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CONTENTS

LIST OF ABBREVIATIONS

A	Atrial muscle strip from left atrium
ADP	Adenosine 5' - diphosphate, disodium
A.P.	Action potential
ATP	Adenosine 5' - triphosphate, disodium
dL/dt	Maximum rate of length change (shortening) of a muscle
dT/dt	Maximum rate of tension development
EDTA	Ethylenediaminetetraacetic acid
EGTA	[Ethylenebis (oxyethlenenitrilo)] tetraacetic acid
F-V	Force-velocity
L max	Muscle length at which the maximum tension is developed
MT	Maximum tension in a twitch or contracture
P	Papillary muscle from left ventricle
PE	Parallel elastic element
SE	Series elastic element
SEM	Standard error of the mean
SR	Sarcoplasmic reticulum
T	Tension
TMdT/dt	Time from onset of tension development to maximum dT/dt
TMT	Time from onset of tension development to maximum tension
TMVce	Time from onset of tension development to maximum Vce
T system	Transverse tubular system; T-tubules
V	Trabeculae or papillary muscle from ventricle
Vce	Velocity of shortening of the contractile elements
CE	Contractile Element
RT	Resting tension
$\underline{P}$	Probability value

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ABSTRACT

Excitation of cardiac muscle membranes leads to actin-myosin interaction which is expressed externally as tension or shortening. In experiments presented here the atria (A) contract and relax with greater velocity and with a shorter time course than the ventricles (V), both isometrically and isotonically. This was observed in isolated heart strips from several species (rat, guinea pig, cat) at various stimulation rates, at different temperatures and also after treatment with reserpine and/or propranolol. Various preloads and afterloads as well as different concentrations of calcium did not eliminate the differences between A and V. An index $\frac{dT/dt}{MT}$ was used to compare A and V contractions and was always higher in A under similar experimental conditions. Throughout the length-tension curves, the resting tension expressed as a % of MT was higher in A than V. The force-velocity curves appear hyperbolic in V but not in A. The % extension of the series elastic component was similar in A and V when measured as % change in muscle length required to return tension to zero from MT.

To identify the level in the excitation-contraction process which might determine the differences in A and V time course and rate of contraction, some cellular structures were altered:

1) EDTA treatment produced high sarcolemmal permeability and eliminated the need for electrical stimulation. Subsequent contractures produced by Ca^{2+} addition exhibited higher rate of tension

development and shorter time to peak tension in A than in V.

2) Long-term glycerination disrupted the membranous systems of the cells. Contractures were produced by Ca^{2+} and ATP. No significant difference in initial rate of tension development or time to maximum tension could be observed between A and V in the following conditions: a) during the first contracture b) during tension redevelopment after quick releases to different lengths or c) during contracture development in presence of creatine phosphokinase.

3) After isolating the myofibrils, ATPase activity was measured and no consistent relationship to the muscle shortening velocity was observed.

Thus, the sarcolemma and contractile proteins do not appear to be the major determinants of the difference in rate and time course of contraction between A and V, assuming that twitch and contractures are controlled by the same subcellular sites. Furthermore, it may be that the transport, uptake and binding of calcium contribute significantly toward the differences between A and V.

I. INTRODUCTION

A series of experiments by Otto Frank (1895) demonstrated the dependence of tension (pressure) on the degree of heart filling (volume) by presenting a family of isometric curves from frog atrium and ventricle (Figures 1a, 1b). Not only may the familiar "Frank-Starling law of the heart" be seen, but also the time course of contraction of atrium and ventricle may be measured. As observed, the atrial time to maximum pressure, measured from start of pressure development, is considerably shorter than in the ventricle. In Figures 1a and 1b the numbers represent increase in fill volume; and in Figure 1a the number 4 represents a preload fill which is over-optimal and results in a pressure decrease in the ventricle. Even with this added load the time to maximum pressure does not appear to vary. By careful examination of other reports such as Graham et al (1967) the same unique features may be observed in intact mammalian hearts.

The difference in contraction times may be seen even when observing cells in an isolated culture preparation. Figure 2 shows typical photo-optical recordings produced by atrial and ventricular cells in an isolated cell culture of mouse heart (Boder and Johnson, 1972). The slope of the contraction and relaxation curves of atrial cells was much greater than that of ventricular cells, although the time interval between contractions was nearly the same. Total

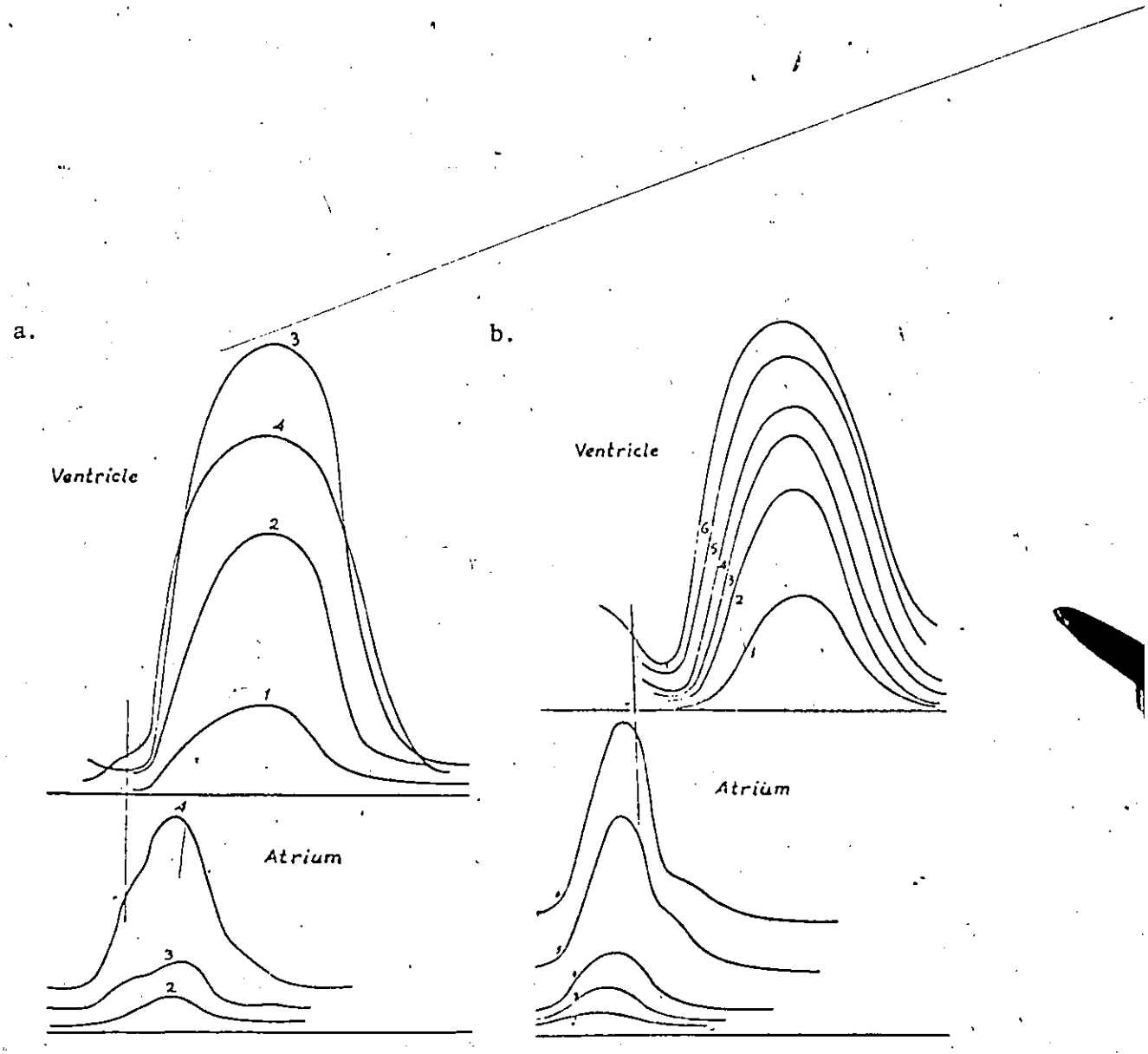


Figure 1 (a and b). Pressure development in the atrium and ventricle of frog heart at various fill volumes. (Frank, 1895).

