be saturated at a $[Ca^{++}]_o$ close to 2 mM. Half saturation of Ca_m appears around 1.3 mM $[Ca^{++}]_o$ while half RSC tension occurs at 0.4 mM. It should be recalled that these muscle calcium determinations were obtained in electrically-stimulated beating papillary muscles. Grossman and Furchgott (1964) found that the calcium content of Guinea-Pig auricles was practically linear between 0.5 - 5 mM $[Ca^{++}]_o$, but was increased by electrically stimulating the muscle. However, the difference between rested muscle calcium and beating muscle calcium corresponded exactly to the relationship between contractile tension and $[Ca^{++}]_o$. In the rat papillary muscle, beating at intervals of 2/minute, a basal calcium content of 1.7 mmoles/Kg. muscle weight is found at zero $[Ca^{++}]_o$ compared to 0.4 to 0.9 mmoles/Kg. Ca^{++} in guinea-pig (Grossman and Furchgott, 1964; Winegrad and Shanes, 1962) and 0.3-0.7 mmoles/Kg. in frog sartorius muscle (Gilbert and Fenn, 1957; Shanes and Bianchi, 1959). The increased basal calcium level in the rat cannot directly be attributed to the activity since this value remained constant between 0 and 0.25 mM.

A word should be said at this point about the measurement of

Ca_m. We have found that the extracellular space is about 43% of the tissue weight. This value appears to be rather high since extracellular space (ECS) measurements in heart tissue has been reported to be about 20 - 30% of the tissue water. There are two main reasons for the discrepancy in our results: a) the 43% ECS represents true ECS + the surface film which was not removed. b) our muscle samples all were less than 10 mg. and we therefore introduced an error in weight. Since we were not really interested in a true ECS value, but rather an indication of how much extracellular fluid was contained in our samples, we have accepted the 43% value as a correction factor for muscle calcium determination.

The tension decay experiments in 0 $[Ca^{++}]_o$, indicated that the $t_{\frac{1}{2}}$ of tension decay was 5.85 minutes. Langer's phase 2 calcium which he defined as contractile calcium had a $t_{\frac{1}{2}}$ of exchange of 6.0 minutes (Langer, 1968). We would also have expected the $t_{\frac{1}{2}}$ to increase in 50% $[Na^+]_o$ since as Langer (1968, 1970) suggests, the Na^+/Ca^{++} ratio would determine Ca^{++} exchange and a decrease in the $[Na^+]_o$ would tend to retain intracellular or cellular bound Ca^{++}. In fact we have observed a $t_{\frac{1}{2}}$ of tension decay of 24 minutes in 50% $[Na^+]_o$ Tyrode.

Summary

1) The relationship between $[Ca^{++}]_o$ and RSC tension is not linear. Maximum tension is reached close to 2.0 mM $[Ca^{++}]_o$. The sigmoid shaped curve can be expressed mathematically by a modification

of the Hill (1910) equation:

$$T_f = \frac{1.8\,[Ca]_o^2}{1.8\,[Ca]_o^2 + 1}$$

2) The relationship between $[Ca^{++}]_o$ and Ca_m is also sigmoid shaped. Below 0.5 mM $[Ca^{++}]_o$, Ca_m remains constant at about 1.7 μmoles/Kg Above 2.0 mM $[Ca^{++}]_o$ Ca_m plateaus at 5.25 mM/Kg. muscle weight. Between 0.5 mM and 2.0 mM $[Ca^{++}]_o$, Ca_m rises practically parallel to the increase in RSC tension.

3) The potentiated tension curve ($PSP_{30"}$ and twin pulse stimulation) shifts the point at which maximum tension is reached to left on the RSC tension/ $[Ca^{++}]_o$ relationship. With $PSP_{30"}$, the highest point at which the plateau is reached is 0.4 mM $[Ca^{++}]_o$.

4) Tension decay in 0 $[Ca^{++}]_o$ Tyrode indicates a $t_{\frac{1}{2}}$ of 5.85 minutes while the $t_{\frac{1}{2}}$ in 0 $[Ca^{++}]_o$, 50% $[Na^+]_o$ Tyrode was 24 minutes and indicates that a decrease in $[Na^+]_o$ tends to maintain $[Ca^{++}]_i$ at a higher level.

B. Tension Recovery Between Rested State Contractions

Introduction

A single stimulus applied to the isolated papillary muscle preparation between rested state contractions always resulted in a contraction producing less tension than the rested state contraction tension. We attributed this to the interval required to fill a tension-determining pool from a stored calcium pool according to our model (Figure IV-23); this corresponds to the recharge of Ca_r from Ca_s. If

we apply stimuli at measured intervals between rested state contractions we should be able to analyze this process. This assumes that the tension developed with each contraction is proportional to the releasable Ca^{++} in Ca_r at the time of stimulation. This analysis should answer the following questions:

a) what relationship does tension have to RSC tension between rested state contractions?

b) does $[Ca^{++}]_o$ affect this relationship?

c) can a knowledge of this relationship account for the interval-strength (T-F) relationship of rat myocardial tissue?

Analysis of Tension Recovery Between Rested State Contractions

The recovery of contractility between the rested state contraction interval was assessed by determining the ratio of the tension produced between the rested state interval (t) to the RSC tension (Figure V-8) which is equal to the fractional tension recovery, T_f. The T_f was obtained at intervals of 0.2 to 30 seconds following an RSC. A plot of log $(1 - T_f)$ against the time of the second stimulus was used to analyze the results. The first experiments were performed at $[Ca^{++}]_o$ = 2.5 mM. When this was analyzed on semi-logarithmic paper, the plot appeared linear suggesting a single exponential. This data was therefore analyzed using a Wang 600 minicomputer program for single exponential fit. This analysis indicated an excellent fit ($r > 0.92$) and the data from further experiments were analyzed in the same manner. However, when $[Ca^{++}]_o$ was reduced below 2.5 mM, it was observed that a

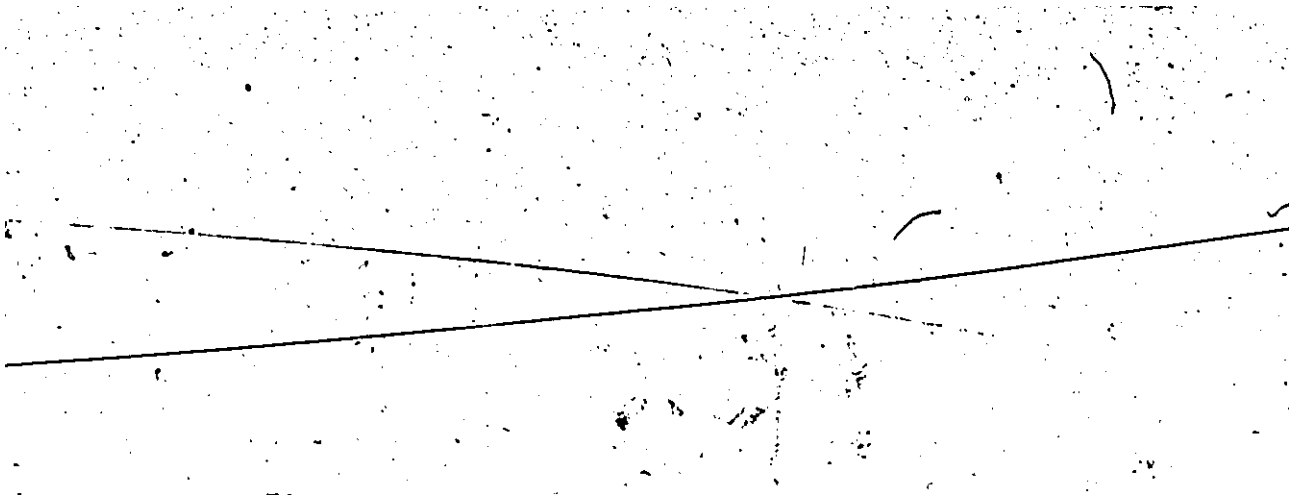
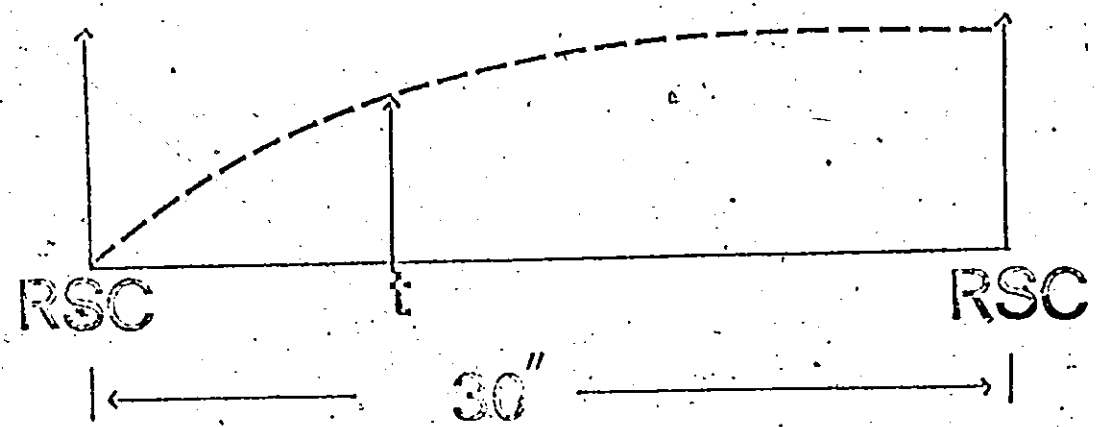


Figure V-8. Diagram of Tension Recovery between Rested State Contractions.

Tension Recovery



$$T_t = \frac{t}{RSC} \text{ Tension}$$

single exponential expression could not adequately describe the recovery of contractility between rested state contractions. At this point, all of the data was analyzed with the Wang 600 minicomputer with a program to resolve a double exponential. This program utilized an iterative technique to reduce the error function in fitting a double exponential equation to the experimental data (Cowan et al., 1973; Heteryi et al., 1973). The printout from this program provided a) the rate constants (and calculated $t_{\frac{1}{2}}$'s), b) the sum of the area's to infinity of the two rate constants (i.e., both exponentials) and c) the proportion of the area contributed by each of two exponentials (i.e., the exponential coefficients).

Results

Figure V-9 shows the effect of $[Ca^{++}]_o$ on recovery of contractility in rat papillary muscle. For the sake of clarity and resolution of the curves, the recovery, T_f, is shown on the ordinate as $(1 - T_f)$ against the recovery time in seconds. At 2.5 mM $[Ca^{++}]_o$ the recovery rate appears much faster (curve C) than the rate at 1.0 mM $[Ca^{++}]_o$ (curve B) and 0.25 mM (curve A). At 2.5 mM $[Ca^{++}]_o$, the curve appears almost linear. Analysis of the recovery by the single exponential computer program indicated that the $t_{\frac{1}{2}}$ of tension recovery was 1.29 ± .316 seconds (Table V-II) while double exponential analysis showed that the curve could best be resolved with two rate constants with $t_{\frac{1}{2}}$'s of .518 ± .059 seconds and 2.95 ± .577 seconds (Table V-III-A). At low $[Ca^{++}]_o$, (0.25 mM), single exponential analysis of tension

Figure V-9. Recovery of Contractility between Rested State Contractions in Isolated Papillary Muscle of the Rat. Effect of $[Ca^{++}]_o$.

A (□) - $[Ca^{++}]_o$ = 0.25mM; B (X) - $[Ca^{++}]_o$ = 1.0mM;

C (○) - $[Ca^{++}]_o$ = 2.5mM.

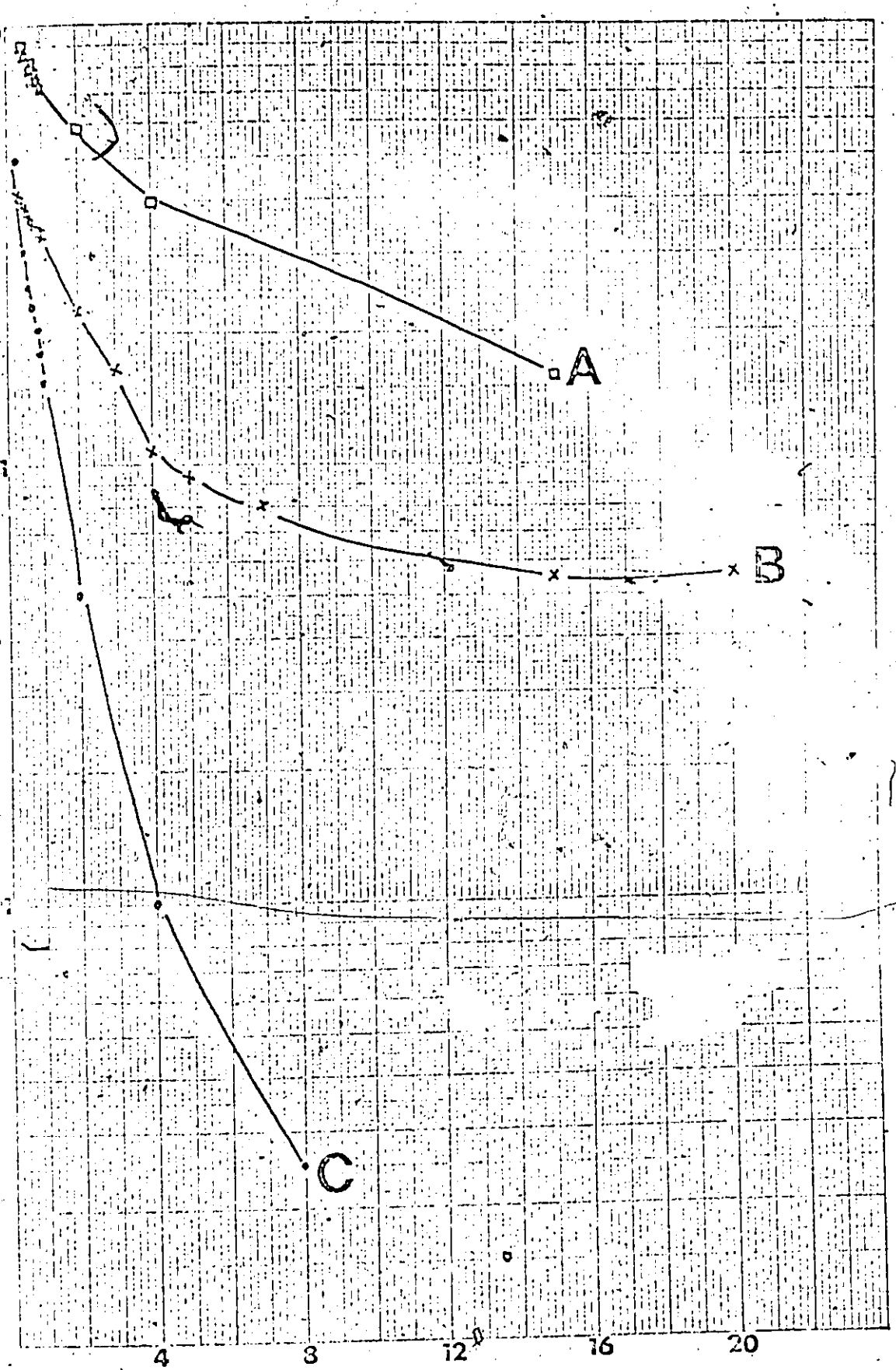

1.0
0.1
A
B
C
4
8
12
16
20
Recovery - sec.

TABLE V-II

RSC Tension Recovery in Rat Papillary Muscle.

Effect of Temperature and $[Ca^{++}]_o$ on $t_{\frac{1}{2}}$ of Recovery.

(Single Exponential Analysis).

$[Ca^{++}]_o$ mM	16°C	26°C	36°C
	$t_{\frac{1}{2}}$ (sec.)		
5	.207 ± .115	.344 ± .060	.132 ± .023
2.5	.207 ± .079	1.29 ± .316	2.06 ± .653
1.0	.663 ± .132	3.11 ± .597	1.55 ± .624
0.25		6.63 ± .983	

TABLE V-III

Double Exponential Analysis of Recovery of Contractility

(A) Effect of $[Ca^{++}]_o$

$[Ca^{++}]_o$ mM	$t_{\frac{1}{2}}$ -1 sec.	$t_{\frac{1}{2}}$ -2 sec.	Area	A_1	A_2
5.0	.078	.446	75	.72	.28
2.5	.518 ± .059	2.95 ± .577	90 ± 7.8	.421	.58
1.0	.704 ± .113	8.80 ± 1.87	298.8 ± 18.9	.19	.81
0.25	.456 ± .055	10.12 ± 4.39	536 ± 117	.077	.923

(B) Effect of Temperature

$[Ca^{++}]_o$ mM	T(°C.)	$t_{\frac{1}{2}}$ -1 sec.	$t_{\frac{1}{2}}$ -2 sec.	Area	A_1	A_2
2.5	16	.499	.849	46	.44	.56
	26	.518	2.95	90	.42	.58
	36	.242	3.40	98	.42	.58
1.0	16	.801	.969	166	.44	.56
	26	.704	8.80	298	.19	.81
	36	.30	5.89	266	.13	.87
0.25	16	.489	1.2	178	.33	.67
	26	.456	10.12	5[illegible]	.077	.923
	36	.525	10.8	583	.08	.920

(cont'd)

TABLE V-III (cont'd)

(C) Effect of Ouabain (0) (10^{-6}M.).

$[Ca^{++}]_o$ mM	Treatment	$t_{\frac{1}{2}}$ -1 sec.	$t_{\frac{1}{2}}$ -2 sec.	Area	A_1	A_2
2.5	-	.518	2.95	90	.42	.58
2.5	0	.157	.354	30	.30	.70
2.0	0	.251	2.74	59	.39	.61
1.0	-	.704	8.80	298	.19	.81
1.0	0	.461	2.73	156	.23	.77
0.25	-	.456	10.12	536	.077	.923
0.25	0	.574	6.22	520	.11	.89

(D) Effect of $[Na^+]_o$ (%)

$[Ca^{++}]_o$ mM	Treatment $[Na^+]_o$ -%	$t_{\frac{1}{2}}$ -1 sec.	$t_{\frac{1}{2}}$ -2 sec.	Area	A_1	A_2
2.5	100	.518	2.95	90	.42	.58
2.5	50	.54	.412	52	.14	.86
2.5	10	.018	.017		.50	.50
1.0	100	.704	8.80	299	.19	.81
1.0	50	.771	5.69	220	.25	.75
0.25	100	.456	10.12	536	.077	.923
0.25	10	.013	.55	80	.09	.91
0.25	10 0-(10^{-6}M.)	.075	.44	75	.17	.83

recovery indicates a $t_{\frac{1}{2}}$ of 6.63 ± .903 seconds while the double exponential analysis indicated that $t_{\frac{1}{2}}$'s of .456 ± .055 and 10.12 ± 4.39 seconds would fit the experimental points. The graphical data indicate that only at high $[Ca^{++}]_o$ can a near linear relationship be observed in the recovery data. Therefore, the double exponential values are to be considered as more descriptive of the tension recovery. Increasing the $[Ca^{++}]_o$ from 0.25 to 2.5 mM mainly affects the second (slower) rate constant and little change is seen in the first (faster) rate constant. The area to infinity under the curves also shows that the overall recovery is faster at higher $[Ca^{++}]_o$. A most interesting aspect is the calculation that the coefficients corresponding to the proportional contribution of the fast and slow recovery phases changes with $[Ca^{++}]_o$.

Thus we may summarize Table V-III-A in the following manner. The recovery of contractility can best be described by a double exponential, i.e., a function having two rate constants. The faster rate constant appears to be fairly unaffected by $[Ca^{++}]_o$, however the slower rate constant (K_2) is increased with an increase of $[Ca^{++}]_o$ from 0.25 to 2.5 mM. The overall recovery time is decreased by increasing $[Ca^{++}]_o$, as indicated by the calculated area to infinity and the relative contribution of each component is altered by $[Ca^{++}]_o$ - an increase $[Ca^{++}]_o$ from 0.25 to 2.5 mM changes the proportional contribution of the fast component from 7.69% to 42% respectively.

Although the single exponential analysis cannot adequately describe the recovery function, the computer analysis indicated that

a single exponential could be fitted to the experimental data with a correlation coefficient 0.90. When the $t_{\frac{1}{2}}$ data from the single exponential data are plotted against $[Ca^{++}]_o$ (Figure V-10) we obtain a curve with a linear portion between 0.5 mEq/L (0.25 mM) and 5 mEq/L. (2.5 mM) $[Ca^{++}]_o$. Linearity was verified by linear regression analysis giving the line depicted in this figure. Extrapolation of the linear portion to 0 recovery time indicates a $[Ca^{++}]_o$ of 5.6 mEq/L. (2.8 mM) very close to the value we obtain for maximum RSC tension. Extrapolation to zero $[Ca^{++}]_o$ indicates a $t_{\frac{1}{2}}$ maximum at around 8 seconds. This graph is obviously a distortion since the single exponential analysis averages both the fast and slow phases of recovery and would underestimate the actual $t_{\frac{1}{2}}$. Figure V-11 shows the $t_{\frac{1}{2}}$ of recovery of the slow phase (open circles) and fast phase (closed circles) as a function of $[Ca^{++}]_o$. The $t_{\frac{1}{2}}$ is linear between 0.25 and 3.75 mM and flattens out at higher $[Ca^{++}]_o$. The fast phase (solid circles) shows little change over the range of $[Ca^{++}]_o$ tested.

Effect of Temperature on the Recovery of Contractility Between Tested State Contractions.