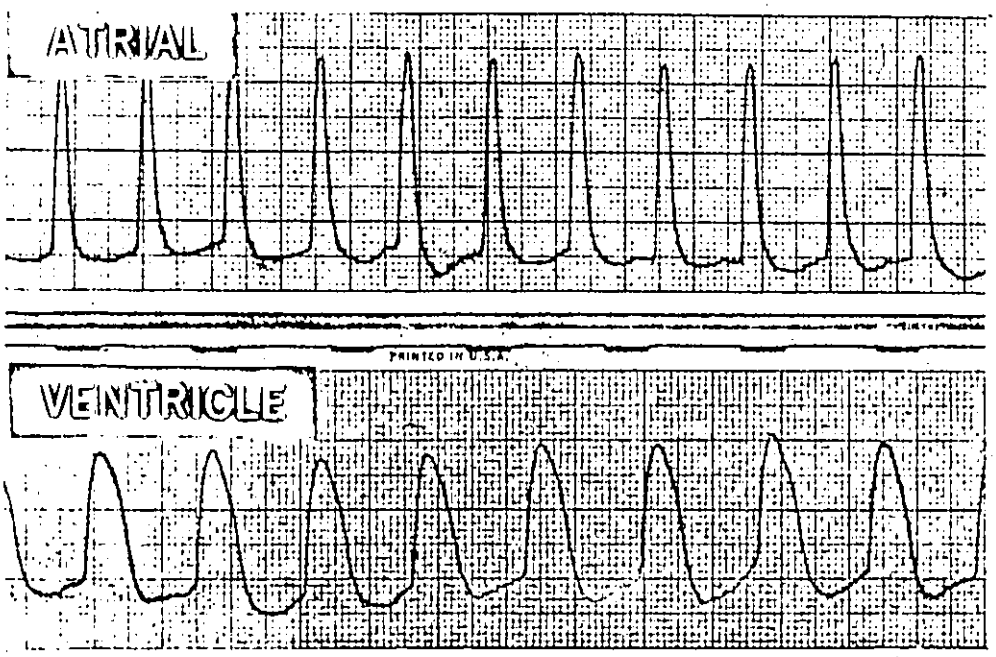


Figure 2. Photooptical recordings from atrial and ventricular cells in an isolated cell culture preparation (Boder and Johnson, 1972).

contraction time also appears less in atrial cells than in those from ventricle.

The third example illustrating the mechanical differences in contraction between atria and ventricles may be seen in Figure 3, which is a record from my initial experiments on rat cardiac muscle. The time from stimulation (at start of trace) to peak tension in atria is much less than the corresponding time in the representative ventricular muscle. Also, the maximum rate of tension development (dT/dt) is higher in the atrium than in the papillary muscle even though atrial developed tension is less. A similar phenomenon has been briefly mentioned by others using isolated electrically stimulated preparations (Blinks and Koch-Weser, 1963; Buccino et al., 1967).

These mechanical contraction differences between atrial and ventricular muscle are present throughout a wide assortment of experimental preparations. The subject of this thesis is the further description and characterization of this phenomenon, as well as an attempt to elucidate some of the underlying mechanisms responsible for the differences in the tension-time relationship. Undoubtedly, there are many major research areas which are worthy of a concerted effort if indeed a totally comprehensive picture is to be gained. Firstly, I have chosen to present a review of the literature relevant to this comparative study of atrial and ventricular myocardium in two of these research areas: 1) electrophysiology and 2) ultrastructure.

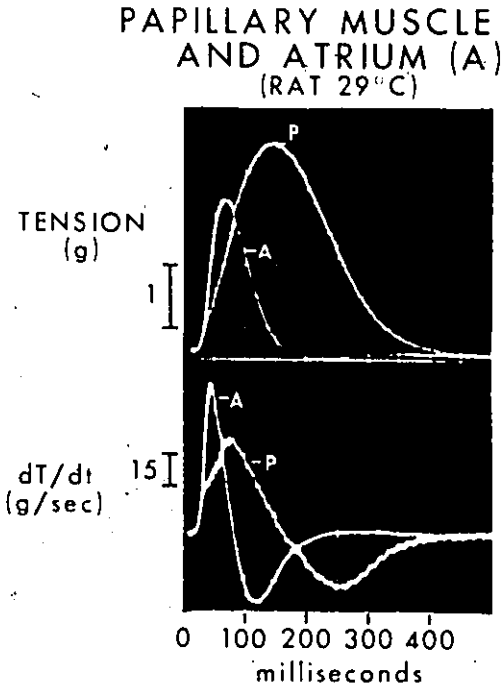


Figure 3. Isometric contraction showing tension (T) and the first derivative of tension (dT/dt) of a rat atrial strip and papillary muscle. Stimulation rate was 5/minute. Muscle weights and lengths were similar.

and biochemical features. This was done so that the reader would have insight into some of the special characteristics of the atrial and ventricular heart regions about which no new experimentation will be presented in this report, but which definitely are related to an understanding of the phenomenon in question.

In addition, the literature review contains relevant background information to the new experiments which will be presented including: isometric and isotonic contraction mechanics, studies involving treatment with ethylenediaminetetraacetic acid (EDTA), glycerol treatment of muscle fibers, and ATPase activity measurement.

This investigation will describe some experiments to characterize the contraction mechanics of isolated strips of atrial and ventricular myocardium under isometric and isotonic conditions. These studies will be carried out under a wide range of experimental conditions such as temperature and stimulation rate alteration. Various attempts will be made to change the shape of atrial twitch contractions to approximate the ventricular characteristics and vice versa. Indices for better comparison of atrial and ventricular contractions will be sought.

The second phase of this study will involve elimination of certain cellular structures in order to ascertain control sites which determine the differences between the atrial and ventricular contraction time courses. The two different methods to achieve this goal will be: 2.) EDTA treatment to disrupt the normal semi-permeable state

of the sarcolemma and b.) Glycerination to disrupt all subcellular structures except the contractile proteins. After these respective treatments and chemical stimulation, the contracture tension development will be analyzed in each case.

The last phase of the experimentation will involve a biochemical analysis to determine the ATPase activities of the contractile proteins from these two heart regions.

In relation to the past knowledge and with the new information generated from these experiments it is hoped that a better understanding of the mechanisms creating differences in atrial and ventricular contraction will be gained, as well as important directions for future research.

II. LITERATURE REVIEW

A. RESTING AND ACTION POTENTIAL

1. Introduction

Membrane potential changes occuring prior to and during muscle contraction are a central concern in an investigation of factors which determine the rate and time course of contractions such as seen in atrial and ventricular muscles.

Although the dT/dt of amphibian (frog) myocardial contractions remain unchanged throughout manipulations of the membrane A.P., changes in the T.M.T. and M.T. are related to the effects of hyper - and depolarizing currents on the A.P. concurrent with a particular contraction (Antoni et al., 1969; Goto and Brooks, 1970; Kowata et al., 1969). Similar measurements in mammalian cardiac muscle seem to show that changes in shape of the twitch are related to alteration of the A.P. which is subsequent to the applied currents; however, considerable data relating mammalian cardiac contraction and the A.P. agrees substantially with results as mentioned above for frog myocardium.

Very few investigators have attempted to measure contraction and/or A.P. of both atrial and ventricular fibers on a comparative basis. Electro-physiological and mechanical studies of these two heart regions might provide a description of the normal state as well as the influence of stimulation rate and temperature on the A.P. and concommitant contractions. Before presenting this information it is

necessary to consider some basic elements of cardiac membrane potentials and methods for analyzing the A.P.

2. Excitation and Permeability

As presently established, the excitation process triggers contraction in both skeletal and cardiac muscle (Sandow, 1965; Huxley, 1971; Langer, 1973). This process is mediated by sequential variation in sarcolemmal membrane permeability to various ions resulting in electrical potential changes across the membrane. These potential changes in sequence is called an action potential (A.P.). It has been suggested that the A.P. may serve not only as a trigger but also as a determinant of the magnitude and duration of the contraction. This may be the case because the cardiac muscle A.P. and twitch contraction have a similar total time duration which lasts several hundred milliseconds. Events which cause the steady-state plateau during the repolarization phase of the A.P. are proposed to be the main determinants of the A.P. duration (Hoffman and Cranefield, 1960).

Similar interplay of changes in membrane permeability to sodium, potassium, and possibly calcium seems to exist in nerve, skeletal muscle, and atrial and ventricular cardiac muscle in so far as sodium and potassium are the main carriers of inward and outward current respectively (Olson and Waud, 1970; Hoffman and Suckling, 1954). Although the same ions may be involved in the above tissues to produce the A.P., its configuration is different and characteristic for each tissue. It is most probable that changes in A.P. shape in

one tissue or from tissue to tissue are the result of variation in ion conductance during repolarization which is both time and voltage dependent (Legato, 1973). In other words, the ion conductance varies quantitatively at several points in time as the A.P. is generated. At present, the complexity of conductance changes during repolarization has not been fully explained. Indeed, Fozzard and Gibbons (1973) state that there appear to be eight separate ionic channels acting in a complex way to produce the distinctive shape of the cardiac action potential.

3. Action Potentials from Atrium and Ventricle

Various phases of the A.P. have been defined to facilitate comparison and are characterized as follows: 0- initial rapid upstroke; 1- early rapid repolarization; 2- prolonged slow repolarization (plateau); 3- terminal rapid repolarization; and 4- diastolic period (Figure 4).

In atrial muscle, separation of phases 2 and 3 becomes a difficult task. Also, phase 4 is characterized by a slow return of the transmembrane potential to the resting value. Compared to the representative dog atrial A.P., the rat ventricular A.P. (not shown) is similar in shape, while the rat atrial A.P. contains considerably less phase 2 and is even more "triangular" than the atrial A.P. illustrated. Therefore, the rat cardiac A.P. is much shorter in duration than the dog, and its phases are consequently more abbreviated.

Membrane potential data has been selected with greatest emphasis placed on investigations where both atria and ventricles were

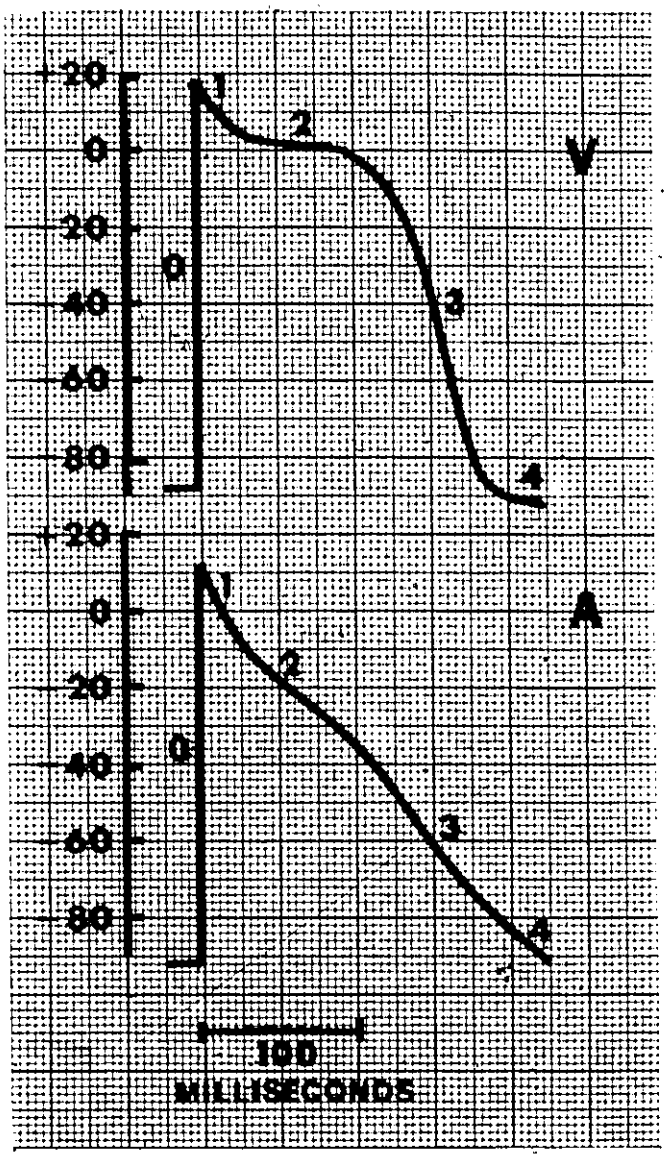


Figure 4. A typical pattern of transmembrane A.P. s from muscle cells of the ventricle and atrium. These were recorded using flexibly mounted intracellular microelectrodes. The records represent the A.P. s measured from dog cardiac muscle fibers.

monitored at the same time (Table 1). It is evident that the atrial and ventricular resting membrane potentials are negative 70-90mV and the A.P. magnitude is 75-120mV with total A.P. duration similar in the two muscles. Furthermore, it has been shown that if surface electrodes are used for stimulation, the electrical excitability of the atrium is similar to that of the ventricular muscle from the same heart. This is expressed in the fact that the resting threshold and strength—duration curves are quite superimposable.

Many experimental alterations produce a change in the magnitude and shape of the A.P. The effect of temperature changes and stimulation rate will be considered.

4. Physical Conditions which Affect Action Potential Shape

a. Temperature

In both atria and ventricles cooling causes a marked increase in A.P. duration, largely due to a decrease in slope of phase 2 (Burgen and Terroux, 1953; Braveny and Sumbera, 1970). Heintzen (1954) analyzed the temperature coefficients of various phases of the frog A.P. as temperature was reduced. He divided the repolarization phase into 10 equal voltage steps and determined the duration of each as a function of temperature. Essentially, the shape of the A.P. was altered such that it could not be represented by the Q_{10} of phases 1, 2 and 3 or by any relatively simple analysis. It follows that A.P. recorded at one temperature could not be superimposed upon that recorded at another temperature merely by replotting the voltage-time course with different scales for each phase.

TABLE 1.

Mammalian Atrial and Ventricular Membrane Potentials

Species	Tissue	Resting Potential (mV)	Action Potential (mV)	Duration of A.P. (msec)	Author
Dog	papillary muscle	70-95	82-125	200-300	Hoffman and Suckling, 1953
	atrium	68-95	82-120	200-300	
Cat	papillary muscle	88	116	150-200	Trautwein et al. 1954
	atrium	35-85	30-95	200-250	Burgen and Terroux, 1953
Rat	ventricle	90	120	50-90	Hoffman and Cranefield, 1960
	atrium 23°C	75	70	93	Hollander and Webb, 1955
	30°C	62	75	62	
	37°C	60	71	27	
	42°C	55	65	20	
Human	ventricle	87	115	350-450	Trautwein et al., 1962.
	atrium	70	75	350-450	

Pertinent observations on the effect of cooling on the cardiac A.P. can be summarized by stating that there is a prolonged A.P. duration in all fiber types from all species and that phase 2 is more sensitive to temperature change than are phases 1 and 3 (Hoffman and Cranefield, 1960). This more sensitive phase 2 may be related to movement of calcium and will be discussed later.

b. Stimulation Rate

Another experimental variable which may have an affect on A.P. is the stimulation rate. An increase in stimulation frequency (6 to 300 /min) applied to atrial and ventricular muscle of dog and cat led to a decrease in A.P. duration (Hoffmann and Suckling, 1954; Trautwein and Dudel, 1954). In fact, a linear relationship between increasing stimulation rate and decreasing A.P. duration occurred in dog papillary muscle. As with higher temperature, phase 2 is shortened and the slope of phase 3 is largely unaltered. In addition, Braveny and Sumbera (1970) demonstrated an increase in repolarization rate concurrent with stimulation rate increase in guinea pig papillary muscle. In dog papillary muscle, where less than 50 to 60 stim/min are used, it appears that the A.P. duration is more directly related to the ionic medium and other experimental conditions because the A.P. is not modified.