Table V-III-B shows the effect of a change in bath temperature on the recovery of contractility. When recovery $(1 - T_f)$ is plotted against time on semilogarithmic paper (Figure V-12) we see that increasing the temperature to 36°C decreases the recovery time by shifting the curve to the right while decreasing the temperature shifts the curve to the left and accelerates the recovery of contractility. This is

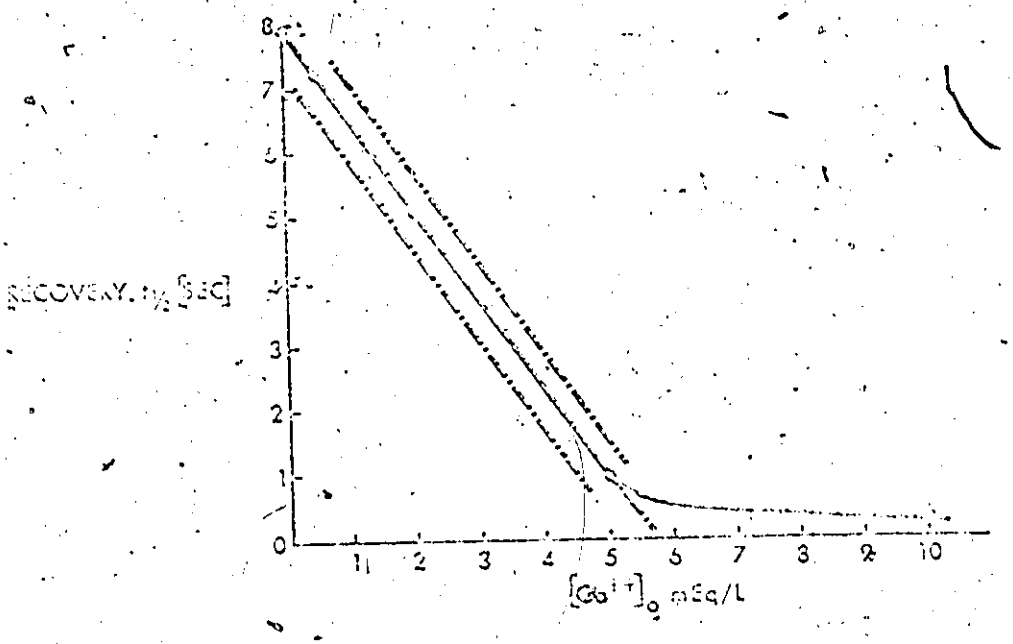


Figure V-10. $t_{\frac{1}{2}}$ of RSC tension Recovery in Isolated Papillary Muscle of the Rat. (Single Exponential Analysis). Effect of $[Ca^{++}]_o$. Broken Line Indicates Standard Error of Estimate of Linearity.

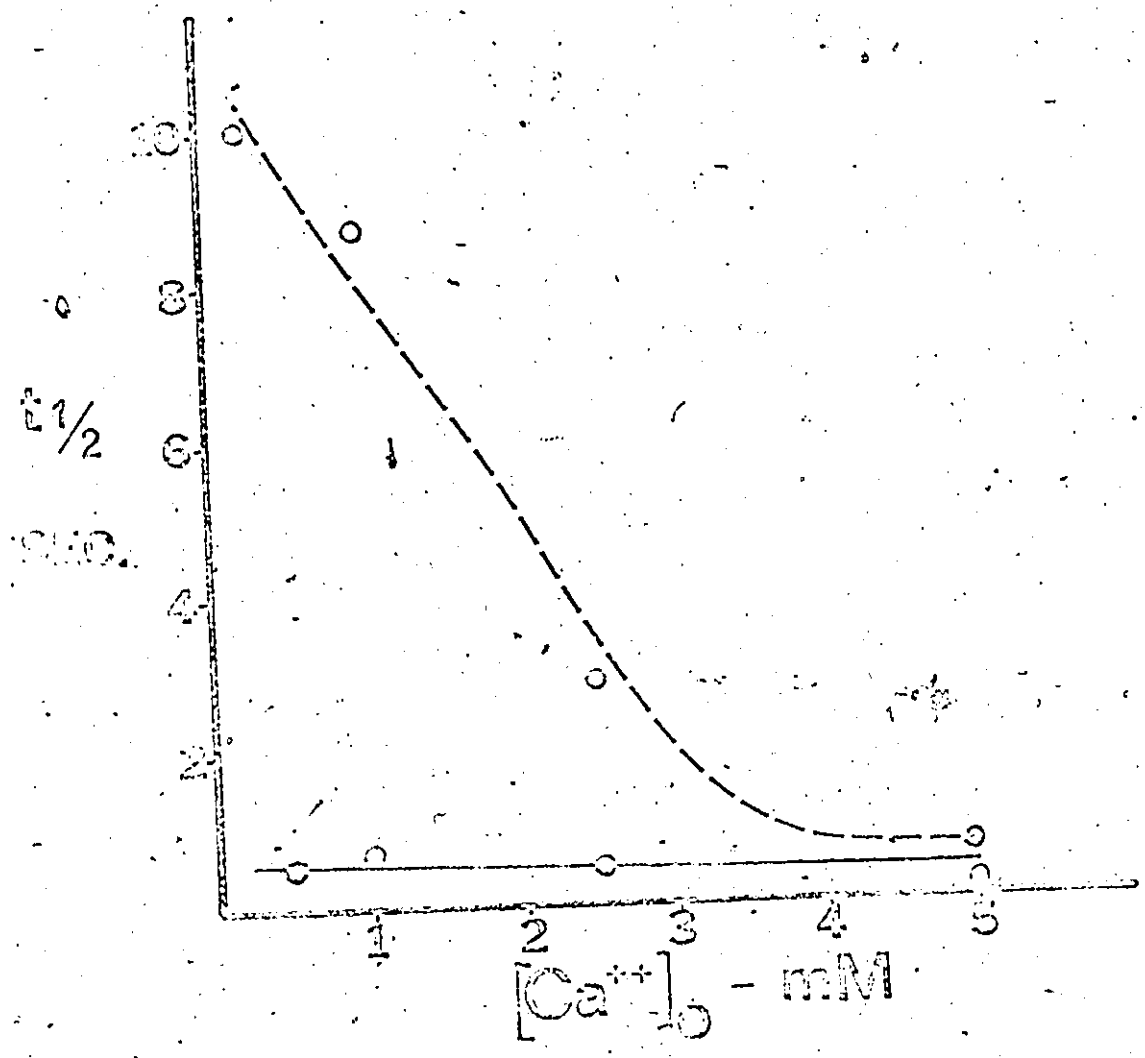
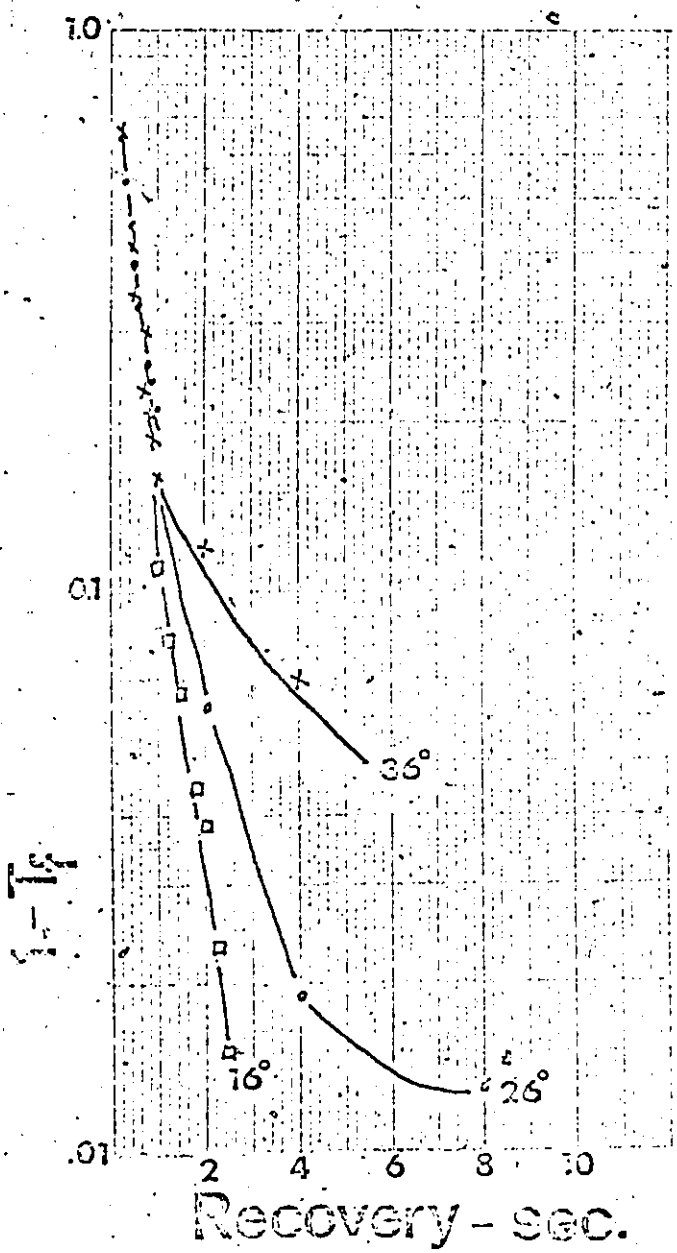


Figure V-11. Effect of $[Ca^{++}]_o$ on $t_{\frac{1}{2}}$ of recovery of RSC tension (0) $t_{\frac{1}{2}}$ -2; (•) $t_{\frac{1}{2}}$-1.

Figure V-12. Recovery of Contractility between Rested State Contractions in the Isolated Papillary Muscle of the Rat. Effect of Temperature. $[Ca^{++}]_o$ = 2.5 mM. (X) - 36°C; (O) - 26°C; (□) - 16°C.



confirmed by the double exponential analysis (Table V-III-B) and single exponential analysis of the data (Table V-II). Temperature has little effect on the fast rate of recovery but the $t_{\frac{1}{2}}$ of the slower phase is increased with an increase in temperature and decreased with a decrease in temperature.

There is as well no change in proportionality of contribution to the recovery by the two rates although there is an overall decrease in the recovery time by 50% by decreasing the temperature to 16°C from 26°C; computed area to infinity decreases from 90 to 46.

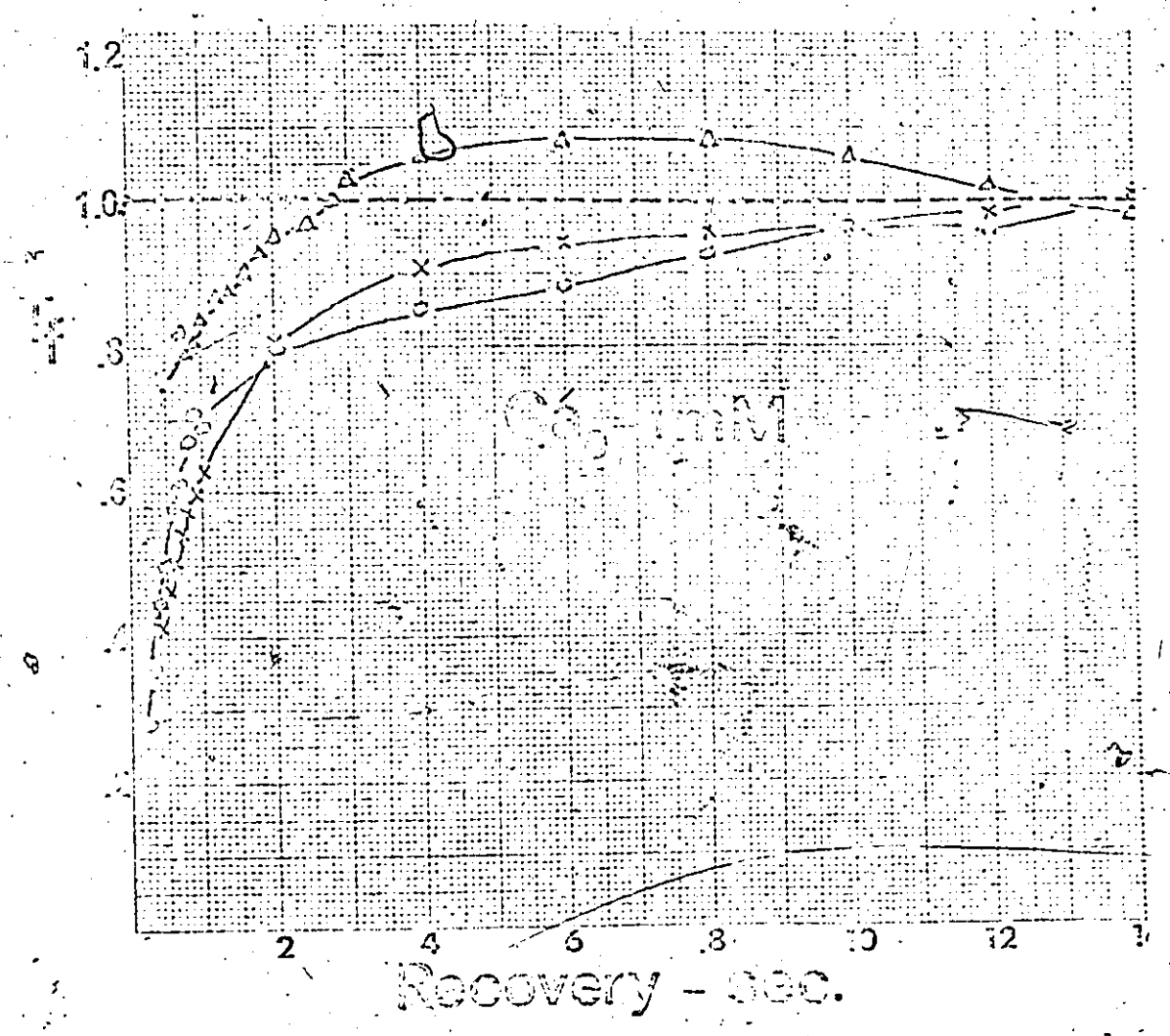
An experiment in which recovery of tension between RSC's was analyzed is shown in Figures V-13 and V-14. Table V-III-B shows the results of double exponential analysis of experiments at 1.0 mM $[Ca^{++}]_o$. Again it appears as if the fast phase of recovery is not significantly altered by an increase or decrease in the bath temperature. However, when the temperature is decreased from 26°C to 16°C, the slow phase of recovery is accelerated. This change in the rate constant is accompanied by a change in proportionality of the rate constants; the proportion of the fast phase contributing to tension recovery is increased from 19% to 44%.

A similar effect of temperature is seen at $[Ca^{++}]_o$ = 0.25 mM.

The effect of temperature on the recovery of contractility between tested state contractions may be summarized as follows:

a) temperature has no significant effect on the fast phase of tension recovery.

Figure V-13. Recovery of Contractility between Rested State Contractions in the Isolated Papillary of the Rat. Effect of Temperature. $[Ca^{++}]_o$ = 1.0mM. (○) - 26°C; (X) - 36°C; (Δ) - 16°C.



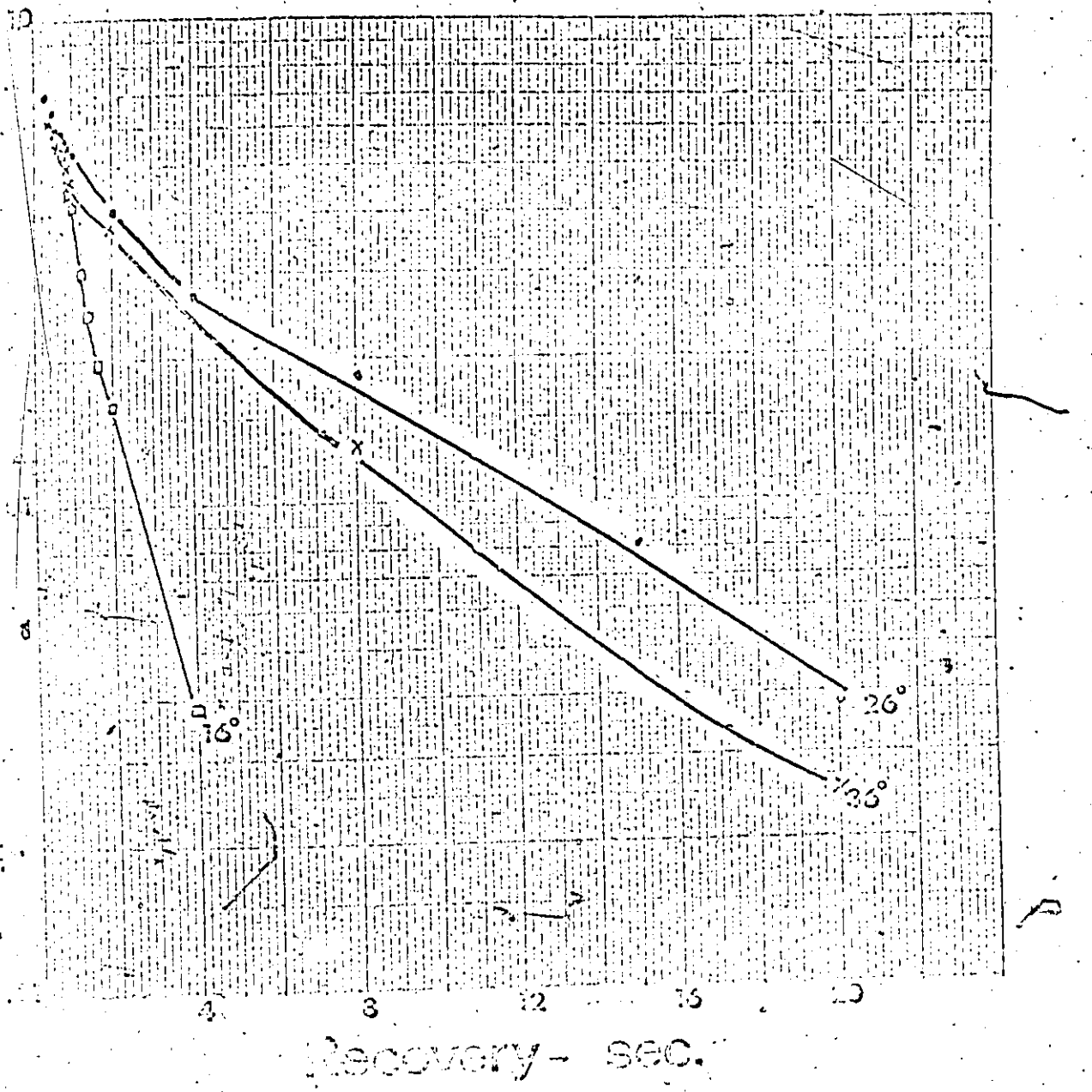


Figure V-14. Recovery of Contractility in the Isolated Papillary Muscle of the Rat. Effect of Temperature $[Ca^{++}]_o$ = 1.0mM. (□) - 16°C; (X) - 26°C; (○) - 36°C.

"b) at 2.5 mM $[Ca^{++}]_o$, a decrease in temperature results in an overall decrease in the recovery time, but both phases of tension recovery do not change with temperature.

c) at 1.0 mM $[Ca^{++}]_o$ a decrease in temperature results in a decrease in the recovery time with the fast phase assuming more of a role as the temperature is decreased. A similar effect is observed at 0.25 mM $[Ca^{++}]_o$.

d) at 16°C, with $[Ca^{++}]_o$ = 2.5, 1.0 and 0.25 mM, the slow phase of tension recovery tends to approach the value for the fast phase rate constant, i.e., they approach a single exponential.

e) the overall tension recovery time is decreased by higher calcium concentration; this is indicated in the calculated area to infinity under the recovery curves.

The Effect of Ouabain on the Recovery of Tension Following RSC's.