No significant shortening of A.P. duration, after stimulation rate increase could be demonstrated by Hollander and Webb (1955) in rat atrium. In this case, they suggested that stimulation rate

primarily affects the contractile processes or energy supply mechanisms instead of the A.P. However, this seems contested by the interesting linear correlation between log stimulation rate and log repolarization rate.

It is quite evident that increasing temperature or stimulation rate of the ventricular muscle of many species causes a decrease in the duration and/or alteration of A.P. shape. However, this is less conclusive in rat atria. The next step will be to investigate the interrelationship between the A.P. configuration and the resulting contraction.

5. The Shape of the Action Potential and Its Affect on the Twitch

a. Duration of the Action Potential and its Affect on the Duration of the Twitch

The relationship between A.P. and twitch duration seems well validated for many species. Brady (1964) summarized a great many correlations between the electrical and mechanical activity of heart muscle. It must be cautioned that knowledge of the specific set of experimental conditions and species is vital in validly interpreting many such experiments. Braveny and Sumbera (1970), Rumberger (1970) and Kavaler (1959) provide evidence that the T.M.T. is directly dependent on A.P. duration in guinea pig papillary muscles. Sumbera's analysis revealed that the duration of applied depolarizing current was linearly related to the area under the contraction curve, the contraction amplitude increment, and the dT/dt. Similarly, maintaining

that the amplitude of developed tension is strongly influenced by the level of depolarization, Morad and Trautwein (1968) described an S-shaped curve between the A.P. duration and T.M.T. The reports agreed that alterations during the first 33-40% of A.P. duration could alter the time course, amplitude and rate of tension development in dog, cat, and sheep ventricles. Furthermore, for the atria and ventricles of guinea pig hearts, Braveny and Sumbera (1968) demonstrated a direct relationship of A.P. duration (measured to $\frac{1}{2}$ repolarization time) and T.M.T., including temperature changes (23-39°C) and Ca^{2+} concentration changes (0.8-7.5mM). Therefore, the interdependence of A.P. duration and contraction time is fairly well validated. Less distinct, however, is the interdependence of the A.P. amplitude or duration and the magnitude of contraction which is discussed in the next section.

b. Magnitude or Duration of the Action Potential and its Affect on the Magnitude of the Twitch.

Wood, et al., (1969) lengthened the A.P. by subthreshold depolarizing currents or by 5mV shifts in membrane potential during the A.P. phase 2 and subsequently recorded positive inotropic effects in sheep ventricular trabeculae. But in the report by Hollander and Webb (1955) cited above for rat atria, no correlation was found between developed tension and action potential height. In addition, Sleator, et al., (1964), using guinea pig atria, noted a lack of cause-effect relationship between A.P. configuration and contraction

strength; some atria even exhibited shortened phase 2 coincident with greater tension development. On the other hand, a longer phase 2 was associated with a weakened contraction in dog papillary muscle (Greenspan et al., 1967). Finally, Mainwood and Lee (1969) found that paired stimuli applied to rat papillary muscle resulted in unchanged A.P.s but altered amplitude of contraction.

6. Action Potential AS INFLUENCED BY CONTRACTILE TENSION

In contrast to the predictability of contraction response from A.P. alteration in frog myocardium, studies with mammalian cardiac muscle suggest a more complex coupling between force and potential change (Johnson and Lieberman, 1971). Spear and Moore (1971) have shown that after an increase in stimulation rates, applied to ventricular muscles of cat, rabbit and guinea pig, the alteration in tension occurred prior to alteration in A.P. shape. Greenspan et al., (1967) suggest that action potential alteration need not initiate contractile change, but both A.P. and contractile change may reflect variations in ionic flux or membrane permeability which is conducive to contractile potentiation. It should be stressed that the A.P. of rat papillary muscle is quite stable except for slight differences in the late phase of repolarization throughout large degrees of mechanical alteration (Spear and Moore 1971).

7. Problems Inherent in Relating the Action Potential to the Twitch

Relating the A.P. configuration with the consequent tension development or shortening involves the universally accepted role of calcium (Ebashi and Endo, 1968; Katz, 1970) as the final activator of

the mechanical event according to the following scheme:

a. membrane potential change stimulates calcium movement via some process (probably simple diffusion);

b. the released calcium moves into the vicinity of the myofilaments, reverses the troponin inhibition, and consequently allows actin-myosin interaction and the subsequent mechanical event.

However, the applicability of this scheme to the A.P.-mechanical output relationship calls for several important considerations. First,"Is the greater part of calcium, which participates in contraction, released through, or from, the sarcolemma across which the A.P. is measured?" The commonly accepted view, as postulated for skeletal muscle, is that the A.P. initiates a series of intermediate steps which in turn release calcium from the sarcoplasmic reticulum (S.R.) (Huxley, 1971). However, Langer (1971) believes that in mammalian heart muscle the sarcotubular (S.R. + T-system) calcium may not play a vital role in the activation process, a view also held by Legato (1973). In addition, the sarcotubular membrane areas per unit of cell volume are considerably smaller in cardiac muscle than in skeletal muscle. Certainly, rates and time courses of the required intermediate steps beginning at the A.P., proceeding through calcium movement, and culminating in contraction must be taken into account when relating the twitch contraction to the previous A.P. shape. Furthermore, an A.P. may not only trigger release of internally stored calcium to produce a

contraction but in addition the shape of the A.P. may strongly influence the amount of calcium available for release by the next A.P. (Beeler and Reuter, 1970).

Second, the mechanical response recorded from the whole preparation should represent the mechanical activity of the cell or group of cells from which the A.P. is measured. Furthermore, the measured depolarization and repolarization may not accurately represent the membrane potential change for a large group of cells. Obviously, the output from a small number of cells could be monitored more precisely as is done on ventricular fibers in voltage clamp experiments (Deck et al., 1964; Fozzard and Hellam, 1968; Reuter, 1967). However, no voltage clamping has been reported for mammalian atrial fibers.

Third, since most of the recordings are isometric, tension includes interplay between the contractile and elastic elements of the muscle (Hill, 1938). Therefore, in comparing the A.P.-contraction relationship in different muscles a knowledge of differences in, for instance, series elasticity is needed. This necessarily follows because series elasticity may affect the rate and time course of the mechanical response.

B. ULTRASTRUCTURAL AND BIOCHEMICAL SIMILARITIES AND DIFFERENCES BETWEEN ATRIA AND VENTRICLES.

The explanation for contraction differences between atria and ventricles may rest on ultrastructural and/or biochemical features of the individual cells or unique variations in intercell connections.

The following morphological descriptions of heart ultrastructure of certain mammalian species, including the rat, will suggest differences and similarities of the two cardiac tissues leading to an appraisal of structure-function correlation aspects.

1. Description of Subcellular Morphology of Cardiac Muscle Fibers.

The essential function of the cardiac muscle cell (cardiocyte) is generation of an orderly contractile response after excitation. The morphologic features of the cells which relate to the excitation, contraction and relaxation phases are of special interest. Structural characteristics for atria and ventricles in three species are summarized in Table 2.

Each cardiac fiber has a nucleus, sarcomeres, mitochondria and sarcoplasmic reticulum (S.R.). The basic anatomical contractile units of the cell are the sarcomeres. Each sarcomere is composed of several different kinds of protein filaments with the primary and best defined being actin and myosin which in rat ventricular muscle is 48% of the normal cell volume (Page and McCallister, 1973). Kovats (1949) found a variation in actin and myosin concentration in pig and rabbit myocardium. After excising the same amount of tissue from atria and ventricles, he extracted less myosin and actin from the former. Along these same lines, Helander (1962) reported less (2/3) myofilament protein nitrogen in beef heart atria than in ventricles. Groups of filaments comprise units called myofibrils in most mammalian cardiac cells. An exception to this was noted in cat (Fawcett and McNutt

TABLE 2.

Comparison of Atrial and Ventricular Cells in Human (*), Cat (x), and Rat (θ).