At 2.5 mM $[Ca^{++}]_o$, Ouabain (1×10^{-6} M.) decreases the overall recovery time and both the slow and fast phases of tension recovery in the double exponential analysis (Table V-III-C) is decreased. At 1.0 mM $[Ca^{++}]_o$ ouabain administration results in a significant decrease in the $t_{\frac{1}{2}}$ of the slow tension recovery phase from 8.80 to 2.73 seconds. The computed area under the recovery curve is also decreased. No significant change in the proportional contribution of the slow and fast phases were observed. When bath calcium was then increased to 2.0 mM by addition of calcium to the bath, the slow recovery phase still had $t_{\frac{1}{2}}$ of 2.74 seconds but the overall recovery time assessed by the computed area decreased from 186 to 59. This is also shown graphically in Figure V-15. At

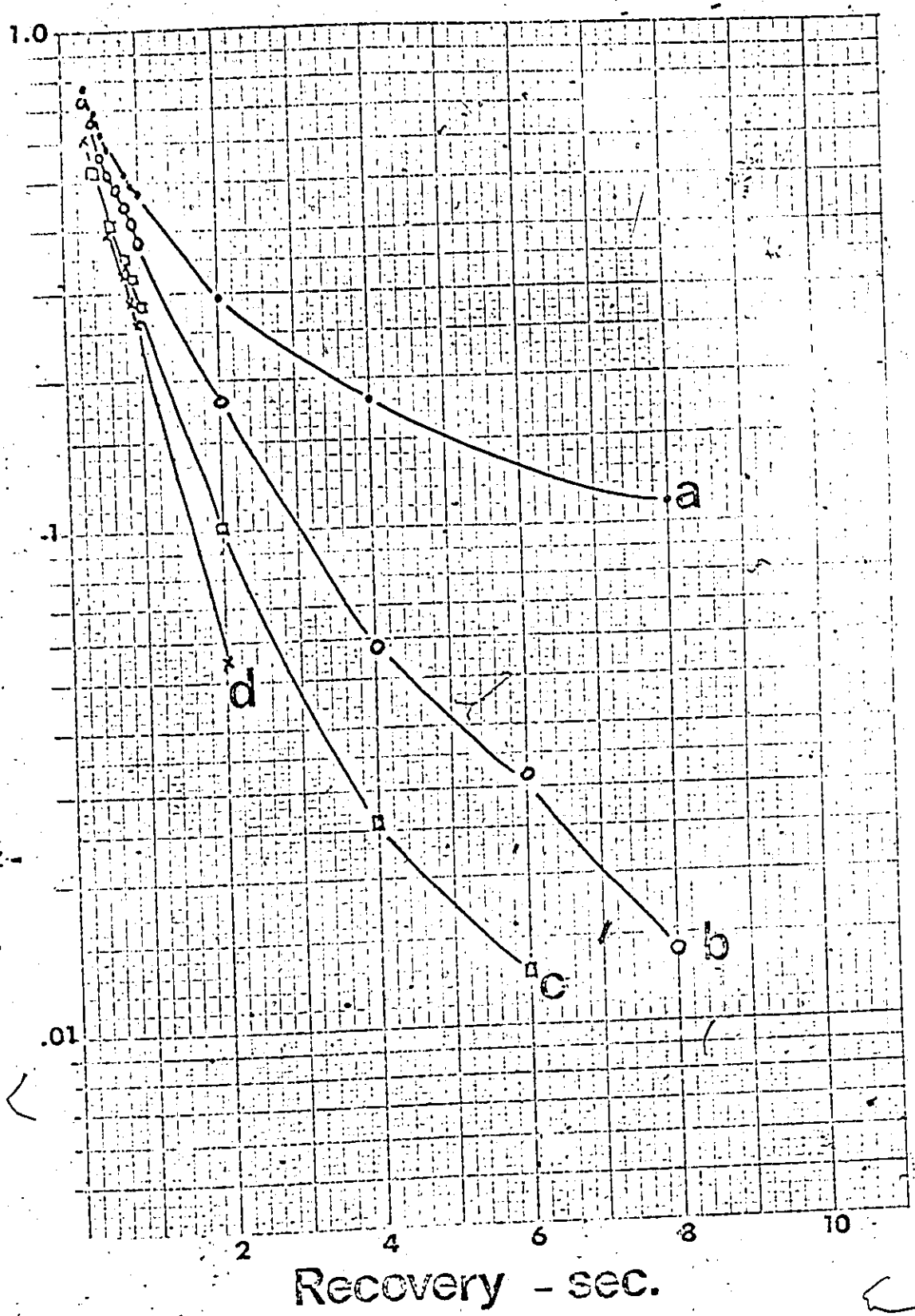
Figure V-15. Recovery of Contractility between Rested State Contractions in the Isolated Papillary Muscle Rat. Effect of Ouabain.

a) $[Ca^{++}]_o$ = 1.0mM.

b) $[Ca^{++}]_o$ = 1.0mM. + 1×10^{-6}M. Ouabain.

c) $[Ca^{++}]_o$ = 2.0mM. + 1×10^{-6}M. Ouabain.

d) $[Ca^{++}]_o$ = 2.5mM.


1.0
.1
.01
a
b
c
d
2
4
6
8
10
Recovery - sec.

0.25 mM $[Ca^{++}]_o$, ouabain administration primarily affected the slow phase of tension recovery, with a decrease in the $t_{\frac{1}{2}}$ from 10.12 to 6.22 seconds.

The Effect of $[Na^+]_o$ on RSC Tension Recovery (Table V-IV-D)

At 2.5 mM $[Ca^{++}]_o$, changing $[Na^+]_o$ to 50% of the normal value resulted in a decrease in the tension recovery time. This appears to be caused by the reduction of the value of the $t_{\frac{1}{2}}$ of the slow phase of tension recovery to the level of the fast phase. That is, we now only have a single fast phase of tension recovery. This is true as well at 10% $[Na^+]_o$. With 50% $[Na^+]_o$ and $[Ca^{++}]_o$ = 1.0 mM. The slow phase of tension recovery is also accelerated, while 10% $[Na^+]_o$ at 0.25 mM $[Ca^{++}]_o$ resulted in a decrease of the $t_{\frac{1}{2}}$ of the slow phase to the value of the fast phase. The overall recovery times assessed by the computed areas under the curves indicates that decreasing the $[Na^+]_o$ increases the rate at which tension is restored between rested state contractions.

Discussion

As our analysis shows, the recovery of contractility between rested state contractions is analogous to the recharge of a pool from a storage site and can be fitted by a double exponential equation. Posner and Berman (1967) found that the recovery of contractility between RSC's in right ventricular strips of the rat was also describable by a second order differential equation. A later paper indicated that the slower phase of tension recovery was most sensitive to changes in $[Ca^{++}]_o$ (Posner and Berman, 1969). This data indicated that their rate

constant, corresponding to $t_{\frac{1}{2}}$ - 2 of our experimental analysis, changed by 60% with an increase of $[Ca^{++}]_o$ from 1.1 to 4.4 mM, while the faster rate constant only changed by 35%; the half-times of tension recovery were both decreased by an increase in $[Ca^{++}]_o$. Our data corroborates these findings, in that we find the major effect of $[Ca^{++}]_o$ is mediated through the slower rate constant. However, we have found that the fast rate constant is not significantly changed by $[Ca^{++}]_o$.

We have found as well that the recovery of tension at low $[Ca^{++}]_o$ is best fitted by a double exponential function but as $[Ca^{++}]_o$ is increased, the slow phase is accelerated to the extent that $t_{\frac{1}{2}}$ -2 approaches the value of $t_{\frac{1}{2}}$ - 1. This is equivalent to having a single exponential to describe tension recovery. We must also question the very fast time constants determined by the computer analysis. The program was designed to provide a double exponential fit, even if a single exponential will fit better, with the result that it may provide an analysis of a double exponential with equal rate constants. Therefore, we may interpret the recovery times at high $[Ca^{++}]_o$ as being a single exponential with a $t_{\frac{1}{2}}$ of 0.5 - 1.0 second. This is also confirmed by graphical analysis of these data.

It may be questioned why a double exponential fit was used to analyze our data. Since Posner and Berman's (1967) analysis suggested that a double exponential described tension recovery, we did not proceed beyond this; the fit of the experimental and computed data matched each other. The values listed under the Area column in Table VIII represent

the sum of the integrated areas to infinity for both components of the curve and was determined by evaluating the ratios: $\frac{\text{intercept -1}}{\text{rate constant -1}}$ + $\frac{\text{intercept -2}}{\text{rate constant -2}}$ = Area. These areas represent the contribution of each component to the overall recovery. Therefore $\frac{\text{area -1}}{\text{Area}}$ or $\frac{\text{area -2}}{\text{Area}}$ are the fractional contributions (with respect to area to ∞) of each phase to the recovery. They were listed in Table V-III as A_1 and A_2.

It is appropriate, at this point, to introduce a model of the system to clarify the results we have obtained for the recovery of contractility between RSC's.

From Figure IV-23 we have proposed that the recovery of tension between RSC's can be modelled by the recharge of Ca_r pool from Ca_s, (Figure V-16).

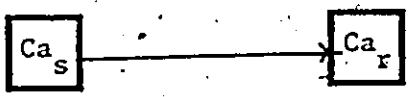


Figure V-16

However, our analysis has shown that a double exponential function can describe the recovery of RSC tension. This can be interpreted as the filling of Ca_r from two pools which we may call Ca_s-1 and Ca_s-2 with rate constants K_1 and K_2 and corresponding $t_{\frac{1}{2}}$s of $t_{\frac{1}{2}}$-1 $t_{\frac{1}{2}}$-2. This is shown diagramatically in Figure V-17.

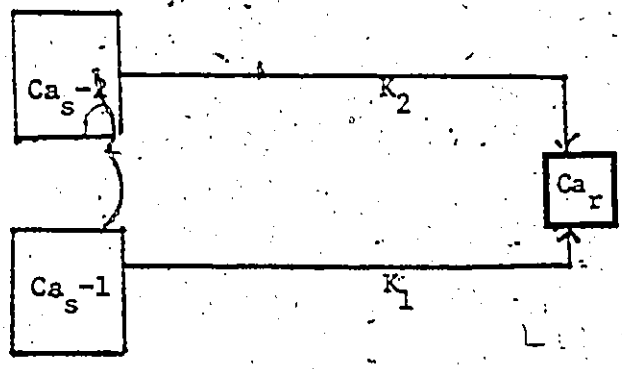


Figure V-17.

We have observed that a) rate constant (or $t_{\frac{1}{2}}$-2) is altered by $[Ca^{++}]_o$ and b) the proportion of contribution of Ca_s-2 is modified by $[Ca^{++}]_o$. At high $[Ca^{++}]_o$, $t_{\frac{1}{2}}$-2 is low while the proportion of calcium transferred to Ca_r is small compared to the amount of calcium delivered to Ca_r from Ca_s-1. At low $[Ca^{++}]_o$, $t_{\frac{1}{2}}$-2 is increased but the proportion of calcium transferred through K_2 to Ca_r from Ca_s-2 is greater than the Ca delivered from Ca_s-1. The proportional contribution of K_1 (A_1) and K_2 (A_2) at each $[Ca^{++}]_o$ tested is shown in Figure V-18. In an attempt to explain these results, the following diagram (V-19) may be helpful. This explanation is purely speculative.

The capacity of Ca_s-2 remains constant, but the concentration in Ca_s-2 changes linearly with $[Ca^{++}]_o$. The capacity (broken line) and concentration (solid line) is shown diagramatically in Figure V-19-A. On the other hand the apparent capacity of Ca_s-1 changes with $[Ca^{++}]_o$ (Figure V-19-B). We also see that the rate constant for recovery of tension in Ca_s-2 is altered by $[Ca^{++}]_o$ and the contribution to tension from Ca_s-2 is only slightly altered by $[Ca^{++}]_o$ (Figure V-19-C) compared

Figure V-18 The effect of $[Ca^{++}]_o$ on the relative contribution of the fast and slow filling components to RSC tension recovery. Ordinate: A_1 (cross hatched area) - contribution of the fast filling component. A_2 (unmarked area) - contribution from the slow filling component.

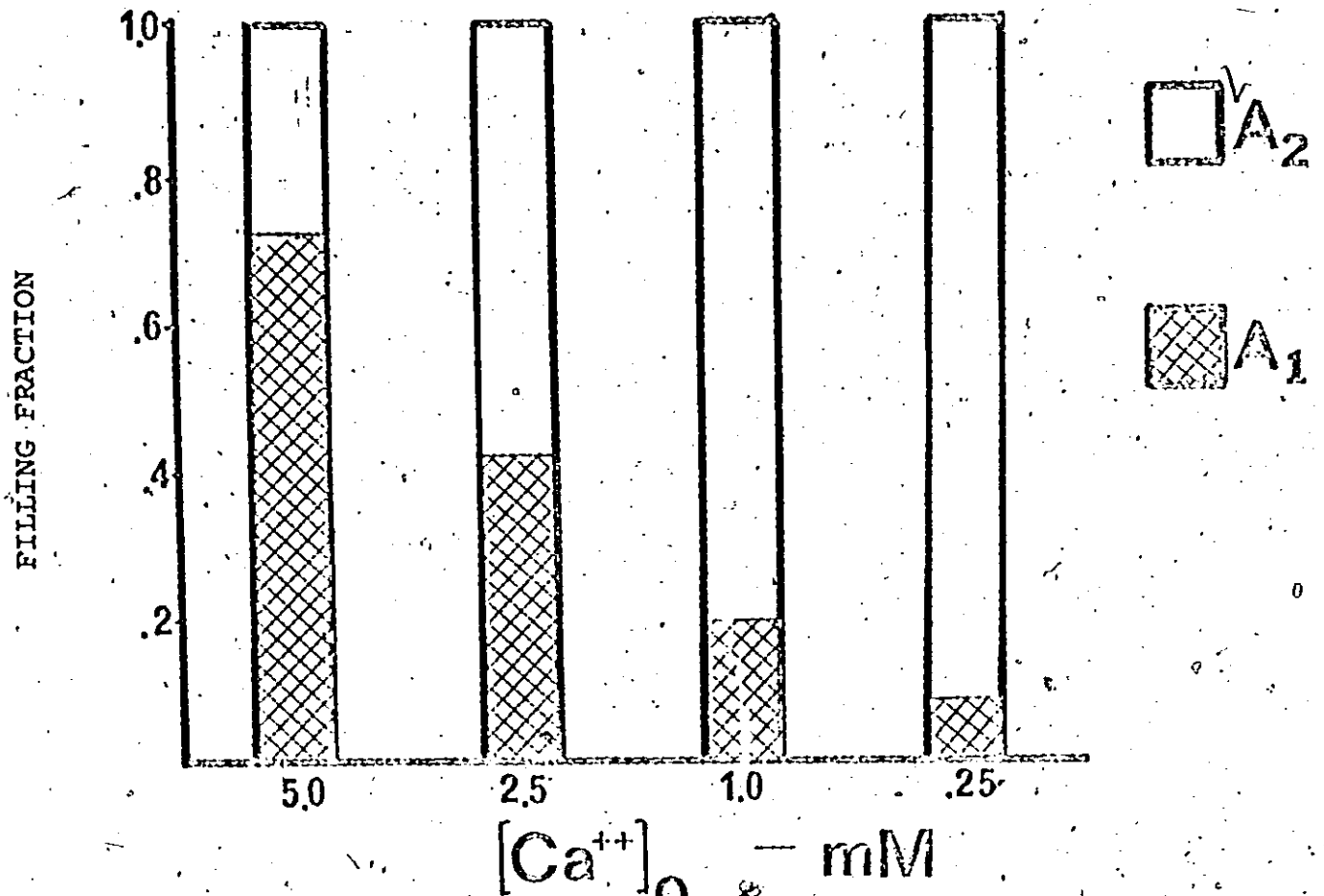
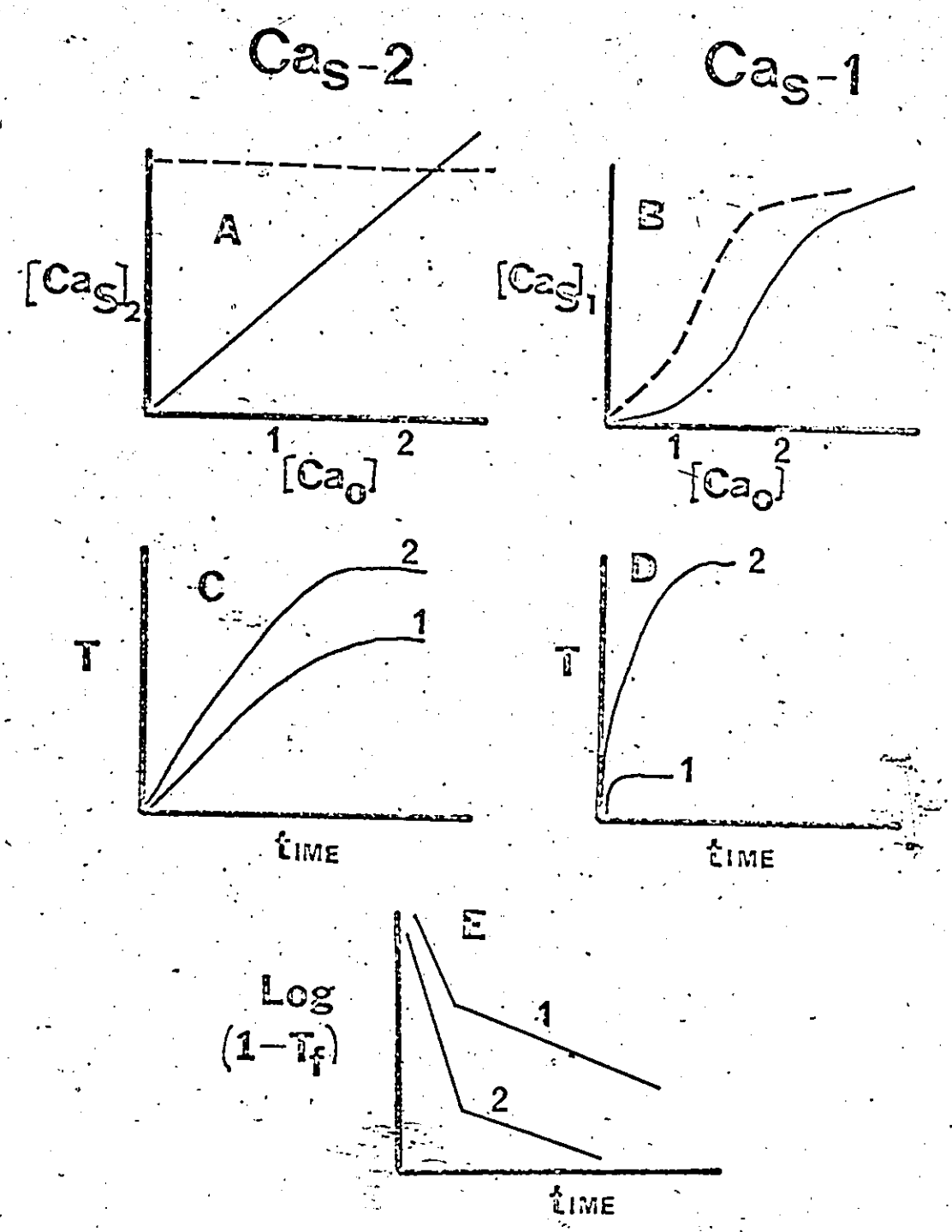

FILLING FRACTION
1.0
.8
.6
.4
.2
5.0
2.5
1.0
.25
$[Ca^{++}]_o$ — mM
A_2
A_1

Figure V-19. Proposed Effect of $[Ca^{++}]_o$ on Ca_s-1 and Ca_s-2 pools their contribution to RSC tension recovery.

A and B. Ordinate - concentration of calcium conta in stored calcium pools Ca_s-1 and Ca_s-2. Abscissa- external calcium concentration (mM). The proposed capacity of the pools is shown by the broken line. contribution of Ca^{++} to fill the tension determining (Ca_r) is proportional to $[Ca_s\text{-}1]$ and $[Ca_s\text{-}2]$.

C and D. Ordinate - Contribution to Tension develope the muscle as a result of filling of Ca_r from Ca_s-1 a Ca_s-2. Tension is shown as function of time (t). Ca releases Ca^{++} slowly and figure C shows the Tension contribution at 1 and 2 mM $[Ca^{++}]_o$. Figure D shows t contribution from Ca_s-1 is fast, but because of the relationship shown in Figure B., the tension contribu is much greater at 2 mM than at 1 mM $[Ca^{++}]_o$.

E - Ordinate - Log $(1-T_f)$. T_f is the fractional tensi recovery between RSC's. Abscissa - (time). This figu combines figures C and D. At 1 mM $[Ca^{++}]_o$ recovery in corporates a small fast phase and larger slow phase fr Ca_s-2. At 2 mM $[Ca^{++}]_o$ the recovery incorporates a gr fast phase from Ca_s-1. Thus recovery at 1 mM $[Ca^{++}]_o$ appears slower than recovery at 2 mM.


Cas-2
Cas-1
A
B
[Cas]2
[Cas]1
1
2
[Cao]
C
D
T
2
1
time
E
Log
(1−Tf)

to the contribution to tension in Ca_s-1 (Figure V-19-D). The rate constant of recovery of tension in Ca_s-1 appears similar (Figure V-19-D). When we combine the recoveries seen in Figures V-19-C and D, we obtain the result seen in Figure V-19-E: At low $[Ca^{++}]_o$, the major contribution to tension recovery is from the slow phase. Conversely at high $[Ca^{++}]_o$ (line V-19-E-2) the major contribution is from the fast phase (Ca_s-1) i.e., we measure a fast recovery rate when $[Ca^{++}]_o$ is high and a slow recovery rate when $[Ca^{++}]_o$ is low.

While the diagram shown in Figure V-19 provides a reasonable explanation for the experimental data on the recovery of contractility, it must be realized that it is only a guess at what may contribute to the recovery of contractility between RSCs.

We may summarize Figure V-19 in the following way:

a) the capacity of Ca_{s2} remains fairly constant; the concentration of calcium in Ca_{s2} is linear with $[Ca^{++}]_o$.

b) Ca_{s2} --recharges Ca_r via rate constant K_2 which is $f\left(\frac{1}{[Ca_{s2}]}\right)$

c) the capacity of Ca_{s1} changes with $[Ca^{++}]_o$. As well, the concentration of calcium in Ca_{s1} is not linear. This results in proportionally larger potential contribution from Ca_{s1} to the Ca_r pool when $[Ca^{++}]_o$ is increased.

d) Ca_{s1} recharges Ca_r with rate constant K_1 which does not change appreciably with $[Ca^{++}]_o$.

e) when we combine the contribution from Ca_{s1} and Ca_{s2} to recharge Ca_r the result is:

i) at low $[Ca^{++}]_o$, overall recovery time is slow, since the major porportion of Ca_r calcium is derived from Ca_{s2}

ii) at high $[Ca^{++}]_o$ overall recovery time is fast since the major proportion of the Ca_r calcium derives from Ca_{s1}.

An Explanation for the Effect of Temperature on RSC Tension Recovery

We have shown that a decrease in the bath temperature generally resulted in a) the reduction in $t_{\frac{1}{2}}$-2 of recovery to a value approaching $t_{\frac{1}{2}}$-1, b) a decrease in the overall recovery time and c) an increase in the proportion contributed by phase 1 to the recovery of contractility. In our model (Figure V-17) this suggests that the concentration in Ca_{s1} increases at low temperature and that the rate constant associated with Ca_{s2} is decreased.

This may be explained in the following manner.

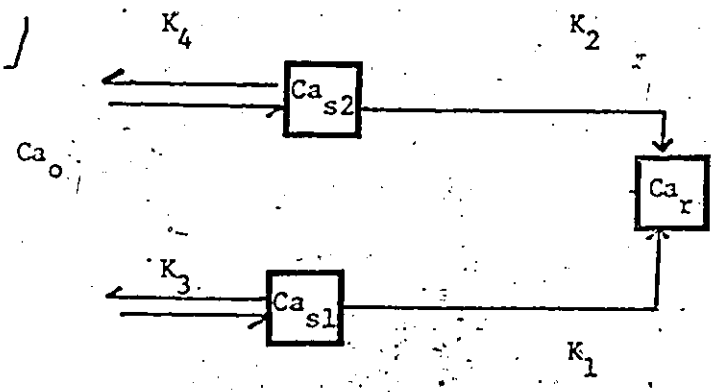


Figure V-20

In Figure V-20 we have two pools contributing calcium to Ca_r, Ca_{s1} and Ca_{s2}. We also suggest that $[Ca_{s1}]$ and $[Ca_{s2}]$ change with $[Ca^{++}]_o$, Ca_s-1 in a non-linear fashion while Ca_s-2 changes linearly. Calcium is exchanged between the Ca_s pools from Ca_o via rate constants K_3 (for Ca_s-1) and K_4 (for Ca_s-2). If we suppose that these rate constants are temperature dependent, then when temperature is changed both K_3 and K_4 are altered. If we then suppose that the temperature coefficient for K_3 is greater than the temperature coefficient for K_4, $[Ca_s\text{-}1]$ would change more than $[Ca_s\text{-}2]$. At low temperatures, $[Ca_s\text{-}1]$ would tend to stay high while an increase in temperature would result in a decrease in $[Ca_s\text{-}1]$. If this is true then we should observe a greater apparent capacity in Ca_s-1 at higher temperatures. This can be tested by PSP. Indeed we find that $PSP_{10''}$ and $PSP_{30''}$ is increased as the temperature is raised from 16 to 36°C.

Effect of Ouabain on Tension Recovery

The effect of ouabain (10^{-6}M.) was not so clearly definable although the primary effect appears to be an acceleration of the rate constant associated with Ca_s-2. The overall tension recovery time was reduced by ouabain administration and at 2.5 mM $[Ca^{++}]_o$, the rate constant associated with Ca_s-2 approached the value for Ca_s-1; this is similar to the effect of an increase in $[Ca^{++}]_o$ on the system, i.e., the recovery of tension can be described by a first order differential equation.

A similar trend is observed when recovery is measured at

2.0 mM $[Ca^{++}]_o$ in the presence of 10^{-6} M. ouabain. The ouabain effect appears to be acting on both of the Ca_s pools, in a manner similar to an increase in $[Ca^{++}]_o$.

The Effect of $[Na^+]_o$ on RSC Tension Recovery

The effect of decreasing $[Na^+]_o$ is analogous to an increase in $[Ca^{++}]_o$ - the slow phase of tension recovery has been completely abolished. This will have important implications in the T-F relationship which we will discuss at later point in the text. However, in summary it appears as if a single exponential (single pool) is more appropriate in defining RSC tension recovery when a) $[Ca^{++}]_o$ is high

b) $[Na^+]_o$ is low

c) Ouabain is administered

whereas a two pool model (double exponential) would be more appropriate in describing RSC tension recovery when:

a) $[Ca^{++}]_o$ is low

b) $[Na^+]_o$ is normal

c) the absence of ouabain.

In between these conditions we would have a mixed contribution of the two pools, Ca_{s1} and Ca_{s2} to tension recovery between rested state contractions. This is shown in Figure V-21 where the proportion of the fast phase (solid line) to the RSC tension (broken line) is shown as a function of $[Ca^{++}]_o$. The effect of a decrease in $[Na^+]_o$ or ouabain administration is to shift the fast phase curve (solid line) to the left.

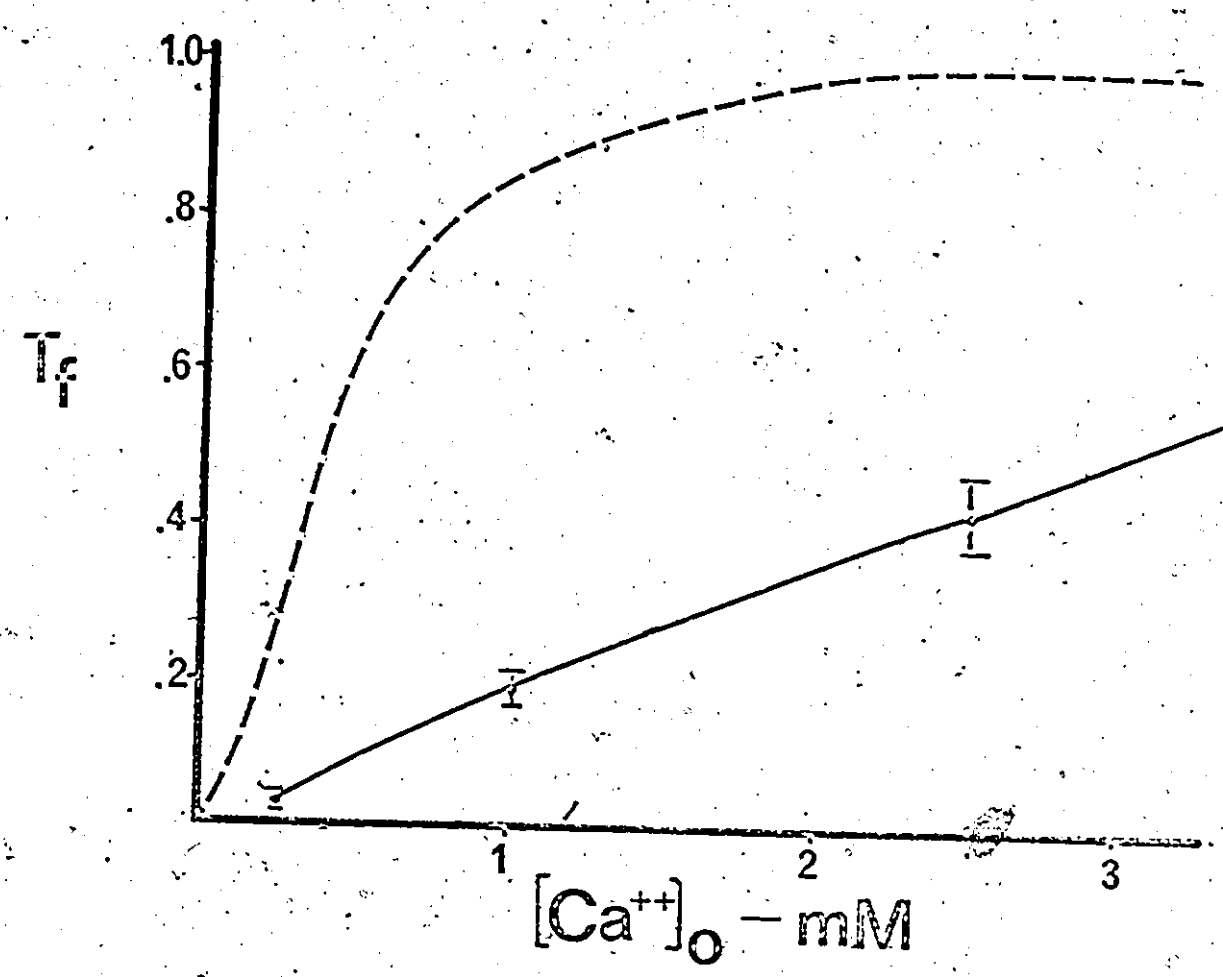


Figure V-21. Contribution of fast phase of tension recovery (●) to RSC tension (broken line).

Mathematical Analysis of the Recovery of Contractility Between Rested State Contractions.

Our experimental data has shown that the recovery of contractility between RSCs can be expressed by a second order or differential equation of the form:

$$T_f = A_1 (1 - e^{-K_1 t}) + A_2 (1 - e^{-K_2 t}) \quad \ldots\ldots(3)$$

where T_f is the fractional recovery of tension

A_1 and A_2 are proportionality coefficients for the exponential terms.

K_1 and K_2 are the rate constants of recovery

and t is the time of recovery between RSCs.

If we could translate this to a hypothetical calcium quantity released from the releaseable calcium pool, $Ca_{r:1}$ we might obtain the following expression:

$$Ca_t = Ca_{max.} \left[A_1 (1 - e^{K_1 t}) + A_2 (1 - e^{-K_2 t})\right] \quad \ldots(4)$$

where Ca_t is the calcium released to the myofilaments at time t from Ca_r and $Ca_{max.}$ is the maximum calcium concentration reached corresponding to $[Ca^{++}]_o$ at $t = \infty$.

We can see from these expressions that the tension recovery will have the same form at all $[Ca^{++}]_o$. We know from our experiments that a) RSC tension is non-linear with $[Ca^{++}]_o$ and b) the rate constants and coefficients are altered with $[Ca^{++}]_o$.

We shall try to account for the recovery characteristics under the various $[Ca^{++}]_o$ conditionsby taking these experimental observations into account in mathematically described RSC tension recovery.

We know that the RSC tension/$[Ca^{++}]_o$ relationship can be described by the expression:

$$T_f = \frac{1.8\,[Ca]^2}{1.8\,[Ca]^2 + 1} \qquad \ldots(5)$$

if we combine expressions 5 and 4 we can define the tension at any point on the RSC tension curve in time between rested state contractions. We assume that the release of calcium from Ca_r will define a relationship such as seen in Formula (5) (Figure V-1) when released and proceeds to Ca_{max}.

Thus

$$T_f = \frac{1.8\,[Ca_{max}\,(A_1\,(1 - e^{-K_1 t}) + A_2\,(1 - e^{-K_2 t}))]^2}{1.8\,[Ca_{max}\,(A_1\,(1 - e^{-K_1 t}) + A_2\,(1 - e^{-K_2 t}))]^2 + 1} \qquad \ldots(6)$$

In equation (6) we follow the convention adopted previously in that K_1 represents a faster rate constant than K_2. We have shown that K_2 is modified by $[Ca^{++}]_o$ and temperature whereas K_1 remains fairly constant. For simple analytical purposes we will assume that A_1 and A_2 are both the same and = 0.5.

Let us begin the analysis of this equation by considering the condition where we appear to have no contribution from K_2 i.e., when the temperature is low or $[Ca^{++}]_o$ is high. Then equation (6) reduces to:

$$T_f = \frac{1.8\,[Ca_{max}\,(A_1\,(1 - e^{-K_1 t})]^2}{1.8\,[Ca_{max}\,(A_1\,(1 - e^{-K_1 t})]^2 + 1} \qquad \ldots.(7)$$

Now if we assume that $A_1 = 1$ since k_1 is the only contributor

to recovery this expression should describe the recovery when $[Ca^{++}]_o$ is high. If we plot this curve when Ca_{max} = 2.5 mM and 0.25 mM with K_1 rate constant corresponding to a $t_{\frac{1}{2}}$ of 0.75 seconds we produce the curves shown in Figure V-22. For comparison we plot a perfect single exponential with the same $t_{\frac{1}{2}}$. This is shown as Line A (broken line). Line B is Formula (7) with Ca_{max} = 2.5 mM while Line C is Formula (7) with Ca_{max} = 0.25 mM. Because the non-linearity in the RSC tension/ $[Ca^{++}]_o$ relationship is incorporated in this expression the recovery does not follow a straight line as shown by the pure single exponential (Line A) but bends to the right during the latter time of the recovery. This is very much like the introduction of a second exponential term. When lines B and C were analyzed for single exponential fit, $t_{\frac{1}{2}}$'s of 0.5 and 0.8 seconds were calculated. A similar effect is observed when we utilize formula (7) but use a $t_{\frac{1}{2}}$ of 5 seconds (Figure V-23).

The introduction of the RSC tension/$[Ca^{++}]_o$ relationship into the recovery expression results in a deviation from the pure single exponential (broken line, A). Line B traces expression (7) when Ca_{max} = 2.5 mM while line C is calculated with Ca_{max} = 0.25 mM. Calculated $t_{\frac{1}{2}}$ for B and C are 2.1 and 5.5 seconds respectively. Both of these simulations indicate:

a) that even when we may be considering a single exponential as appropriate to describe the recovery of tension between RSCs, incorporation of the relationship between RSC tension and $[Ca^{++}]_o$ will

Figure V-22. Tension Recovery Simulated with Formula (7) $t_{\frac{1}{2}}$ = 0.75 seconds.

Line A - Pure Single Exponential.

Line B - Ca_{max} = 2.5 mM.

Line C - Ca_{max} = 0.25 mM.

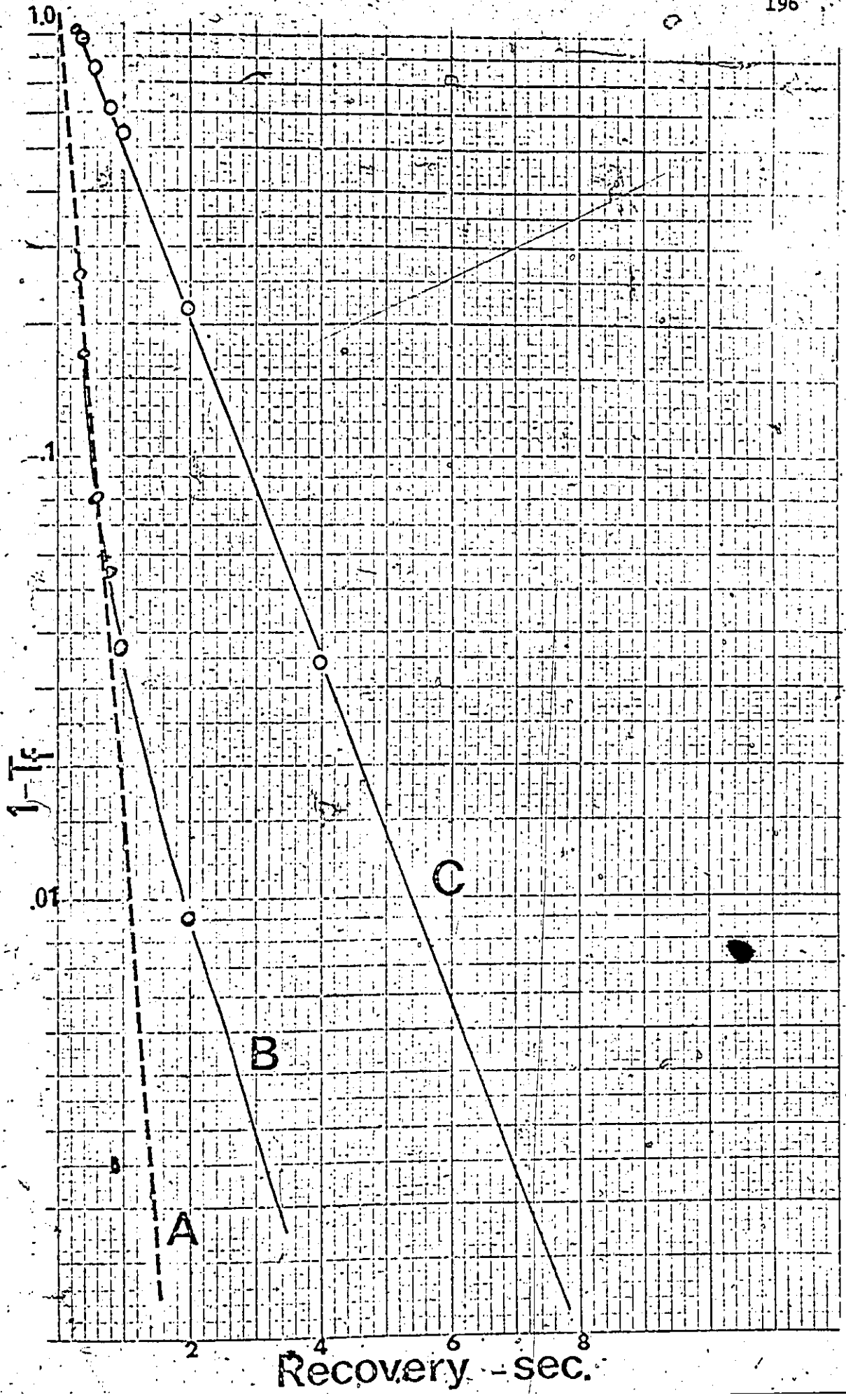

1.0
.1
.01
1-T
A
B
C
2
4
6
8
Recovery - sec.

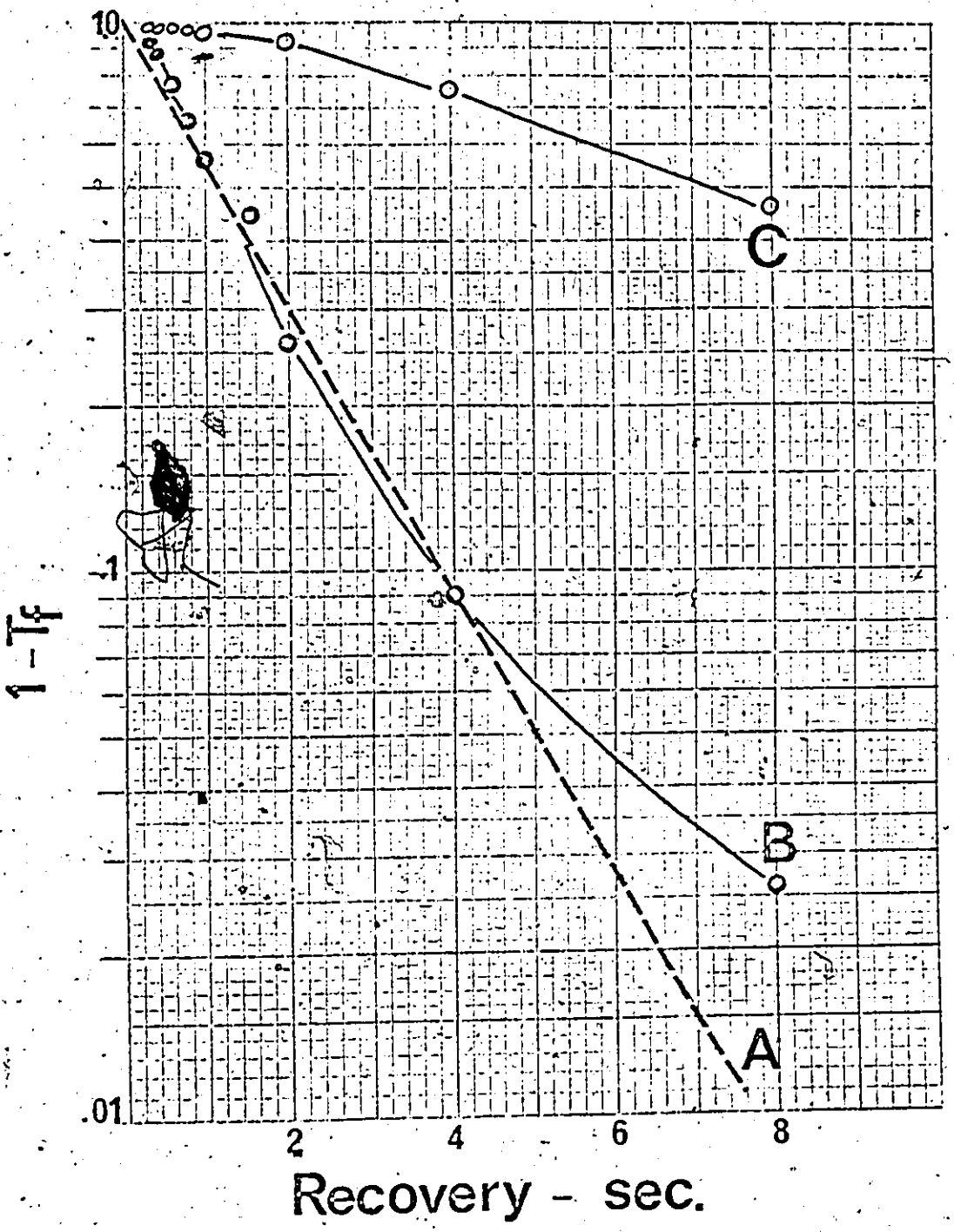
Figure V-23. Simulation of Recovery of Contractility Using Form

$t_{\frac{1}{2}} = 5$ seconds.

Line A - Pure Single Exponential.

Line B - $Ca_{max} = 2.5mM$.

Line C - $Ca_{max} = 0.25mM$.


10
1
.1
.01
1 - Tf
2
4
6
8
A
B
C
Recovery - sec.

distort this so that it appears that a double exponential may be involved.

b) because of the RSC tension/$[Ca^{++}]_o$ relationship a decrease in Ca_{max} results in an apparent decrease of the recovery time constant.

These simulations suggest that at least part of the recovery of tension between rested state contraction may be associated with the non-linearity in the RSC tension/$[Ca^{++}]_o$ relationship. We must emphasize again, that we cannot attribute the non-linearity to any particular anatomical or hypothetical location in our model since we can only measure $[Ca^{++}]_o$ and tension.

Let us now see if the expression incorporating the non-linearity of the RSC tension/$[Ca^{++}]_o$ response and the double exponential can simulate tension recovery at low $[Ca^{++}]_o$. Figure V-24 shows a plot of the curve produced by formula (6). The values of A_1 and A_2 have been arbitrarily set at 0.5 so that they both contribute equally to the recovery of tension. Line A describes a pure double exponential with $t_{\frac{1}{2}}$'s of 0.7 and 30 seconds.. Line B was computed with a Ca_{max} of 2.5 mM while line C was calculated with a Ca_{max} of 0.25 mM. It is evident that line B describes a faster recovery phase than line C. Furthermore, the incorporation of the non-linearity in tension/$[Ca^{++}]_o$ relationship results in a marked bend to the right in the simulated recoveries, i.e., the double exponential is exaggerated.