FEATURE	ATRIAL CELL	VENTRICULAR CELL
Size and Shape	(*) elliptical, width 6-8 , length 20 u (x) width 5-6u, length-no value (θ) width-no value, length-no value	(*) cylindrical, width 15-20u, length 100u. (x) width 9-10u, length-no value (θ) width 20u, length 100u. (unpublished, Korecky)
Organization of Contractile Material	(*) arranged in long parallel rows. (x) myofilaments not arranged into units. (θ) wider range of myofibril densities from one cell to another	(*) same (x) same (θ) less myofibril density variation
Mitochondria	(*) less abundant (x) more abundant (θ) more spherical, less closely packed	(*) more abundant (x) less abundant (θ) volume appeared less
Granules	(*) "atrial" granules with distinctive composition, Type A,B,C and D. (θ) many 0.2-0.4 u granules	(*) "residual" bodies with high catecholamine content, Type C granules. (θ) absent
Transverse Tubular System	(*) absent (x) very few (θ) none in comparison to that seen in ventricle	(*) abundant (x) abundant (θ) abundant
Sarcoplasmic Reticulum	(*) well developed and abundant (θ) not as well organized	(*) well developed and abundant (x) more organized
Intercellular	(*) intercalated discs (I.D.) mainly oriented horizontal to the long axis of the cell forming side-to-side connections. (x) I.D. often oblique to myofilament axis (θ) more loosely arranged and less regularly oriented cells	(*) I.D. oriented with specialized areas perpendicular to long axis. (x) I.D. not often oblique to myofilament axis. (θ) less loosely arranged cells

Major Sources: McNutt and Fawcett, 1969; Fawcett and McNutt, 1969; Hibbs and Ferrans, 1969; Legato, 1973; Forssmann and Girardier, 1970; Berger and Rona, 1971.

1969; McNutt and Fawcett, 1969) where no definite myofibrils could be seen, only a homogeneous mass of filaments. The dark Z-bands which bound the sarcomere are the points of attachment of thin actin filaments. These actin filaments, at either end of the sarcomere, slide toward each other as cross-linkages are sequentially made and broken with the myosin filaments (Huxley and Taylor, 1958). The so-called regulatory proteins, troponin and tropomyosin, under the influence of available calcium appear crucial in initiating and/or inhibiting the actin-myosin interaction (Ebashi and Endo, 1968).

Mitochondria are profuse in cardiac muscle fibers occupying an estimated 36% of rat ventricular fibers (Page and McCallister, 1973), 39% of rabbit ventricular fibers and 28% of rabbit atrial fibers (Pratt, 1967). Situated between the fibrils, mitochondria assume a great variety of shapes and sizes. For the most part, they are randomly oriented, 2-9μ in length and cylindrical. However, some may have both lateral and longitudinal projections and lobes (Fawcett and McNutt, 1969; McNutt and Fawcett, 1969). In the ventricle of mouse heart, mitochondria were found to be 1.65 times larger than in atrium (Herbener, 1966).

The sarcolemma of the ventricular cells gives rise to hollow, branching invaginations called transverse tubules, or collectively the T-system. There is a virtual absence of T-tubules in atrial cardiocytes (Sommer et al., 1972; Hibbs and Ferrans, 1969; McNutt and Fawcett, 1969). Consequently, the typical skeletal and ventricular muscle triad arrangement of one T-tubule surrounded by 2 cisternae of the S.R. is very rarely seen in atrial muscle. In ventricular cells, the T-

system communicates openly with the extracellular space and generally the tubules range from 1500-2000 Å in diameter, a size considerably larger than in skeletal muscle (McNutt and Fawcett, 1969). From the lateral cisternae of the S.R., tubules branch to encompass the myofibrils totally. The S.R. tubules do not appear to be continuous with the T-system but are continuous with other tubules, viz., the longitudinal tubules (L-tubules). Z-tubules have been described in atrial and some ventricular fibers and regularly occur in register with the Z-lines. In rat left ventricular fibers, the fraction of cell volume occupied by T-system and S.R. is approximately 5% (Page and McCallister, 1973). Table 3 summarizes morphologic units of myocardial fibers relevant to contraction and suggests the major physiological actions during muscle activity.

2. Granules in Cardiac Muscle Cells.

Besides lack of a T-system, atrial cells have a prominent quantity of granules. Histochemical investigation and electron microscopy (E.M.) of mammalian myocardial granules as yet do not provide a base on which to conclude function. After density gradient centrifugation and E.M., Susa-Lucero (1969) isolated fractions of granules for biochemical analyses without a loss of ultrastructural detail.

Presence of norepinephrine and epinephrine was determined by relative fluorescence of these compounds in preparations of granules. In rat atria, the results furnished an indication of a norepinephrine-binding protein in association with a specific granular layer, while

TABLE 3

Structure - Function Relationship of Myocardial Fibers

Structural Component	Function	Mechanism
Sarcolemma	Excitation	Depolarizing impulse generated
Transverse tubular system	Excitation	Transmission of depolarizing impulse into fiber
Lateral cisternae of sarcoplasmic reticulum	E-C Coupling	Storage, release of calcium
Myofilaments	Contraction	Actin-myosin interaction in presence of calcium
Tubules of sarcoplasmic reticulum	E-C Uncoupling	Removal of calcium from myofilament space
Mitochondria	Energy liberation, E-C uncoupling	Oxidative phosphorylation, Calcium uptake

Major sources: Langer, 1973; Legato, 1973; Dhalla, et al, 1970; Johnson and Lieberman, 1971.

this was absent in ventricle. On the other hand, work by Hibbs and Ferrans (1969), Jamieson and Palade (1964), and Bold and Bencosme (1972) suggests that the specific granules do not constitute a storage site for catecholamines. No degranulation occurred after 1 mg/kg reserpine administration. However, there was a significant increase in granule number after large doses of norepinephrine and a reduction after atropine. Bencosme and Berger (1971) point out that many of the investigations have not specified size and type of granule studied aside from relating them to catecholamine distribution.

To date, then, it is known that a substantial proportion of mammalian atrial cells contain granules, whereas ventricular cells hold very few. Various types of granules have been distinguished by size as A, B, C and D. Types A, B and D reside abundantly and exclusively in atrial cells, with type C common in both atrial and ventricular cells (Berger and Rona, 1971). Functionally, these membrane-bound, circular or elongated 0.2 -2 μ diameter granules are still being explored.

3. Distribution of Various Compounds in the Myocardium.

Spectrofluorometric analysis and fluorescence histochemistry have been utilized in determining the catecholamine content of various regions of the mammalian heart in many laboratory animals. There is widespread agreement that the atria have a higher concentration of norepinephrine than ventricles and generally higher content in the right than in the left side of the heart (Angelakos, 1969). Variation

in norepinephrine concentration roughly follows the density of catecholamine - containing nerve fibers.

The distribution of one of the other neural elements, acetylcholine (ACh) and two enzymes (cholinesterase and cholinacetylase) involved in its metabolism, has been evaluated in hearts of several species (Vlk and Tucek, 1965 - see Table 4). Specifically, ACh was higher in A than V while the cholinesterase and cholinacetylase was higher in the right than in the left atria. Content and metabolism of ACh was great in atria possibly because:

a. larger numbers of cholinergic nerve endings

b. substantially more ACh per unit of nerve endings, or

c. more extraneural storage sites

James and Spence (1966) localized large amounts of esterase in the human heart conduction system, less in the right atrium and none in the ventricle.

Lower levels of oxidative enzymes, such as succinic dehydrogenase, have been reported in atrial cells but most other compounds are distinctly more concentrated in the atrium than in cells of the ventricle (Table 4). The fact that mitochondrial size may be smaller in atrium could account for the enzymatic difference.

4. Extracellular Space

It was noted that collagen was higher in atrial cells than in ventricle (Table 4) and further consideration of the extracellular space contents may be useful. Estimation of tissue space by inulin

TABLE 4.