When formula (6) is used with $t_{\frac{1}{2}}$'s of 0.7 and 3.5 (Figure V-

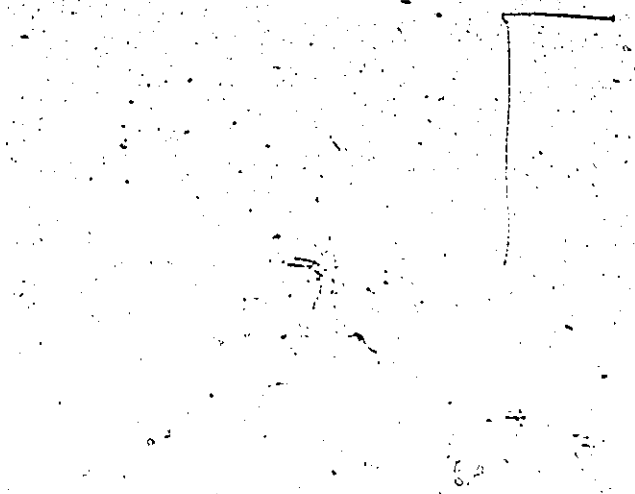


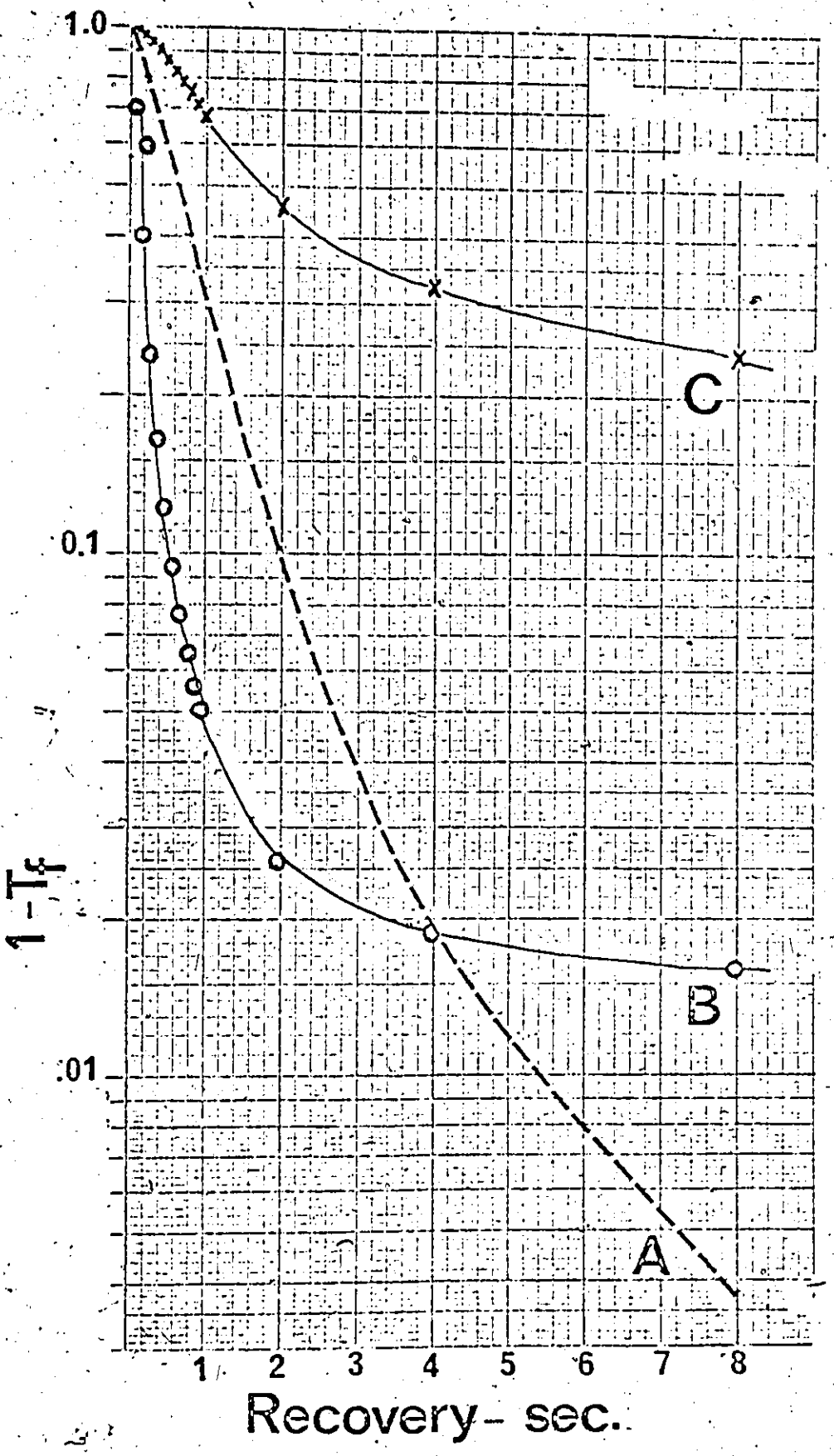
Figure V-24. Simulation of Recovery of Contractility in Isolat
Papillary Muscle using Formula 6.

$t_{\frac{1}{2}}$ = 0.70, 30 seconds.

Line A - Pure Double Exponential.

Line B - Ca_{max} = 2.5 mM.

Line C - Ca_{max} = 0.25 mM.


1.0
0.1
.01
1 - Tf
C
B
A
1
2
3
4
5
6
7
8
Recovery - sec.

25) a similar result is observed. Line A describes the pure double exponential, line B is computed with a Ca_{max} of 2.5 mM while line C is calculated with a Ca_{max} of 0.25 mM. The rate of recovery of line B is obviously faster than line C and line A.

It would be useful to quantify the abberation from the true rate constants of tension recovery caused by the assumption that the RSC tension/ $[Ca^{++}]_o$ relationship contributes in tension recovery measurement.

This was done in the following manner: We generated tension recovery data using formula 6. This provided us with a simulated tension recovery incorporating the RSC tension/ $[Ca^{++}]_o$ relationship. Since we believed that the double exponential recovery is most applicable at 0.25 mM $[Ca^{++}]_o$ we used this value of Ca_{max} and since we found that the value of A_1 was around 42% we arbitrarily set the value of A_1 and A_2 at 0.5. We then generated tension recoveries with $t_{\frac{1}{2}}$'s of 1,1; 1,2; 1,5 and 1,15-seconds; these curves are shown in Figure V-26 and labelled A, B, C and D respectively. We then applied both a single exponential and double exponential computerized analysis to this data. The results are shown in Table V-IV. When both $t_{\frac{1}{2}}$ were the same (1,1) the computer program could not separate them into two $t_{\frac{1}{2}}$ of the same value. Instead, it calculated $t_{\frac{1}{2}}$ of 0.48 and .98. So it underestimated on a fast component and computed one value correctly.

When $t_{\frac{1}{2}}$'s of 1, and 2 were utilized (curve B), the computer program for double exponential underestimated by 25% on the fast phase

Figure V-25. Simulation of Recovery of Contractility in Isolated Papillary Muscle using Formula 6.

$t_{\frac{1}{2}}$ = 0.70, 3.5 seconds.

Line A - Pure Double Exponential.

Line B - Ca_{max} = 2.5 mM.

Line C - Ca_{max} = 0.25 mM.

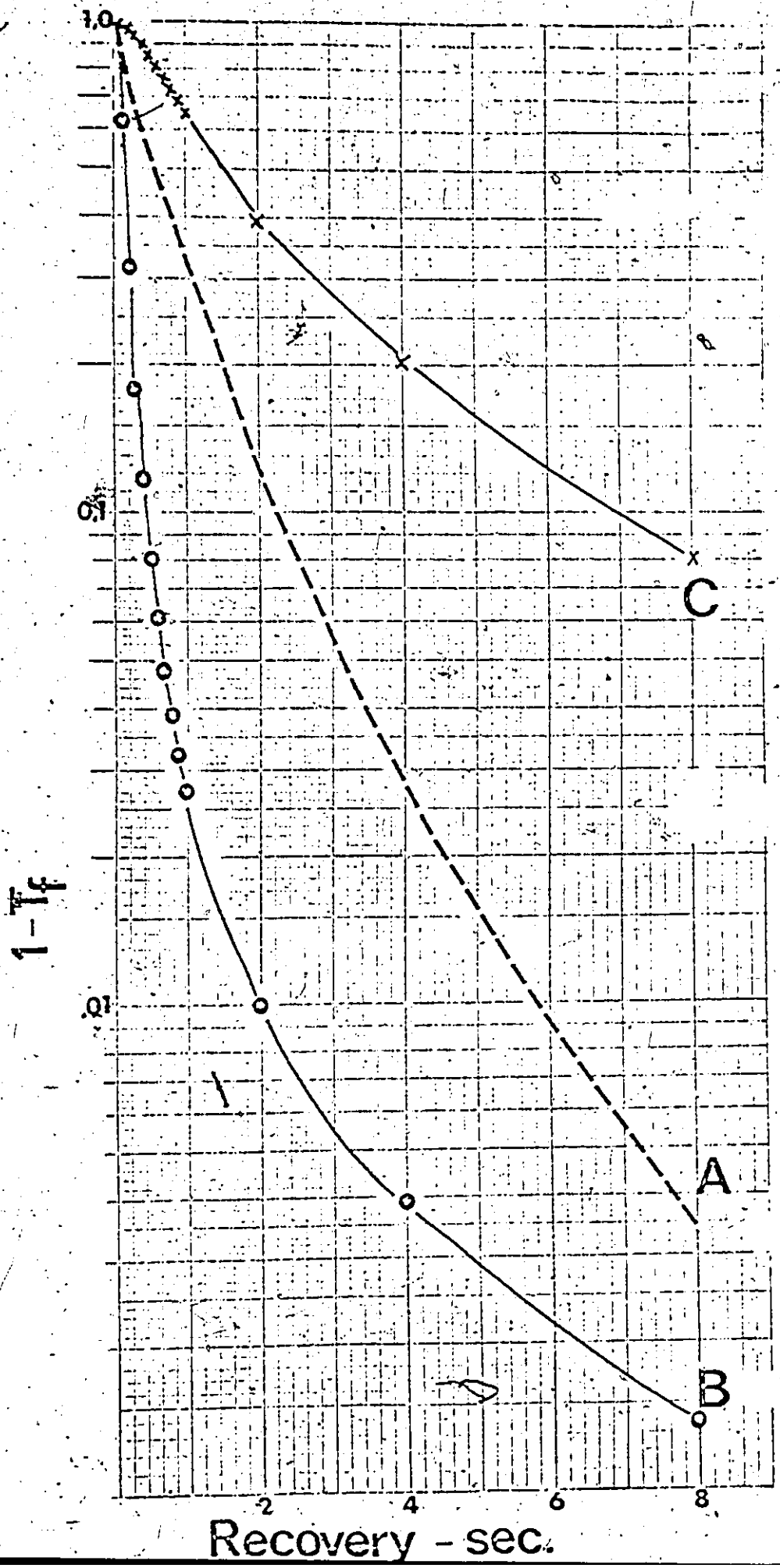

1.0
.2
.1
1 - Tf
C
A
B
2
4
6
8
Recovery - sec.

Figure V-26. Simulated Recovery of Contractility between Rested State Contractions. $[Ca^{++}]_o$ = 0.25mM. Using Formul

Line A (●) - $t_{\frac{1}{2}}$ 1, 1 sec.

Line B (□) - $t_{\frac{1}{2}}$ 1, 2 sec.

Line C (0) - $t_{\frac{1}{2}}$ 1, 5 sec.

Line D (Δ) - $t_{\frac{1}{2}}$ 1, 15 sec.

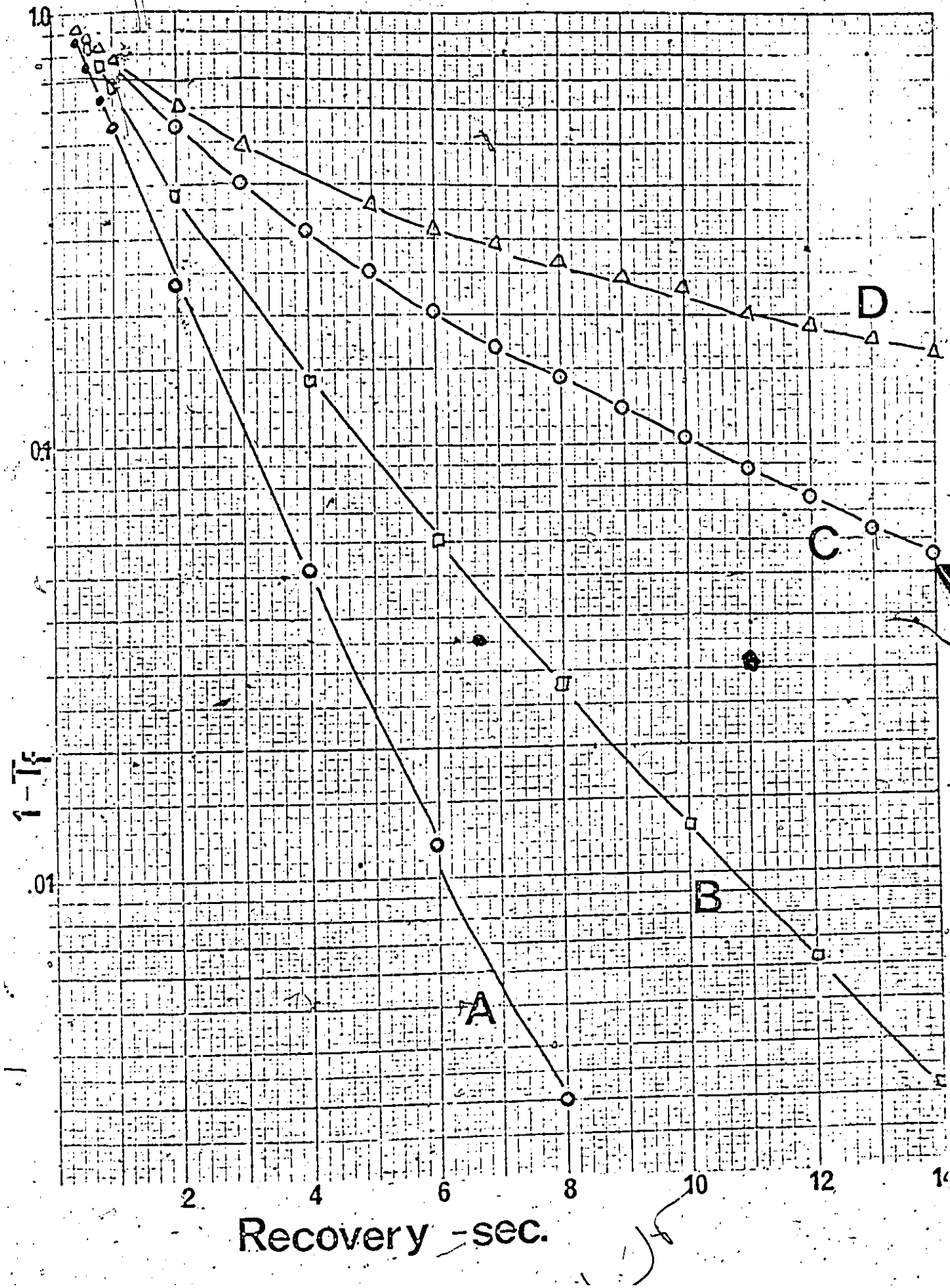

1.0
0.1
.01
1 - T
A
B
C
D
2
4
6
8
10
12
Recovery - sec.

TABLE V-IV

Estimation of Error in Assessing True RSC tension Recovery $t_{\frac{1}{2}}$'s

Curve	A	B	C	D
Actual $t_{\frac{1}{2}}$'s used in Equation 6^1(sec)	1,1	1,2	1,5	1,15
$t_{\frac{1}{2}}$'s calculated by Double Exponential Analysis (sec.)	0.48,0.98	0.75,1.86	1.04,4.3	0.98,7.88
$t_{\frac{1}{2}}$ calculated by Single Exponential Analysis (sec.)	0.94	1.61	3.2	5.14

$$T_f = \frac{1.8\left[Ca_{max}\ 0.5\ (1 - e^{-K_1 t})\ \ 0.5\ (1 - e^{-K_2 t})\right]^2}{1.8\left[Ca_{max}^{\circ}\ 0.5\ (1 - e^{-K_1 t})\ \ 0.5\ (1 - e^{-K_2 t})\right]^2 + 1} \quad \ldots(6^1)$$

where $Ca_{max} = 0.25$ mM (0.5 mEq/L.).

and only 7% on the slow component. However when the actual $t_{\frac{1}{2}}$'s were 1 and 5 seconds, the computer analysis only underestimated on the slow component. With the longest $t_{\frac{1}{2}}$ used (line D), (1, 15 seconds), the computer analysis was true for the fast component but underestimated by 40% on the slow phase of recovery.

Therefore if we assume a non-linearity is involved in the recovery of RSC tension, we must therefore take into account that our computer analysis would be underestimating the true $t_{\frac{1}{2}}$'s of recoveries involving double exponentials with the magnitudes of time constants used in this experiment. Hence in any true quantitative model of calcium movement we must correct for true recovery time constants involved with Ca_s-1 and Ca_s-2.

Finally we would predict that a single exponential would adequately describe RSC tension recovery at high $[Ca^{++}]_o$ (Figure V-27) and a double exponential would describe tension recovery at low $[Ca^{++}]_o$ (Figure V-28).

Summary

In summary we may make the following conclusions regarding the recovery of contractility between rested state contractions:

a) the recovery of contractility between rested state contractions can be described by a single exponential term with $t_{\frac{1}{2}}$ of around 0.5 seconds at $[Ca^{++}]_o$ levels of 2.5 mM and greater.

b) below 2.5 mM $[Ca^{++}]_o$, the recovery can best be described

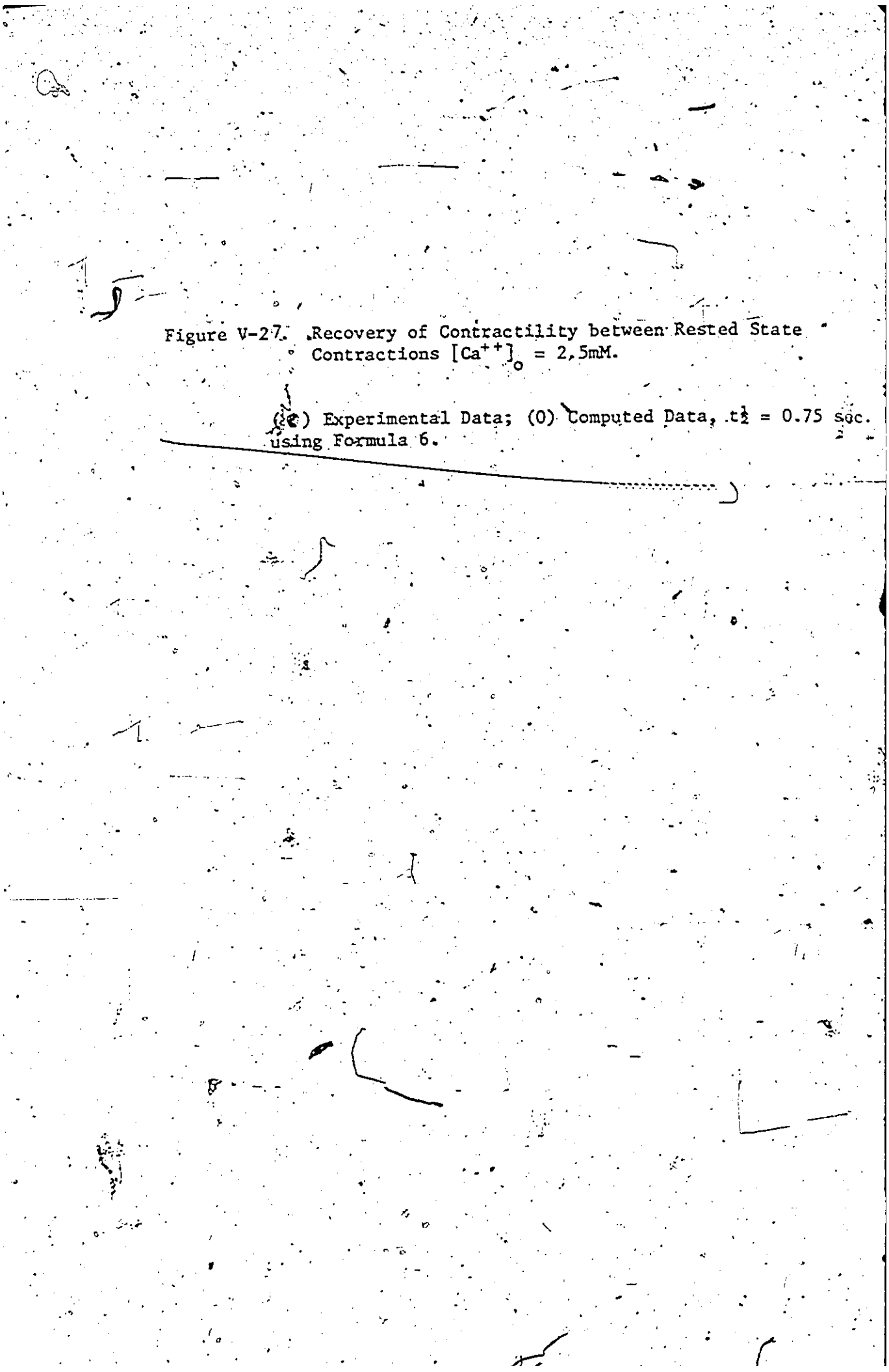
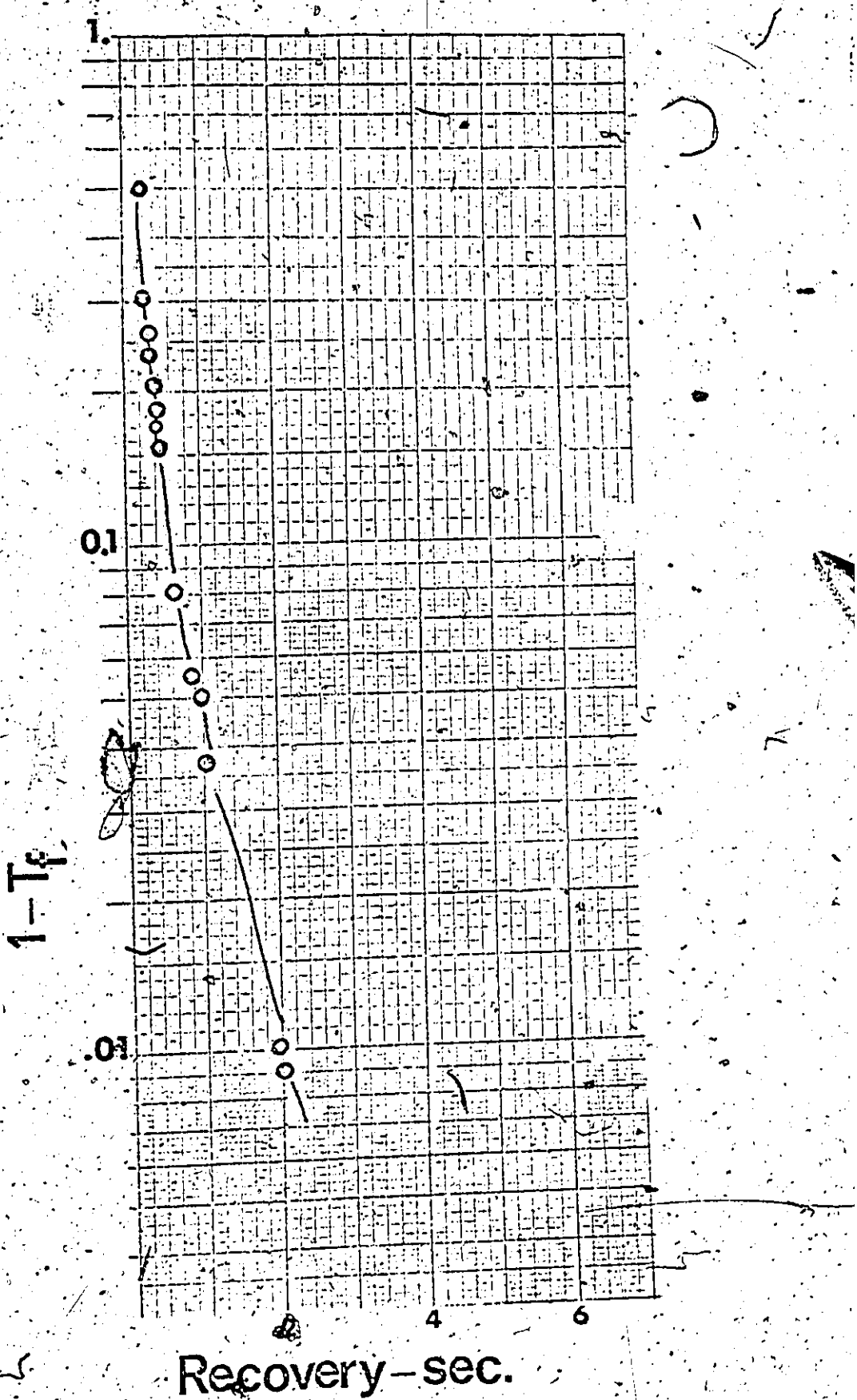


Figure V-27. Recovery of Contractility between Rested State Contractions $[Ca^{++}]_o$ = 2.5mM.

(●) Experimental Data; (O) Computed Data, $t_{\frac{1}{2}}$ = 0.75 sec. using Formula 6.


1.
0.1
.01
$1-T_c$
4
6
Recovery–sec.

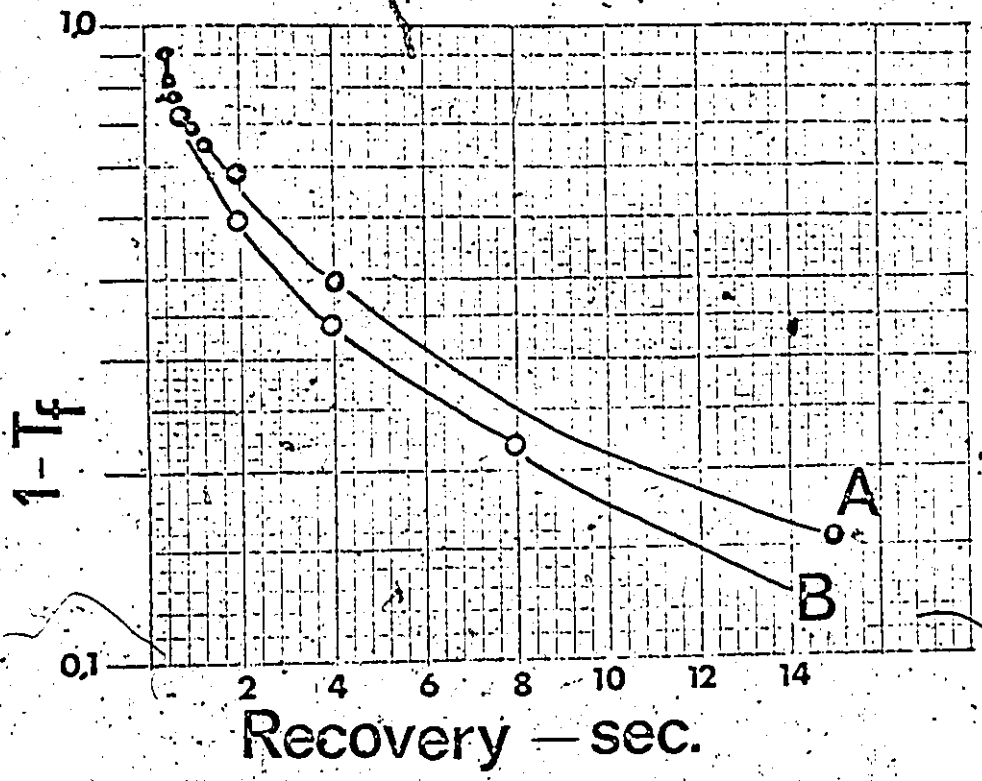


Figure V-28. Recovery of Contractility between Rested State Contractions $^{\circ}[Ca^{++}]_o$ = 0.25 mM.

Line A ( ) Experimental Data;

Line B (○) - Computed Data;

$t_{\frac{1}{2}}$ = 0.7, 10 sec. using Formula 6.

by a double exponential term with a fast $t_{\frac{1}{2}}$ of 0.5 seconds and a slow $t_{\frac{1}{2}}$ which is affected by $[Ca^{++}]_o$; this term increases to approach 0.5 seconds as $[Ca^{++}]_o$ is increased to 2.5 mM. As well, the proportion of the fast component (A_1) decreases to a very small value as $[Ca^{++}]_o$ is reduced.

c) as the temperature is reduced, the time constant for the slow recovery phase is reduced, and the contribution (A_1) from the fast phase is increased.

Section VI

A Model of Calcium Movement to Account for the Interval-Strength Relationship in Rat Myocardium.

The simplest hypothesis consistent with the facts from the data in the preceding section is that a calcium fraction which determines tension (Ca_r) is regenerated or refilled by a first order process from two pools. One of these pools (Ca_s1) fills Ca_r with a fast rate constant, K_1, while the other pool (Ca_s-2) fills Ca_r with a slower rate constant, K_2. Ca_s1 has the following characteristics in the rested state condition:

a) it decreases more than Ca_s-2 as $[Ca^{++}]_o$ is reduced

b) it is reduced by an increase in temperature (eg. from 16°C to 26°C).

We may illustrate this diagrammatically as:

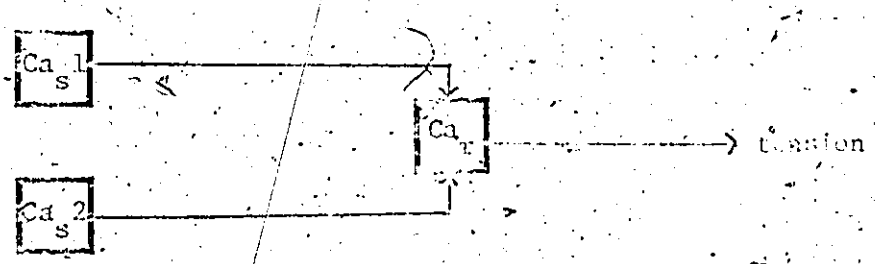


Figure VI-1

From this simple model (Figure VI-1) a tension-frequency curve could be reconstructed since at filling time $t_f(\frac{1}{F})$ the filling of Ca_r can be predicted for both Ca_s-1 and Ca_s-2 contributions. If T_{ca} represents the calcium contribution to tension from these pools than at frequency F:

$$T_{caF} = T_{ca} \quad (1 - e^{-1/F\tau})$$

where τ is the time constant at that frequency.

A plot of this would have the following characteristics (Figure VI-2). It is clear that the fast filling phase is much greater

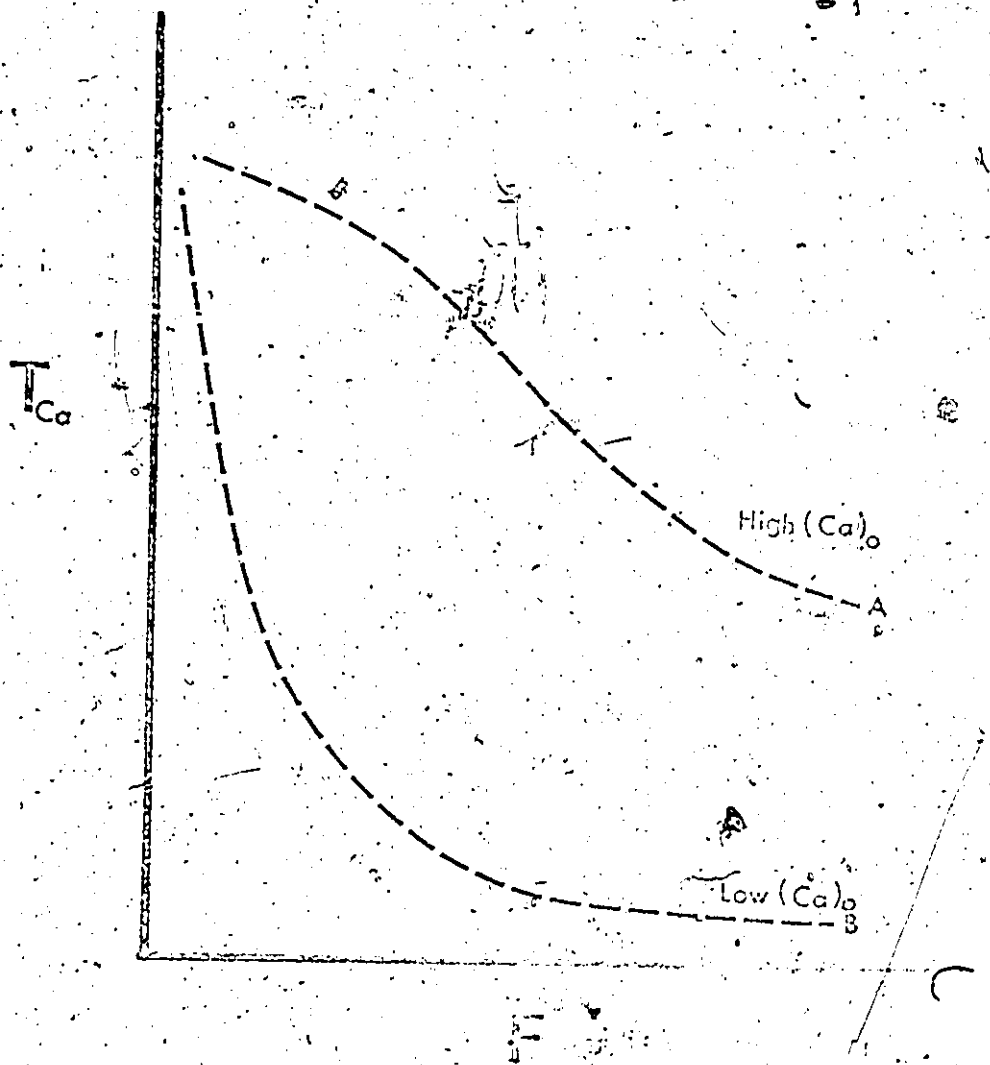


Figure VI-2. Simulated T-F relationship at high $[Ca^{++}]_o$ (A) and low $[Ca^{++}]_o$ (B) without Ca^{++} recycling. A curve generated with τ = 0.5 sec. corresponding to Ca_s-1 donation. B curve generated with τ = 5 sec. corresponding to Ca_s-2 donation.

than predicted under steady state conditions at frequencies above about 30/minute since the response of T_{ca} is not as shown in Figure VI-2 at low $[Ca^{++}]_o$ (curve B). We have only measured recovery between rested state contractions. But we have shown that the shape of the tension/ $[Ca^{++}]_o$ curves are altered from the RSC tension/ $[Ca^{++}]_o$ curve by a) an increase in stimulation rate, b) twin pulse stimulation, c) $PSP_{30''}$.

Figure VI-3 shows the expected tension response to frequency (F) at low (curve B) and high (curve A) calcium, based upon RSC tension recovery. However we observe that we actually have the curve shown in C. i.e., a positive inotropic response. This must be contributed by an increase in the fast filling fraction to the Ca_r pool. Therefore we might assume one further characteristic to the Ca_s-1 pool: the Ca_s-1 concentration must be increased by repetetive activity. There are several ways in which this may occur:

a) Ca^{++} is gated into Ca_s-1 as a result of electrical activity from the external calcium pool.

and b) a recycling of Ca^{++} which is released into the cytoplasm from the myofilaments and then "pumped" into Ca_s-1.

There is some experimental evidence for both of these suggestions.

a) a slow inward current thought to represent an inward Ca^{++} current has been shown to occur with depolarization of myocardial tissue (Beeler and Reuter, 1970, a, b; Tarr, 1971). Thus each action potential

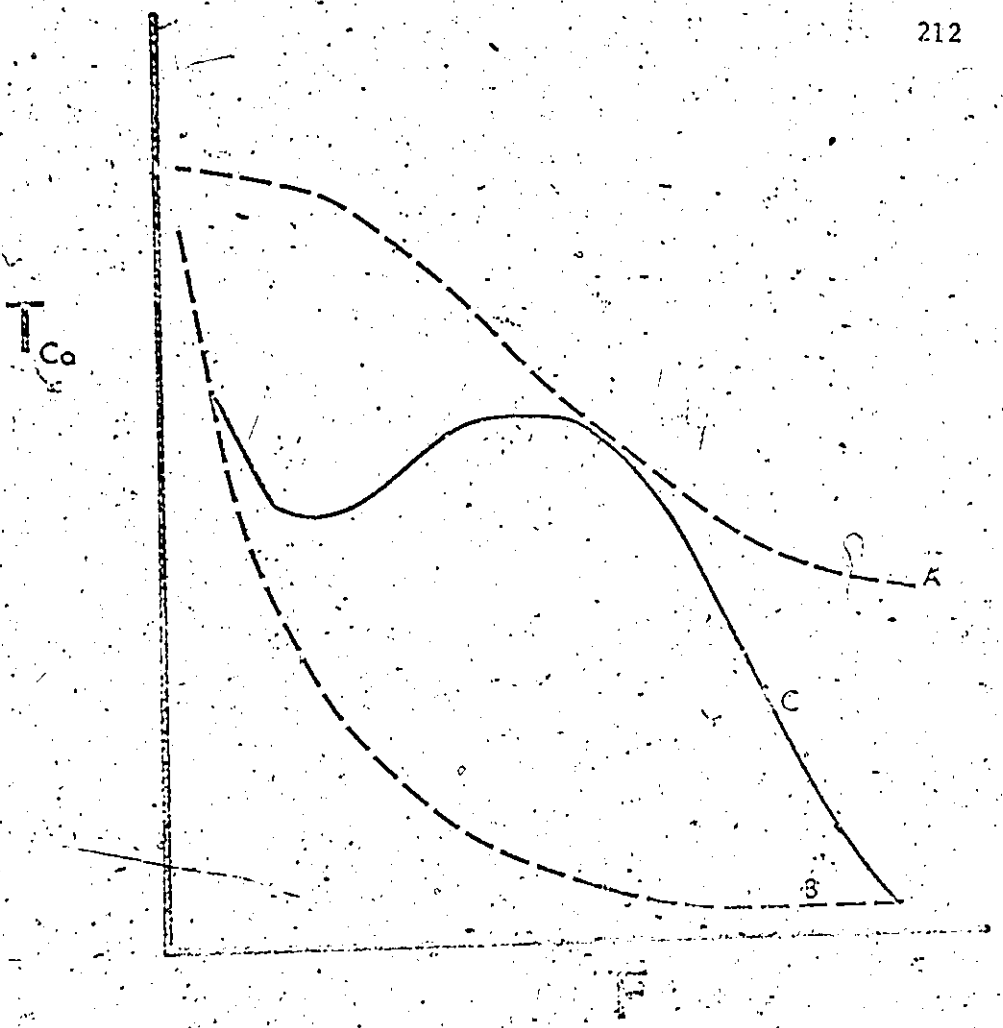


Figure VI-3. Simulated T-F relationship at high (A) and low (B) $[Ca^{++}]_o$, low $[Ca^{++}]_o$ with recycling (C). τ_B = 3 seconds. τ_A = 0.5 seconds.

is believed to contribute some Ca from $[Ca^{++}]_o$ to the sarcoplasm. In addition several studies have shown that there is a linear relationship between $[Ca^{++}]_o$ and inward Ca^{++} current (Reuter, 1967; Beeler and Reuter, 1970, a, b; Reuter and Scholz, 1968; Kohlhardt et al., 1972).

b) it has been suggested that the sarcoplasmic reticulum of heart participates in the relaxation of muscle by accumulating Ca^{++}, transporting it and releasing it to be a) passed out of the cell or b) recycled within the cell (Katz, 1970; Langer, 1968).

These two points are reinforced by our own experimental data which shows that:

a) a brief burst of repetetive stimulation at a high rate results in an increase in tension (PSP) at low $[Ca^{++}]_o$.

b) paired-pulse stimulation results in an increase in tension at low $[Ca^{++}]_o$.

We may be able to quantify the "extra" calcium utilized by the cardiac tissue in potentiation ($PSP_{30"}$). To do this we must make several assumptions.

i) peak tension is proportional to calcium released internally (Ca^{++}).

ii) ½ peak tension is reached when free $[Ca^{++}]_i$ = 25 μmoles/Kg (Weber et al., 1963) which also is the amount of calcium released from the internal pool.

iii) all of the calcium gated into the cell during the brief burst of rapid stimulation becomes available for the next potentiated

contraction.

iv) there would be only one reason for the increase in tension after the burst of stimulation (PSP) and this would be due to the increase in free Ca^{++} from the releaseable pool (Ca_r) due to Ca^{++} gated in.

Therefore $\frac{\Delta T_f}{\text{pulse \#}} \times 50 = Ca^{++}$ gated in (μmoles/Kg/pulse)

The results of these computations are shown in Table VI-I. Ca "entry" from 0.2 to 1.5 mM $[Ca^{++}]_o$ appears to be between .0246 to 4.65 μmoles/Kg/pulse. Beeler and Reuter (1970,b) estimated entry per beat at around 1-50μmoles/Kg while Grossman and Furchgott (1964), Niedergerke (1963), and Winegrad and Shanes (1962) estimated Ca^{++} entry at 0.5 - 2.7 μmoles/Kg per contraction. Our values are similar to these latter values. However, our Ca^{++} entry calculations indicate that this would be less than 10% of the Ca^{++} needed to fully saturate the troponin. Langer (1968, 1970) suggests less than 2%. Therefore it is clear that gating alone is not adequate to explain the T-F curve. We would lose more than we gain with an increase in frequency unless some of the free Ca released during contraction is recycled to the releaseable sites. Furthermore, it becomes apparent that the recycled Ca^{++} must be directed to the fast filling fraction (to Ca_s-1) so that as the frequency of stimulation is increased Ca_r receives a greater amount of its input from Ca_s-1 in spite of a shorter recovery cycle.

We therefore believe that calcium released from the myofilaments

TABLE VI-I

Estimated Increase in Internal Free Ca^{++} during Potentiation.

$[Ca^{++}]_o$ mM	$T_{PSP30''}$ s.	T_F	$[Ca^{++}]_i$ μM/Kg.	ΔCa_i μM/Kg.	Pulses	$\Delta[Ca^{++}]_i$/pulse μM/Kg./pulse
0.1	.13	.039	1.94	2.00	15	.0246
0.1	.27	.081	4.03		100	
0.2	1.01	.301	15.07	4.78	7	.266
0.2	1.33	.397	19.85		25	
0.3	.91	.272	13.58	26.72	2	2.05
0.3	2.70	.806	40.30		5	
0.4	2.23	.367	18.35	23.44	2	3.91
0.4	2.80	.836	41.79		8	
0.5	1.78	.531	26.56	20.44	2	3.41
0.5	3.15	.940	47.00		8	
0.75	2.50	.746	37.30	9.3	2	4.65
0.75	3.12	.931	46.60		4	
1.00	2.95	.88	44.00	6.0	2	3.0
1.00	3.35	1.00	50.00*		4	
1.5	3.20	.955	47.70	1.4	2	0.7
1.5	3.29	.982	49.10		4	

* Value of Maximum Activation of Actomyosin System (Weber et al., 1963).

is cycled to the $Ca_s 1$ pool, which would allow more calcium to enter Ca_r between stimuli. It is obvious that the increase in available Ca^{++} must be associated with a time dependent process since the increase in tension occurs between certain frequencies (30 - 100 stimuli/min.).