Distribution of Various Compounds in the Myocardium

	Animal	Region 1	Region 2	Concentration of Region 1 compared to Region 2	Author
Norepine-phrine	rabbit, dog, rat, guinea pig	A	V	higher	Angelakos et al. 1969
		RA	LA	higher	" 1963
		RV	LV	higher	
Acetylcholine	rabbit, rat	A	V	higher	Abdon and Borglin 1945
	dog, rabbit, rat, guinea pig	RA	LA	higher	Rothschuh, 1954 Vlk and Tucek 1965
Cholinesterase	human	RA	V	higher	James and Spence 1966
	rat	A	V	higher	Hegab and Ferrans 1966
					Ord and Thompson
Cholinacetylase	dog, rabbit, rat, guinea pig	RA	LA	higher	Vlk and Tucek 1965
Succinic dehydrogenase	rat (fetal, young adult)	A	V	lower	Cooper, 1955
	rabbit	A	V	lower	Pratt, 1967

(continued)

	Animal	Region 1	Region 2	Concentration of Region 1 compared to Region 2	Author
Nucleoside phosphatase	rat				
	a) Golgi apparatus	A	V	same	Ferrans et al, 1970
	b) reactive cisternae	A	V	higher	
Nucleotide	rabbit, ox	A	V	lower	Davies et al, 1947
Phosphocreatine	rabbit, ox	A	V	higher	"
Glycogen	rabbit, ox	A	V	higher	"
	rat	A	V	higher	Weisberg and Rodbard 1958
Collagen	human	A	V	higher	Oken and Boucek 1957
		A	RV, LV	higher	Lowry et al, 1941

or sucrose revealed that ventricular tissue spaces were very much smaller than auricular tissue spaces (Barclay et al., 1960). In contrast, an E.M. study of rat myocardial tissue space by Melax and Leeson (1972) indicated no noticeable difference in the contents or extent of spaces between atrial and ventricular fibers. Unit fibrils of collagen, but no elastin, were embedded in the amorphous extracellular material.

C. MECHANICS OF CONTRACTION

Cardiac muscle as well as other types of muscle tissue develop active tension to an amount which is a function of length prior to the onset of contraction. The tension generated at several lengths describes the familiar length-tension curve of isolated muscles and is manifested as the Frank-Starling mechanism of the intact heart. Another feature of heart muscle is its ability to alter the MT or dT/dt at any initial muscle length. This has been termed "contractility change" and may be induced by inotropic interventions such as increase in frequency of stimulation or norepinephrine. The measurement of cardiac inotropic state separated from the influence of muscle length (preload and/or afterload change) has been a major endeavour by many recent authors and will not be discussed in detail here.

1. Muscle Models

Most investigators in the field of muscle mechanics operate within the framework of the three component muscle model of Hill (1938). In this model the contractile element (CE) shortens during

isometric contractions and stretches the series elastic element (SE) while the parallel elastic element (PE) supports resting tension. In addition, viscous elements have been included in many conceptual models of muscle and are evidenced by a phenomenon such as stress relaxation which has been accredited to viscous elements in the PE (Alexander, 1962; Little and Wead, 1971). The dT/dt derived from isometric contractions reflects the velocity of the CE provided that the modulus of elasticity of the SE remains unchanged: dT/dt = (dL/dtCE) x (dT/dLSE). Two variations in arrangement of these basic components of the model have been described: a) the Maxwell model where the SE is attached in series to the CE, both of which are parallel with the PE, and b) the Voigt model where the SE is attached in series to both the CE and PE. The assumptions necessary in using these particular models have been criticized by Pollack (1970) and at least partially rebuffed by Sonnenblick (1970); a recent review of these arguments is presented by Ross and Sobel (1972). One requirement suggested by Pollack was that force in the muscle must be corrected for transfer of preload from the PE to the CE if the Voigt model was assumed, but this is not so with the Maxwell model since PE length remains constant and the SE is presumed not altered by resting length. Since this dissertation is not designed to be a study in muscle modeling, suffice it to say that no model has thus far been universally valid but the Maxwell model seems to have been most successful and will be the assumed model in this study.

2. Length-Tension Relationship

The length-tension relationship is a basic property of muscle. It can be demonstrated by increasing the muscle length prior to contraction and observing the increase in force developed in the subsequent contraction. There is a critical length beyond which developed force will decrease. As the muscle is stretched there is an increasing amount of passive tension produced. The relation of length to passive tension appears to be exponential at first, then becoming linear as length is further increased (Brady, 1967; Grimm et al., 1970).

There is relatively high passive (resting) tension at lengths where MT is developed in cardiac muscle as compared to passive tension near zero at optimal length in skeletal muscle. Cardiac muscle apparently produces resting tension all along the ascending part of the length-tension curve. Therefore, the total tension is a combination of active and passive tension (Grimm and Whitehorn, 1968). To achieve as little RT as possible, many cardiac muscle studies used very low preloads; however one criticism is that these shorter muscle lengths may be outside the normal physiological range. The high resting tension in cardiac muscle has been attributed to the high content of collagen. However, the sarcolemma and intracellular elements have also been postulated as sites for residence of resting tension.

It will be of interest to compare A and V length-tension

curves since A curves have not been reported.

3. SE Component

Although measurements of the stress-strain of the SE of skeletal muscle and heart papillary muscle have been made, atrial SE compliance has not been quantified. The assumptions necessary in normalizing per cross-sectional area remain argumentative in atria. Assuming a situation where a muscle has no SE component the peak force developed by the muscle would theoretically be instantaneous. Therefore functionally the SE may act as a safety device or buffer. The consequences of an added SE compliance on the twitch time course and rate have been investigated in at least 2 studies. This is an important consideration because the difference in twitch time course between A and V may be at least partly attributed to a different SE component. Hill (1951) showed that after placing a compliance in series with a frog skeletal muscle there was a delay in reaching MT as well as a smaller T developed. Using cat papillary muscles Parmley and Sonnenblick (1970) added springs with varying compliance in series with the muscles. Both dT/dt and MT were reduced in proportion to the compliance and the TMT was slightly prolonged. Hill (1950) established a technique for measuring the characteristics of the SE by quickly releasing a muscle to zero force from peak isometric tetanus. Thereby, a tension-extension curve of the SE in frog skeletal muscle was obtained by plotting the change in length against the change in force during the release. The values for compliance

(mm/g) or stiffness (g/mm) may thus be calculated.

One way to compare the SE of different muscles is to compare the change in length (as a % of L max) which is necessary to reduce the tension to zero from MT. This measures the amount of stretch produced in the SE as the muscle develops MT. Values between 1 and 10% have been calculated for frog skeletal muscles with the lower range, 1-3%, calculated after taking into account the stray compliance of the equipment, etc. For ventricular cardiac muscle the SE compliance ranged from 6% (Abbott and Mommaerts, 1959) to 10% (Sonnenblick, 1962) and 5% (Parmley and Sonnenblick, 1967). During early experiments designed to measure the SE, a great deal of the compliance was actually in the recording apparatus, tendons, knots, etc.

Recently, a new concept of the SE and its location has been submitted by Huxley and Simmons (1970, 1971). A "spot follower" device allowed continuous measure of the length of a middle segment of a muscle fiber and a 1 msec release time allowed improved time resolution. Results from their experiments on frog striated muscle suggest that most of the series compliance lies in the cross-bridges which generate the relative force between thick and thin filaments. It is assumed that there is an elasticity associated with each crossbridge. Rotation of the myosin crossbridge "head" with successive attachment to the actin filament generates a tension which is transmitted through the elastic element of the crossbridge to the myosin filament. With this postulated mechanism, tension is generated with no filament

sliding; however sliding of the filaments would occur during isotonic contraction.

4. Isotonic Contraction

The need to correct for the SE component is largely eliminated in an isotonic contraction (Wilkie, 1956) where the length of the SE remains constant as the load is lifted. At this time the shortening velocity of the CE equals the velocity of muscle shortening. In the ventricular cardiac muscle the velocity of CE has been directly measured in isotonically contracting papillary muscles (Ullrick, 1964; Henderson et al., 1969; Noble et al., 1969). At present, the velocity of the CE has not been measured in the isolated atrial muscle of the rat and there is no reported analysis of relationships between length (l), its rate of change (dL/dt) and time course of these events.