This can be shown diagramatically as (Figure VI-4):

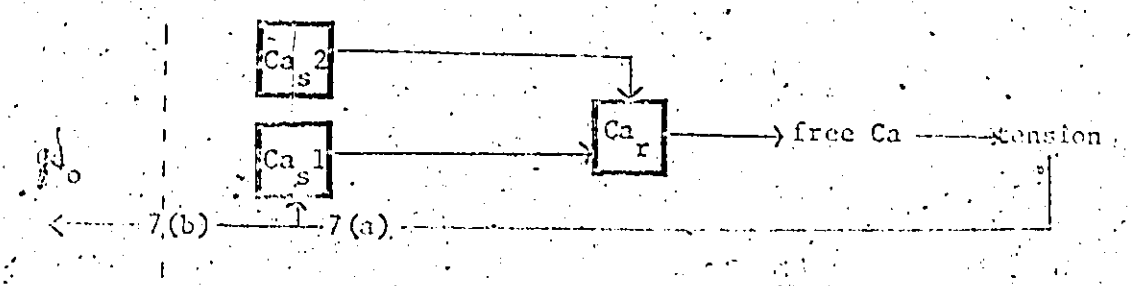


Figure VI-4

Ca_s-2 and Ca_s-1 recharge the releaseable Ca^{++} pool Ca_r. Upon stimulation the Ca_r pool is completely released and becomes free Ca - tension is developed and relaxation is accomplished by sequestering: part of this is taken up through process 7(a) to Ca_s-1, while the remainder, passes out of the cell.

Now given the appropriate conditions, with an increase in frequency we could increase the amount of Ca^{++} transferred from $Ca_s 1$ to Ca_r if process 7(a) is operative and we gate an amount of Ca^{++} into $Ca_s 1$ with each depolarization.

The Complete Model

Figure VI-5 is a diagram of the model which incorporates all of the elements necessary to account for our experimental data.

K_3 and K_4 are the rate constants governing $Ca_s 1$ to $[Ca^{++}]_o$

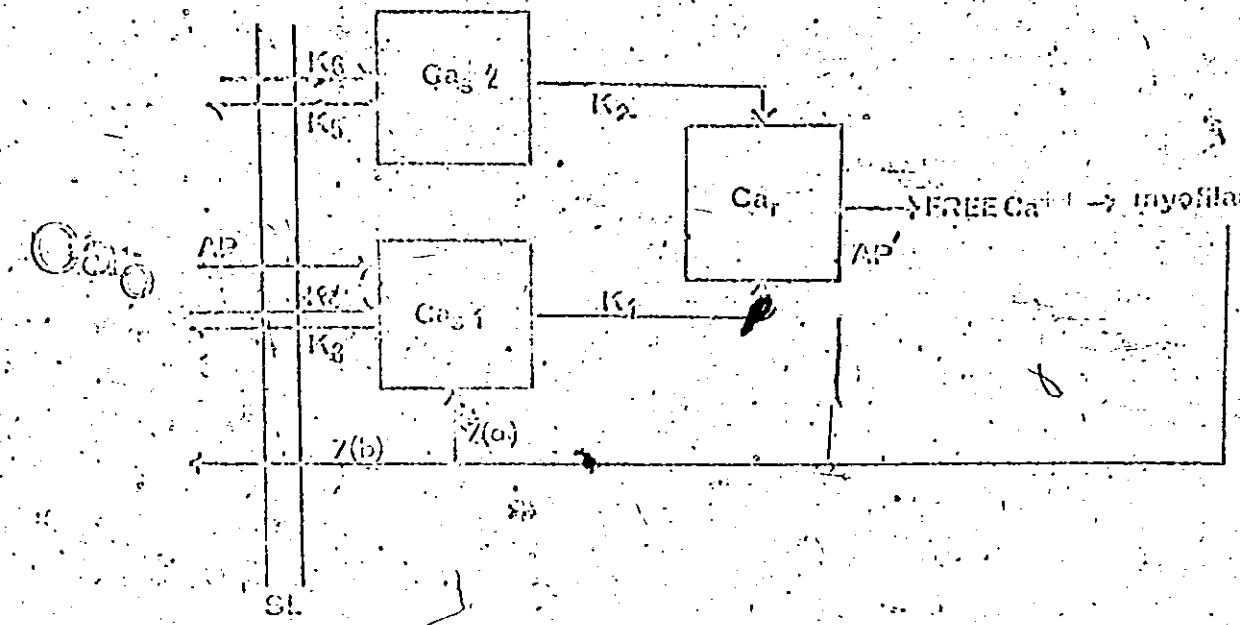


Figure VI-5. Model of Calcium Movement in Rat Cardiac Muscle.

while K_5 and K_6 are those concerned with Ca_s-2 . The term AP close to Ca_s1 . indicates gated calcium entry with each action potential. K_2 and K_1 are the rate constants governing the refilling of the Ca_r (releaseable Ca^{++}), pool and process 7 represents recycled (7a) or non-recycled (7b) Ca^{++}. AP near Ca_r is action potential induced release of Ca^{++} from Ca_r.

The temperature coefficient for rate constant K_3 is greater than that for K_5; thus $[Ca_s\text{-}1]$ changes more than $[Ca_s\text{-}2]$ during a temperature change. At RSC intervals, when $[Ca^{++}]_o$ is low, $[Ca_s\text{-}1]$ is reduced and the major contribution to tension recovery (Ca_r filling) is from Ca_s-2. On the other hand, when $[Ca^{++}]_o$ is increased, $[Ca_s\text{-}1]$ increases and the major contribution to Ca_r filling is from Ca_s-1 via K_1.

At low $[Ca^{++}]_o$, $[Ca_s 1]$ can be increased by decreasing the period between stimuli and by gating Ca^{++} into Ca_s-1. Similarly, a brief rapid burst of stimuli, with an appropriate wait until Ca^{++} is recycled through 7(a) will indicate PSP. Therefore we have the observation that tension always reflects events which preceded the contraction.

Analogy of the Model with Ultrastructural Characteristics

The sequence of calcium movement believed to occur in cardiac muscle may be summarized as follows:

a) Calcium enters the cell with each action potential (Beeler and Reuter, 1970). This calcium is thought to cross the sarcolemmal membrane into the sarcoplasm, or bound to sarcolemmal sites. The calcium

which enters the sarcoplasm may be taken up either by mitochondria or other organelles or the sarcoplasmic tubules (Langer, 1973).

b) the calcium entering the cell may act as a trigger to release Ca^{++} stores which are thought to exist i) in the terminal cisternae of the T system or ii) the sarcoplasmic reticulum.

c) the released free Ca^{++} activates the troponin system producing contraction. This Ca^{++} is taken up by the sarcoplasmic reticulum, and transported to the region of junction with the T system where it is i) released out of the cell or ii) kept close to the terminal cisternae for recycling in the contraction cycle.

The Ca -1 Pool

In a recent review article, Langer (1973) suggested that internal membranes such as the T system and its continuation, the longitudinal system may be involved in excitation contraction-coupling. This evidence is derived from the studies of Bailey and Drosel (1968), Shine et al., (1971), Saari and Johnsen (1971) and Little and Sleator (1969) which indicated that calcium bound at surface or subsurface sites was responsible for initiating contraction. These studies are also supported by experimental evidence using La^{+++} which has been shown to displace Ca^{++} from superficial sites (Langer and Frank, 1972; Sanborn and Langer, 1970). It has been revealed that the surface of myocardial cells are coated with a basement membrane composed of mucopolysaccharide which stains when "attached" to La^{+++} (Fawcett and McNutt, 1969; Shea, 1971). Page and McCallister (1973) have shown that this membrane coating

extends from the surface through the T system to the area of the longitudinal projections (Bockman et al., 1973). These mucopolysaccharides apparently have a high affinity for Ca^{++} (Scott, 1968) and it is possible that calcium will be tightly bound or at least more tightly bound in the T system compared to the longitudinal system. One might therefore conceive of the longitudinal system as a source of labile calcium.

The longitudinal tubular system has been shown to be in close proximity to the sarcoplasmic reticulum (Page and McCalister, 1973). We may speculate that these longitudinal projections of the T system may be considered as the Ca_s-1 pool of our model (Figure VI-6).

How does this match with the tension characteristics of our model?

a) the filling of Ca_r by Ca_s-1 is relatively fast. The longitudinal system would be in close proximity to the releasing site (cisternal region) in the cell. Thus Ca^{++} in the longitudinal tubule diffuses very quickly to releasing sites.

b) Ca_s-1 changes with $[Ca^{++}]_o$: The longitudinal tubule is continuous with the surface via the T system. This provides for a direct route between Ca_s-1 and the surface. Winegrad (1968, 1970) has suggested that calcium is passed out of the cell via the T system in skeletal muscle.

c) the Ca_s-1 is affected by recycled Ca^{++}: Since the SR is in close proximity to the longitudinal tubules, calcium removed from the myofilaments will pass out of the cell via the terminal cisternae to the T system, i.e., Ca^{++} will be recycled to Ca_s-1.

d) $[Ca_s-1]$ increases with a decrease in temperature. This observation is difficult to explain. Therefore one may imagine that a process which has a high temperature coefficient controls calcium loss from Ca_s-1. Although it is speculative, it is possible that the decrease in temperature results in less Ca pumped from the releasing sites of the SR-longitudinal tubules maintaining a higher concentration in these regions.

The Ca_s-2 Pool

We believe that the Ca_s-2 pool would be associated with the surface membrane. This Ca would be tightly bound to the surface and would be displaced only at a slow rate to the myoplasm or to releasing sites ($t_{\frac{1}{2}} < 10$ sec.). This calcium pool does not change with $[Ca^{++}]_o$ as much as Ca_s-1 since it is more tightly bound. The alteration in the time constant associated with the Ca_s-2 pool can be reconciled by assuming that at higher $[Ca^{++}]_o$, Ca^{++} is more easily displaced from the surface sites to the releasing sites i.e., the affinity coefficient for the membrane is sensitive to $[Ca^{++}]$.

The Ca_r Pool

The logical location for the Ca_r pool is in terminal cisternae of the T system where longitudinal tubules abutt the SR. This has also been suggested in the review by Langer (1973).

Role of the SR in the Model

It is now widely accepted that the SR of cardiac muscle

participates in the relaxation of cardiac muscle protein by rapid uptake of Ca^{++} (Inesi, 1973). We believe that process7of our model (Figure VI-6) represents Ca^{++} uptake and transport.

It is appropriate at this point to discuss some of the characteristics of SR in order to clarify this model. We may explain the positive T-F relationship and the PSP response in terms of a sequestering of Ca^{++} by the SR and transfer to releaseable sites i.e., Ca^{++} would be removed from myofilaments, taken up by SR and transferred to the longitudinal elements of the T system in the region of the terminal cisternae.

Winegrad's (1968, 1970) experiments on skeletal muscle suggest that the SR uptake and transport does not occur instantaneously. In fact, Connolly et al., (1971) found that the post-tetanic period in frog skeletal muscle was characterized by processes with $t_{\frac{1}{2}}$'s of 2-3 seconds and 2-3 minutes. Although we may not have "movement times" of the same magnitude in cardiac muscle, the principles may still apply. Palmer and Posey's (1967) data on the accumulation of Ca^{++} by SR indicated three rate constants, associated with transport, storage and binding of Ca^{++}. At least three factors are said to be involved in limiting the maximum levels of calcium accumulation by SR: a) the pCa of the medium (Weber et al., 1966) and the saturation of the transport system, b) the rate of passive efflux (Johnson and Inesi, 1969), and c) the intravesicular (intra SR) Ca^{++} concentration (Weber et al., 1966; Inesi, 1971; Weber, 1971). This can be restated that the rate of passive

efflux from the SR is determined by pCa of the medium, saturation of the transport system, intravesicular Ca^{++} concentration and calcium accumulation by the SR. Furthermore Connolly et al., (1971) postulated that movement of calcium following muscle activation can be divided into calcium at relaxation sites and calcium at release sites. Early in relaxation there would be less calcium in the latter than the former site while the opposite condition would obtain in later stages of the relaxation cycle.

Our data on the effect of temperature on the Ca_s-1 tension recovery constant indicates that an increase in temperature generally caused an increase in the recovery time rather than the expected decrease associated with a temperature rise. Lee and Shin (1969) found that the active transport of calcium out of red cells was slowed by a decrease in temperature, resulting in calcium accumulation in the cells. One could suppose that an increase in SR calcium at lower temperatures through a similar process could accelerate recovery by allowing more calcium to be transported to the releasing sites. That is, the decreased loss of calcium at low temperature would more than offset the decreased Ca^{++} accumulating effect on the ATPase of the SR. Although the Ca^{++} accumulation by SR is known to be energy (ATP) dependent (Ebashi and Lipmann, 1962; Ebashi, 1961; Hasselbach and Makinose, 1961), a Ca^{++} binding protein fraction has been isolated from SR with an apparent dissociation constant of 4×10^{-5} M. and not requiring ATP (MacLennan

and Wong, 1971). This would suggest that part of the sequestration of Ca^{++} by SR could proceed independently of energy considerations even at low temperature.

There is conflicting evidence on the ability of Ouabain to inhibit the ATPase of the sarcoplasmic reticulum. Several studies indicate that cardiac glycosides could influence the active calcium transport system of the sarcoplasmic reticulum (Dutta et al., 1968; Dutta and Marks, 1969).

Later work indicated that the cardiac glycosides did not influence the active calcium transport system, but altered the calcium binding properties of the cardiac SR (Klaus and Lee, 1969; Lee and Klaus, 1971; Lee et al., 1969). This was also confirmed by Entman et al., (1969) who showed an increased calcium binding. On the other hand, Carsten (1967) found a diminished Ca^{++} binding after glycoside treatment. Pretorius et al., (1969), and Besch et al., (1970) found no change in SR Ca^{++} uptake after treatment with glycoside even though positive inotropism was observed. Akera et al., found an inhibition of microsmal Na^{+}-K^{+}-ATPase by Ouabain.

It appears as if there is no clear cut evidence in the literature as to the effects of Ouabain on isolated sarcoplasmic reticulum. This is probably because of the difficulty in obtaining a pure preparation of sarcoplasmic reticulum without contamination from other cellular components especially the plasma membrane (Hajdu, 1957; Kamat and Wallach, 1965; Wallach and Kamat, 1964; Wallach and Ullrey, 1964).

Although these results do not exclude the possibility that Ouabain might affect the binding state of Ca^{++} to the SR. It is clear that there is no unanimous understanding of the terms uptake, binding and transport with respect to Ca^{++} in the SR since these words are used almost interchangeably. This may be the result of the fact that SR has almost out of necessity been studied in isolated conditions rather than in situ where it is postulated that cyclical changes occur in its state of Ca^{++} activity.

The Na^{+} K^{+} ATPase has not been reported in SR (Wolienberger, 1967), but Palmer and Posey (1967) found a rapid release of Ca^{++} by Na^{+} in isolated rabbit cardiac reticulum suggesting that a Na^{+}/Ca^{++} competetion may operate at the level of the SR. Smith et al., (1972) have indicated that the primary receptor site for Ouabain and other cardiac glycosides is associated with the plasma membrane and deep within it. However, they also suggested that lesser concentrations of glycoside might be attached to other areas of the cell namely the so-called microsomal fraction.

Recently Gibbons and Fozzard (1971) have suggested that Ca^{++} taken up by cardiac SR is bound and removed by an active pump to another region of SR which is not identical to the uptake region. Beeler and Reuter (1970a, b) and Gibbons and Fozzard (1971) have shown that this translocation is both voltage and time dependent and that it would involve shifting the Ca from a primary storage site of SR into another.

This would correspond in our model to the movement of Ca^{++} from uptake site to release site in the SR, and in the case of the fast recovery component, accelerate the filling of Ca_s-1.

We may summarize these observations in the following manner:

a) it appears as if SR is capable of accumulating Ca^{++} from the myoplasm.

b) the role of the SR does not appear limited to Ca^{++} accumulation, but may transport Ca^{++} from one portion (uptake portion) of the SR network to another (a releasing portion).

c) the transport process may be time dependent.

d) there is no clear-cut evidence that ouabain, or Na^+, affects the SR transport, uptake or release of Ca^{++}.

These data suggest that a likely ultrastructural analogue for the model in Figure VI-5 is the one shown in Figure VI-6. We should point out here where the effect of ouabain might be occuring in our model. It has been shown that Ouabain inhibits the membrane ATPase (Lee and Klaus, 1971). It is possible that our Ca_s-2 pool might be affected, i.e., Na^+ transport across the membrane is inhibited, an increase in $[Na^+]_i$ would occur close to the membrane and Ca^{++} might be displaced from Ca_s-2. This Ca^{++} in turn will be taken up by the SR and pumped into Ca_s-1. Thus the administration of ouabain would result in the shift of Ca^{++} in Ca_s-2 to Ca_s-1. The effect of low $[Na^+]_o$ might result in the same process i.e., a shift of Ca from sarcolemmal sites to the site corresponding to Ca_s-1 (the terminal cisternae).

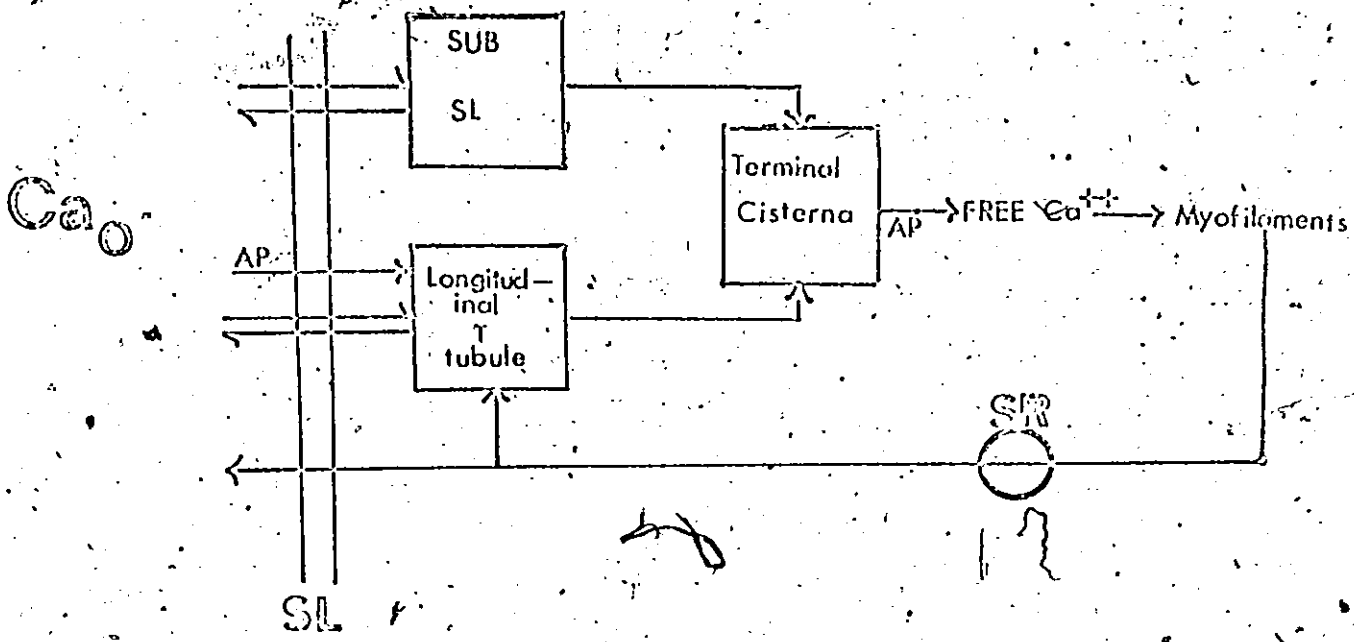


Figure VI-6. Ultrastructural Model of Calcium Movement in Rat Cardiac Muscle.

Figure VI-7 is a diagramatic representation of the calcium movement in rat cardiac muscle. Ca^{++} can cross the sarcolemma (SL) and is attached to membrane sites. The Na^+, K^+, ATPase would be located in the membrane as well. Ca^{++} which enters the cell with each action potential enters the myoplasm and is taken up actively by the SR or the longitudinal elements of the T system in the terminal cisternal region. Sr Ca^{++} is transported to releasing sites in the terminal cisternae where it approaches the longitudinal elements of the T system. Ca^{++} can diffuse out of the cell through the T system or can diffuse to releasing sites in the terminal cisternae. The release of Ca^{++} from the terminal cisternae activates contraction and the uptake of Ca^{++} by SR achieves relaxation. Calcium recycles from the myofilaments to the SR to the terminal cisternae (releasing area) in the main although Ca^{++} from the membrane sites also participates in contraction. At low $[Ca^{++}]_o$, and high temperatures, the major contribution is from surface Ca^{++} while at low temperatures, Ca^{++} is derived mainly from cisternal elements (the terminal cisternae releasing area).

When ouabain is administered it blocks sodium transport at the SL level; Na^+ builds up on the inside of the membrane and a Na^+/Ca^{++} competition displaces this Ca^{++} to the longitudinal elements of the T system. At low pH, the Ca^{++} in either the membrane or the internal sites may be displaced and RSC tension will decrease.

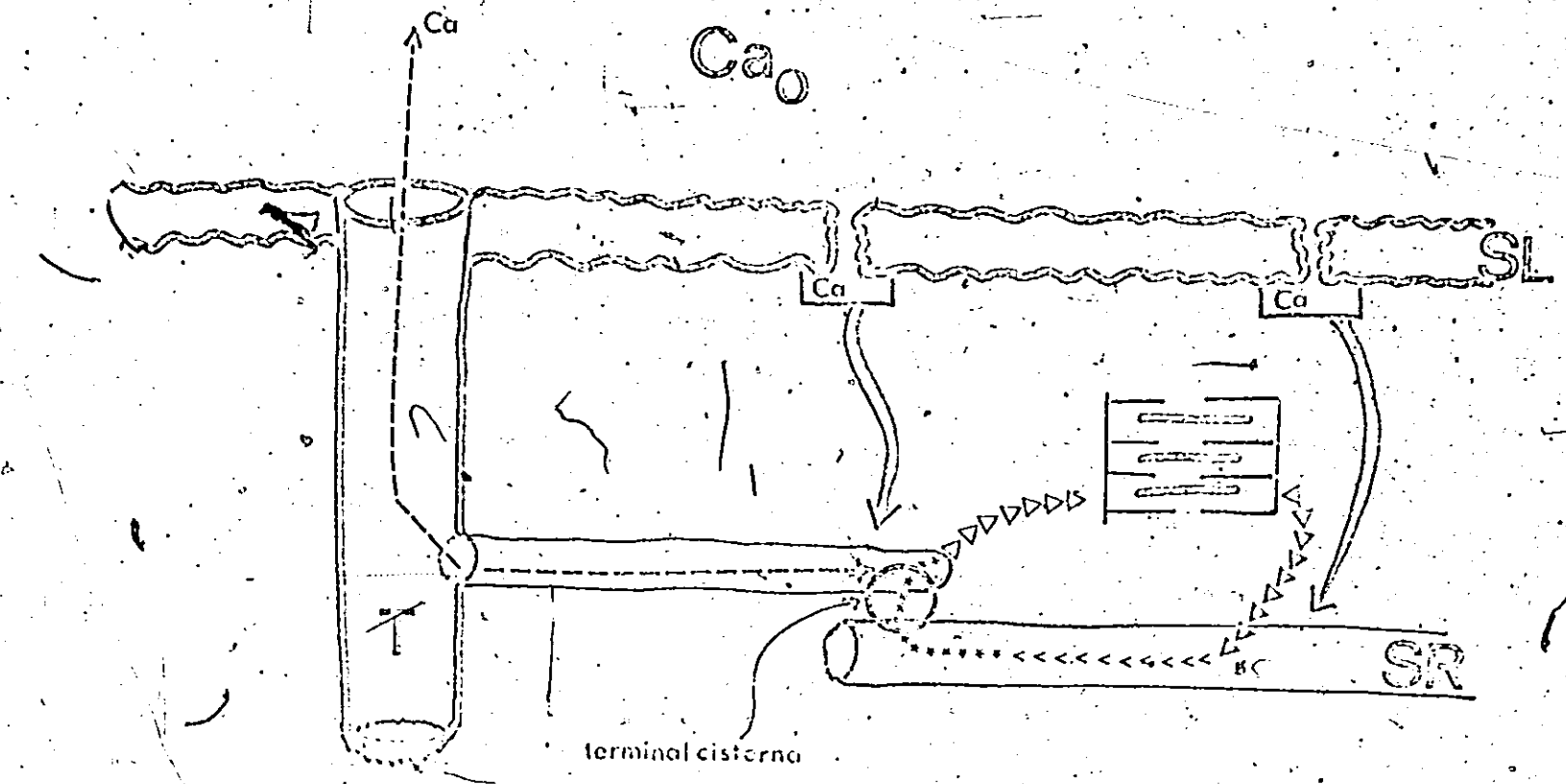


Figure VI-7. Diagramatic Representation of Calcium Movement in Rat Cardiac Muscle.

In conclusion, we have presented a model of calcium movement in rat myocardium which suggests that contractile calcium is derived from three pools. Because the kinetics of calcium movement between these pools appears to be rather fast it does not appear feasible that conventional washout and efflux/influx techniques can be used to assess the absolute sizes of these pools. However, if one guessed that the size of the Ca_s pools were 5-10 times larger than the Ca_r pool (the tension determining pool) the total amount of Ca_s calcium would be 250-500 μmoles Ca^{++}/ Kg when muscle is fully activated. We have found the total calcium content of the muscle, Ca_m, is many times in excess of the contractile requirements.

The calcium in rat myocardium must therefore be very tightly compartmentalized between non-contractile and contractile pools which would make direct measurement of the contractile calcium even more difficult.

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APPENDIX

AN EXPRESSION FOR THE RELEASE OF CALCIUM FROM INTERNAL STORES DEPENDENT UPON STIMULATION FREQUENCY.

Let us consider a situation where we would have only a single storage pool contributing to the recovery of a tension determining pool. For example we would have such a situation when $[Ca^{++}]_o$ approaches 2.5 mM.

In Figure A-1 we have calcium stored in calcium pool Ca_s having a volume V_s and thus C_s is concentration and Q_s is amount of calcium. Similarly this calcium refills pool C_r (concentration) with volume V_s with an amount Q_r. Ca_s exchanges calcium between outside calcium (C_o) with rate constants K_1 and K_2.

The transfer of calcium to Ca_r from Ca_s is frequency dependent. Therefore, the calcium that can be released from Ca_r is $F \times Q(\frac{1}{F})$. This calcium is fed back to Ca_s as fraction α or is lost from the system $(1-\alpha)$. An amount of calcium can be gated into the cell and is a fraction (A) of the concentration difference between C_o and C_s. Thus the gated calcium is $F \times A\ (C_o - C_s)$. In the steady state when $t > \frac{1}{F}$:

$$K_1 C_o + FA\ (C_o - C_s) + \alpha FQ(\tfrac{1}{F}) = FQ(\tfrac{1}{F}) + K_2 C_s \quad \ldots\ldots 1$$

$$FAC_s + FQ(\tfrac{1}{F}) + K_2 C_s - \alpha FQ(\tfrac{1}{F}) = FAC_o + K_1 C_o \quad \ldots\ldots 2$$

When calcium is released from Ca_r

$$Ca_r(t) = C_s\ (1 - e^{-1/FT}) \quad \ldots\ldots 3$$

and so $Q(\frac{1}{F}) = C_s V_r (1 - e^{-1/F\tau})$

Now $FAC_s + K_2C_s + F(1-\alpha) C_s \cdot V_r(1 - e^{-1/F\tau}) = C_o (FA+K_1)$ 5

So

$$C_s = \frac{C_o (FA+K_1)}{FA+K_2+F(1-\alpha) V_r (1 - e^{-1/F\tau})} \quad \text{.....6}$$

and solving for $Q(\frac{1}{F})$

$$Q(\frac{1}{F}) = \frac{V_r (1 - e^{-1/F\tau}) C_o (FA + K_1)}{FA+K_2 + F(1-\alpha) V_r (1 - e^{-1/F\tau})} \quad \text{.....7}$$

This is the solution for calcium released from Ca_r when we have a single pool determining tension development. However, we have found that a two-pool system is more descriptive to Ca_r filling. To simplify the mathematics of this system we can imagine that we have two parallel systems - one which operates as in Figure A-1 and a second which is parallel to it but does not have feedback (α) and has no gated calcium (A). This is shown in Figure A-2.

Thus equation 7 becomes equation 8 for the second pool and $Q(\frac{1}{F})$ describes calcium released from Ca_r-2 in the steady state.

$$Q(\frac{1}{F})\text{-}2 = \frac{V_{r2} (1 - e^{-1/F\tau_2}) C_o K_i'}{K_2' + FV_{r2} (1 - e^{-1/F\tau_2})} \quad \text{.....8}$$

But now Ca_s-1 receives the calcium released from Ca_r-2 which is

$$Q(\frac{1}{F})\text{-}2 \times F \times \alpha \quad \text{.....9}$$

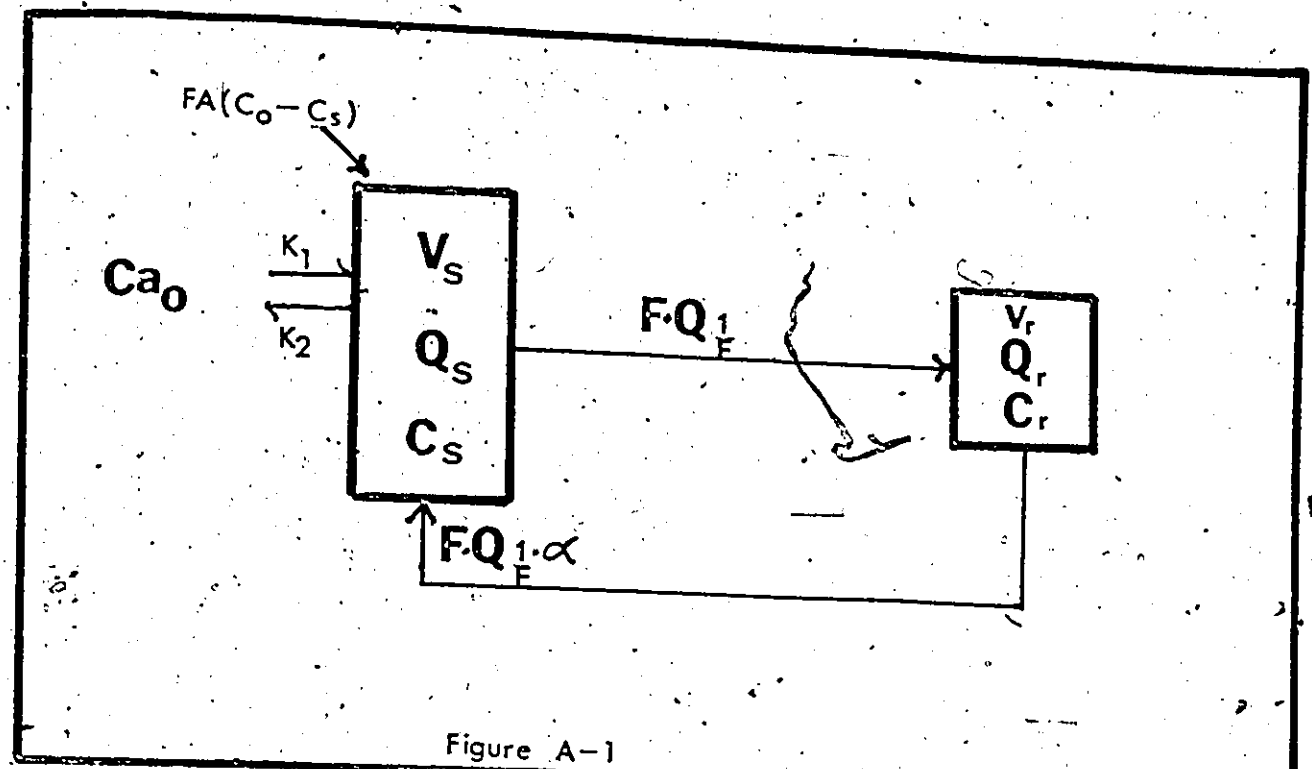


Figure A−1

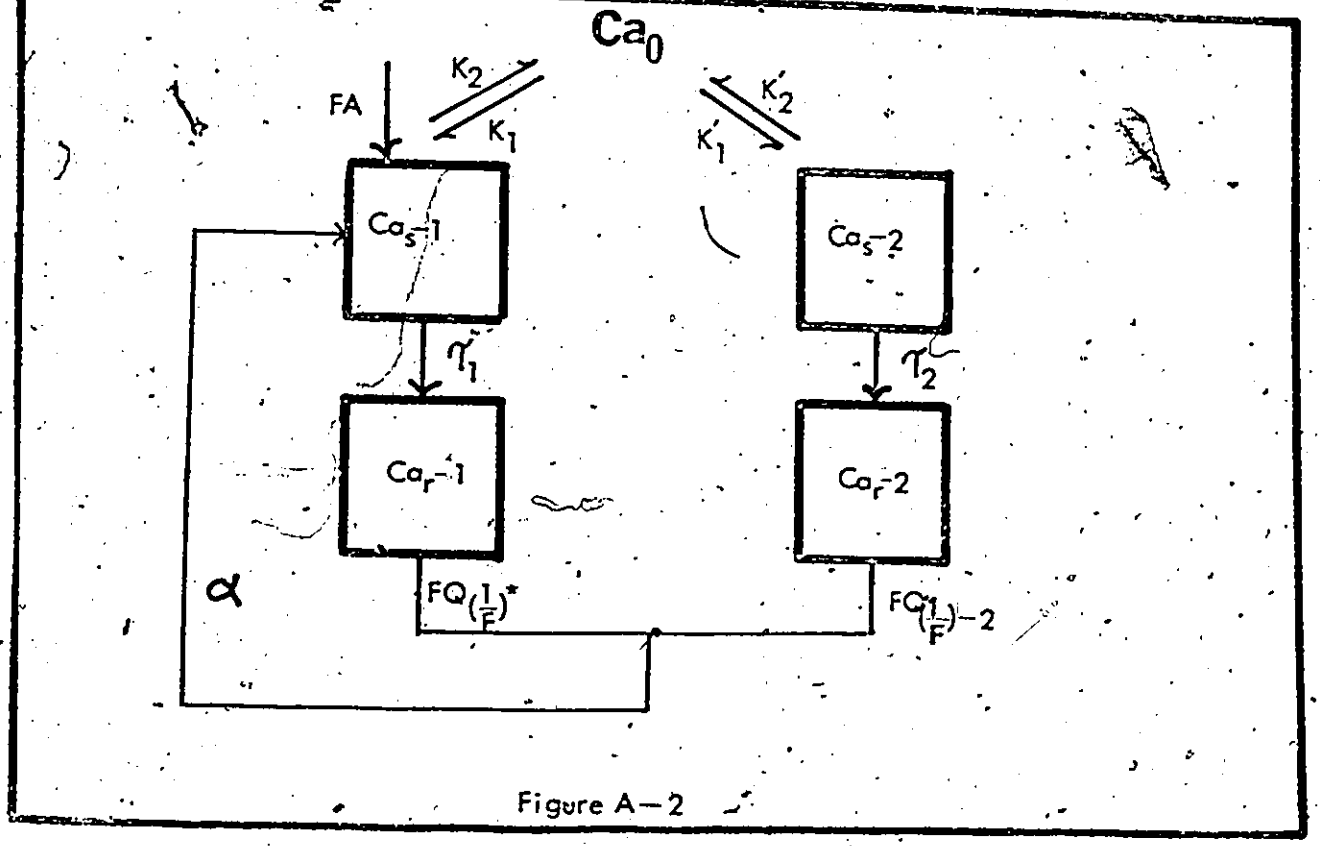


Figure A−2

Now the equation for Ca_s-1 is

$$FQ\left(\frac{1}{F}\right)-2 + FA\,(C_o - C_s) + K_1 C_o + \alpha FQ\left(\frac{1}{F}\right) - 1 = FQ\left(\frac{1}{F}\right)-1 + K_2 C_s$$

and

$$FAC_s + FQ\left(\frac{1}{F}\right)-1 - \alpha FQ\left(\frac{1}{F}\right)-1 + K_2 C_s = FAC_o + K_1 C_o + FQ\left(\frac{1}{F}\right)-2 \quad \ldots\ldots\ldots 10$$

$$C_s^* = \frac{C_o\,(FA + K_1) + FQ\left(\frac{1}{F}\right)-2}{FA + K_2 + F\,(1-\alpha)\,V_{r1}\,(1 - e^{-1/F\tau_1})} \quad \ldots\ldots\ldots 11$$

and the new $Q\left(\frac{1}{F}\right)$ -1 is

$$FQ\left(\frac{1}{F}\right)^* = C_s^*\,(1 - e^{-1/F\tau_1}) \quad \ldots\ldots\ldots 12$$

and the amount released from Ca_r will be

$$FQ\left(\frac{1}{F}\right)^* + FQ\left(\frac{1}{F}\right)-2 = Q_T \quad \ldots\ldots\ldots 13$$

Now with the appropriate values for the rate constants and values of γ we should be able to simulate a condition where:

$Q\left(\frac{1}{F}\right)$-2 begins at a high value and falls quickly with F,

and $Q\left(\frac{1}{F}\right)^*$ increases with F as F increases because of feedback from side 2 of the system.

Results

Table A-1 and Figure A-3 show the results of a simulation where we obtained a positive inotropic response. Although the Q_T value does not fall off abrubtly above 120/min (F= 2.) we can modify the equation to suppose to $A \rightarrow 0$ as $F \rightarrow 2.0$ sec. i.e., as F increases less calcium per beat is gated in. We would then have a fall in the contribution from A and Q_T would fall.

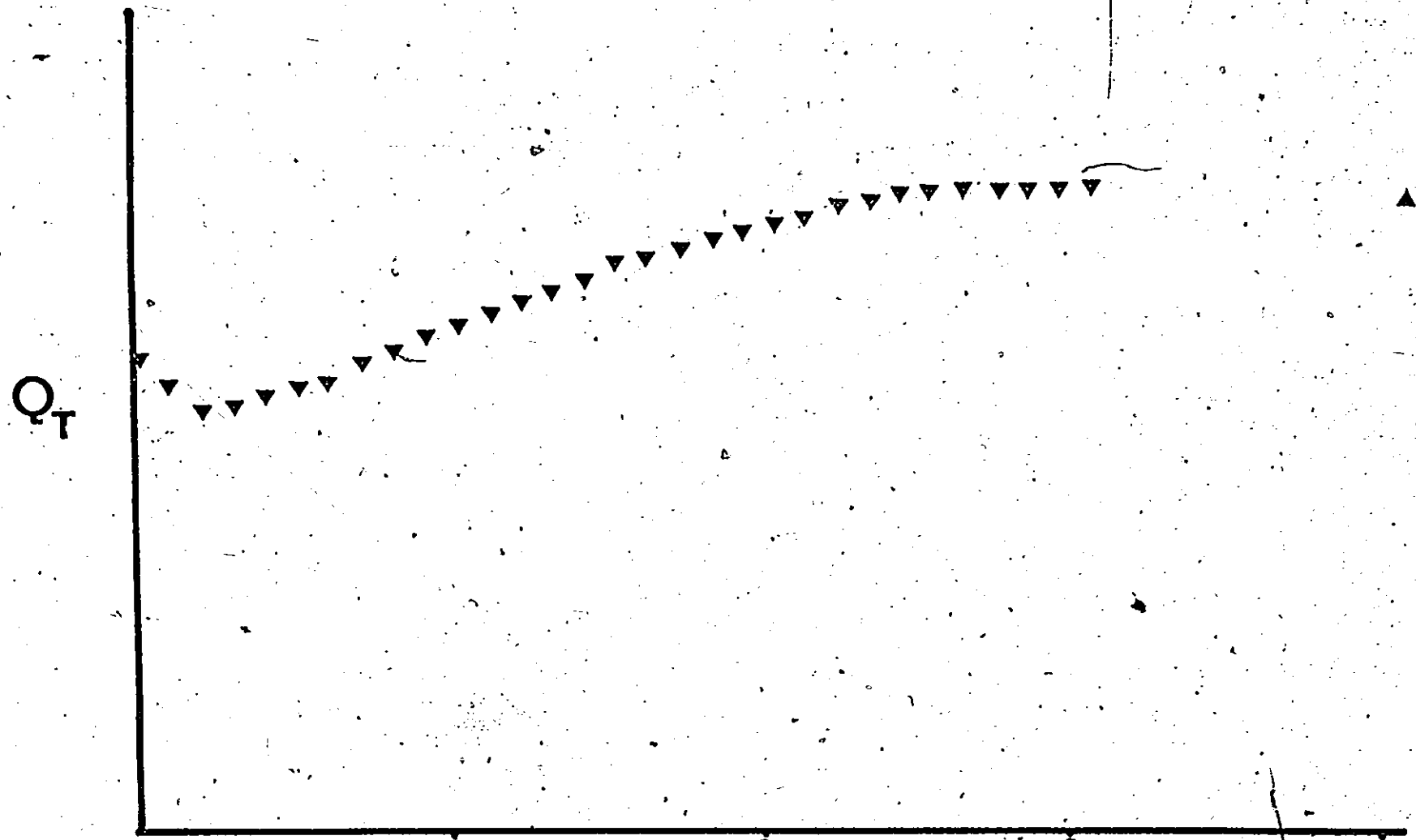
Table A-1

Computed Release of Calcium in T-F Response of Papillary Muscle

Frequency (F) (sec.)	$Q(\frac{1}{F})$-* Ca units	$Q(\frac{1}{F})$ -2 Ca units	Q_T Ca units
0	.25	.075	.325
.1	.275	.036	.311
.2	.285	.021	.306
.3	.292	.015	.307
.4	.299	.012	.310
.5	.305	.009	.315
.6	.311	.008	.315
.7	.317	.0069	.323
.8	.322	.0060	.328
.9	.327	.005	.333
1.0	.333	.0048	.337
1.2	.342	.004	.346
1.4	.351	.0034	.354
1.6	.358	.003	.361
1.8	.366	.0026	.369
2.0	.372	.0025	.375
3.0	.395	.0016	.397

V_{r2}	0.1	τ_2	15 sec.	V_{r1}	0.3
C_o	2.5 units	K_1	.02 $sec.^{-1}$	τ_1	0.1 sec.
K'	.03 $sec.^{-1}$	K_2	0.2 $sec.^{-1}$	A	.01
K_2'	0.1 $sec.^{-1}$		0.9		

Figure A-3. Simulation of T-F Response of Rat Papillary Muscle Ordinate Q_T - amount of Calcium Released from Ca_r Pool at Frequency F (sec.).


Q_T
1
2
3
4
Frequency (F) sec

The example we have shown results only from preliminary testing of these equations. We have included these results in an appendix simply to demonstrate that with the appropriate kinetics of calcium contribution from the two hypothetical pools Ca_s-1 and Ca_s-2 we could mimic the triphasic T-F relationship we observe in the rat with low external calcium concentration.*

* The equations in the appendix are based upon the assumption that any oscillations or changes in the Ca_s pools are small compared to the pool sizes and therefore the results from these equations can only be considered as rough approximations of the true steady-state values.

A truer estimation of the system is now in the process of being formed in co-operation with the Computer Science Department using an analogue computer.