The data which have been derived in cat papillary muscle (Brutsaert and Sonnenblick, 1971) indicate that the time interval from stimulation to peak dL/dt in isotonic contractions is a linear function of the load. The extrapolated intercept of peak dL/dt on the time axis approximates the first instance at which the maximum velocity of the CE (Vmax) is attained at zero load. The ratio of this time to Vmax in isotonic contraction to the time to maximum tension in the isometric twitch is 19% to 21%, and is independent of inotropic interventions. Studies to determine this extrapolated time to peak shortening velocity of the CE in rat atrium and ventricle

may yield further insight into some characteristic features of these two muscles.

5. Force-Velocity Relationship

The fundamental relationship between force and velocity in skeletal muscle was elaborated by Hill (1938) and extended to cardiac muscle by several workers including Sonnenblick (1962). In heart muscle this relationship, characterizing the CE, produces an inverse curvelinear form. Whether or not the curve is a true hyperbola as described by Hill's equation is still debated (Noble et al., 1969, Brady, 1968).

A common method for measuring the force-velocity relationship is by recording the velocity of isotonic shortening under conditions of increasing afterload. This method has been criticized because the measurements are made at various times during the contraction cycle and at various lengths. However, corrections for the shortening of the CE were made (Brutsaert and Sonnenblick, 1969) and subsequently a true hyperbolic curve was claimed.

A second method, quick-release during isometric contraction, allows generation of families of force-velocity curves at a constant time during the contraction as well as at constant CE length. There are also divergent results with this method.

In the intact heart a technique incorporating a logarithmic amplifier has recently been used to measure the force-velocity relationship. Specifically, it was shown that the ratio of the rate of rise of ventricular pressure (dP/dt) to the simultaneous ventricular

pressure (P) approximates the contractile element velocity (Vce) with acceptance of certain assumptions which are critical when dealing with the intact heart. (Hugenholtz et al., 1970). Plots of this ratio $(dP/dt)P^{-1}$ on the ordinate versus P on the abscissa gave an equivalent of the force-velocity relation as seen in isolated muscle. It will be interesting, therefore, to compare not only the $(dT/dt)T^{-1}$ versus time for atrial and ventricular contractions of isolated strips but to at least qualitatively observe the force-velocity plots from these isometric contractions.

6. Ionic Changes and Duration of the Contraction

As the intensity of the active state is reflected by the dT/dt, the duration of the active state is directly proportional to the TMT of cardiac muscle (Buccino et al., 1967). The duration of the active state is believed to be determined by the time period during which the Ca^{2+} level in the myoplasm is sufficient to maintain the respective binding sites of troponin saturated with Ca^{2+} (Brady, 1968). Thus any factor which would delay the sequestration of Ca^{2+} from the myoplasm should prolong the duration of contraction and vice versa. Indeed, the duration of contraction in the isolated myocardium may be altered by external interventions. For instance catecholamines shorten its duration while some ionic changes in the external medium, such as the addition of Sr^{2+} (Buccino, et al., 1967) or combination of high Na^{+} with low K^{+}, favour its prolongation. Alkaloids such as caffeine or ryanodine also alter the duration of contraction. The former compound liberates Ca^{2+} and the latter initiates the removal

of Ca^{2+} from skeletal muscle (Hajdu, 1970) as well as prolongs the latent period and the initial part of tension development in the ventricular muscle. (Penefsky and Kahn, 1970). Thus if the difference in duration of contraction between the atria and the ventricles could be reduced or completely abolished by one of these external interventions, certain mechanisms underlying the regional differences may be revealed.

D. ISOLATED CONTRACTILE SYSTEMS

1. Chemically "Skinned" Myocardial Fibers

By careful dissection of the sarcolemma the interior of skeletal muscle fibers can be directly exposed (Natori, 1954, 1955; Hellam and Podolsky, 1969). These "skinned" muscle fibers have been used extensively to study the response of the contractile apparatus to controlled changes in the composition of bathing fluid. In heart muscle the small size and diverse arrangement of the fibers forestall such a preparation.

In 1960, Thomas observed that ethylenediaminetetra-acetic acid (EDTA) destroyed the selective permeability of muscle fiber membranes. One of his experiments involved perfusing frog heart with EDTA. There was an increase in the sulfate ($S^{35}O_4$) space from 25 to 70% indicating an increase in extracellular space. After EDTA treatment Goto and Abe (1964) reported disappearance of contraction and greatly prolonged A.P. in rabbit ventricular muscle which was assumed associated with calcium availability. Recently, EDTA application to frog and rabbit

cardiac fibers has resulted in a possible analogue to the skinned fiber (Winegrad, 1971; Fabiato and Fabiato, 1972). This chemical procedure increased the sarcolemmal permeability but did not impair intracellular function. After the calcium buffer system (EGTA-CaEGTA) was added, the tension developed per unit of Ca^{2+} concentration resembled that observed in an isolated myofibril preparation. Rate of tension development in EDTA-treated fibers follows a time course which indicates the absence of major diffusion barriers. This method offers a valuable alternative to the glycerinated fiber, which will subsequently be described, with the important exception that only the surface membrane appears modified.

2. Glycerinated Muscle Preparations

a. Short-term Glycerination

A great deal of new information on excitation-contraction coupling in muscle has been generated by soaking fibers for short periods in glycerol, i.e., a mild treatment. A mild treatment consists of adding glycerol to the bath (300-500mM) for up to one hour while the muscle is being electrically stimulated. This technique was used by Yamaguchi, et al., (1962), who immersed frog sartorius muscle in glycerol-Ringer solution and subsequently transferred it into normal Ringer. The mechanical response remained unchanged in glycerol-Ringer, but diminished after transfer to normal Ringer and subsequently was restored with time. This diminution or abolition of mechanical response and restoration with time has been termed the

"glycerol effect".

It should be restated that in heart ventricle and skeletal muscle the essential structures connecting excitation with contraction appear to be the T-tubules and the sarcoplasmic reticulum. It has been suggested that this is the pathway for transmitting the action potential "message" at the surface membrane to the contractile proteins in the fibers (Huxley and Taylor, 1958; Legato, 1969). The effect produced by glycerol treatment and removal may be the result of action on the T-system.

Frog skeletal muscle contains large numbers of T-tubules. After a glycerol-soaking and return to normal Ringer solution, the T-system was disrupted and almost none of it was connected to the extracellular space (Eisenberg and Eisenberg, 1968; Howell, 1969). It should be emphasized that the sarcolemma remained intact and the myofilaments were normal. After T-system disruption, Gage and Eisenberg (1967) passed depolarization currents through the fibers and caused action potentials which were threshold-dependent and propagated; however, no contraction occurred. The conclusion was that an intact transverse tubular system was necessary for an action potential to cause myofibrillar contraction. In an experiment to determine the effect of glycerol treatment on "slow" muscle fibers, the iliofibularis from muscle was dissected into "fast" (phasic) and "slow" (tonic) portions (Stefani and Steinbach, 1968). After glycerol addition and subsequent removal the tonic part responded with an action potential but with no contraction. In the non-tonic bundles membrane excitation

was not "uncoupled" from contraction and a normal twitch resulted when stimulation was applied. If one assumes that the T-tubules were destroyed, then these results suggest that the T-system does not play a significant part in the initiation of excitation-contraction coupling in frog slow muscle fibers.

Cardiac muscle appears more resistant to the effects of hypertonic glycerol. After treatment of sheep and rat cardiac fibers with a glycerol-electrolyte solution, (400 and 1000 mM) contractility never disappeared and action potentials were not influenced. The T-system appeared intact during glycerol treatment and even after return to normal tyrode solution (Niemeyer and Forssmann, 1971). This unique insensitivity of cardiac fibers was attributed to two morphological properties:

(1) the T-system membrane in heart is coated with a 200Å thick basement membrane, which is absent in skeletal muscles.

(2) T-tubules are much larger diameter in heart muscle than in skeletal muscle.

In contrast, experiments on rabbit atria (Schmidt et al., 1972) showed ultrastructural changes such as swelling and disruption of the sarcotubular system and mitochondria coincident with contractile changes following glycerol treatment. Furthermore, similar observations were made by Porter-Sanders et al., (1969) using rabbit heart atria; however, ventricular muscle did not show the "glycerol effect" or

uncoupling of excitation from contraction.

Guinea pig atria and papillary muscles were compared following treatment with hypertonic glycerol (400 mM) for 2 hours (Strossberg et al., 1972). Return to the normal solution resulted in temporary loss of contractile response in atrial muscle but not in papillary muscle. No significant ultrastructural alterations were produced in papillary muscles. The electrical-mechanical uncoupling in atria can not be attributed to T-system disruption as in skeletal muscle because no T-tubules are present in atria.

b. Long-Term Glycerination

Glycerinated muscle fibers have been widely used in studying muscle contraction since Szent-Gyorgyi (1949) first developed the technique. The isolated muscle fibers are treated with glycerol at a cold temperature in order to disrupt the membranes and thus render the organized contractile system of actin and myosin permeable to divalent cations, large anions and ATP. Many variations of this procedure have been used both on skeletal muscle (Nanninga and Kempen, 1971; Ruegg, 1971; Wise et al., 1971) and heart muscle. Refer to Table 5 for a composite summary of methods used in glycerinating heart muscle. No study with glycerinated atria was reported.

Rigorous treatment of muscle fibers with glycerol causes the destruction of muscle membranes and release of glycolytic enzymes thereby exposing the intact contractile proteins. An example of this treatment was described by Taeschler and Bing (1953) who treated dog heart ventricular strips with 50% glycerol for 3 days at $0^{o}C$, and

TABLE 5.

Summary of Methods Used in Glycerinating the Myocardium

Author	Animal	Muscle	Extraction Medium and Temp.	Extraction time	Unique features
Taeschler and Bing, 1953	dog	trabeculae of left ventricle (2mmx2cm)	0°C for 3 days; then to new -20°C, 50% glycerol	10-14 days	Strips tied by ends to wooden sticks; .4-.7 mm diameter strips dissected from bundles.
Hawkins and Smith, 1956	bull-frogs	whole hearts	50% glycerine at -10 to 20°C	48 hr-5 days	heart perfused with various solutions.
Ranney, 1954	dog	trabeculae of ventricle	2.9 M glycerol, 10^{-2}M EDTA Na_2 - 15°C	7-25 days	fibre bundles dissected to 0.1 mm^2 X-S area.
Benson et al, 1958	dog	trabeculae from lateral wills of ventricles	50% glycerol at 0°C for 72 hrs., then to fresh at -20°C.	14 days-6 mos:	strips tied on glass rods in extended positions; final bundles .1x.2x8-20mm
Briggs, 1958	dog	trabeculae of left ventricle	-18°C in 50% glycerol buffered to pH 7.4 with phosphate.	30-70 days	optically measured diameter and X-S area based on cylindrical shape.
Lee, 1961	dog	trabeculae from lateral walls of ventricles (1x2-3x20 mm)	0°C for 24 hrs; then to new 50% glycerol 0.1M histidine buffer, pH 7.0 at -20°C.	2-35 days	strips tied to wooden rods; later cut by chopper to .1x.1x20mm
Bozler, 1968	turtle	rings of stomach (.4mm) and aorta (.2mm)	35% glycerol, -15°C	1 day-several weeks	mounted on frames and washed in 2mM $MgCl_2$ on an automatic shaker before storage.
Cherney et al, 1971	hog & rabbit	wall of hog carotid and rabbit psoas	50% glycerol, 8.75 mM imidazole buffer, pH 7.0 at 4°C for 24 hrs; then to -18°C	4-60 days	arterial bundles tied to glass rods at 10% stretch; smallest thickness used as diameter.

then for 10 to 14 days at -20°C . Electrical stimulation failed to excite these glycerinated fibers, however tension was developed (contracture) by a solution of ATP at proper electrolyte concentration. In dog ventricular strips, ATP concentrations between 1 x 10^{-2} M and 5 x 10^{-4} M were about equally effective in generating similar degrees of tension (Briggs, 1958). Properties of these glycerinated myocardial fibers were quite analogous to the glycerinated skeletal fibers and very useful in studying the chemomechanical process separated from the remainder of the cell.

E. ATP HYDROLYSIS BY CONTRACTILE PROTEINS

One of the conclusions from the work of Hill (1938, 1964) on skeletal muscle was that the rate of heat release from muscle was a function of the velocity of contraction. In 1939 Engelhart and Ljubimova showed that the main structural protein of muscle was also an ATPase. Therefore, it was postulated that the ATPase activity of the contractile proteins may be an index of energy release. Since these experiments, a great number of studies have shown that the ATPase activity is directly related to contraction velocity not only in skeletal muscle from different species (Barany et al., 1965; and Barany, 1967) but also in smooth and cardiac muscle. For a summary of studies on cardiac muscle, see Table 6, where ATPase activities are measured using preparations of myofibrils, actomyosin or myosin.

Mechanical properties of "fast" and "slow" skeletal muscles differ as reflected by their maximal velocity of shortening (Vmax) which

TABLE 6.

ATPase Activity - Cardiac Muscle Contractile Proteins

Species and Region	Preparation	Conclusion	Author
cardiac and skeletal muscle	myofibrils, actomyosin myosin	All ATPases higher in skeletal than cardiac muscle	Barany et al., 1964
dog, rabbit: heart, red and white skeletal muscle	myosin	Two types of myosins: a) white skeletal b) red skeletal and cardiac; cardiac and white skeletal actins not different	Katz et al., 1966
rat, right and left ventricle	myofibrils (normal and glycerol-extracted), actomyosin	ATPase activity decreased with age of the animal	Alpert et al., 1967
cat, right and left ventricles	myofibrils	Right ventricular failure causes significant depression of ATPase of both right and left ventricles	Chandler et al., 1967
rat, ventricle	myosin	Ca^{2+}-activated ATPase from hearts of hypophysectomized rats was reduced compared to normal rats	Hjalmarson, 1970
cat, right and left ventricle	myofibrils, actomyosin, myosin	Significant reduction of all ATPase activities after adrenalectomy	Rovetto et al., 1970

(continued)

Species and Region	Preparation	Conclusion	Author
rat, ventricle	myofibrils	No significant difference in activity/unit protein between hyper-and normotensive hearts	Aoki et al., 1971
cat, left ventricle	myofibrils	Alteration of contractile state produced by norepinephrine or digitalis or by changes in hormonal status such as hyper-thyroidism are not associated with change in ATPase activity	Seagren et al., 1971
rabbit, right and left ventricle	glycerinated papillary muscles, myofibrils, actomyosin	All ATPase activities decreased in fibers from hyperthophied hearts compared to normal	Henry et al., 1972
rat, right and left ventricle	myosin	No difference in Ca^{2+} -and Mg^{2+} -activated ATPase activities comparing right and left ventricles; light myosin fragments similar electrophoretically	Kleid et al., 1972
rat, ventricle	sarcomeres (1 wide by 5-24 long)	Relationship between shortening and ATPase activity varied with sarcomere length, concentrations of substrate, Mg^{2+} and Ca^{2+}, and drugs	Takauji et al., 1972

is less in "slow" than in "fast" muscles (Close, 1971). It has been shown that low molecular weight fragments isolated from myosin, after treatment with a variety of structure - disrupting agents, also differ when prepared from "slow" and "fast" skeletal myosins (Locker and Hagyard, 1968). Furthermore, the characteristics of myosin light fragments may determine the level of myosin ATPase activity and thus the Vmax (Stracher et al., 1972).

Mammalian heart has many characteristics of a "slow" muscle, i.e., low shortening velocity and ATPase activity (Kleid et al., 1972). In addition, light fragments from cardiac myosin are similar to those of "slow" muscle. These studies refer to ventricular cardiac muscle and no study has ever reported the characteristics of myosin from atria or its ATPase activities. Furthermore, there has been no measurement of myofibrillar or actomyosin ATPase from atrial muscle. Since atrial contraction velocity is several times faster than the ventricle, the ATPase activities may be correspondingly higher.

In retrospect, information is quite convincing that the myosin molecule is associated with varying ATPase activity. However, other molecular interactions, such as actin binding by myosin, may also determine the final mechanical event. In order to take advantage of a higher level of structural organization, ATPase activity will be compared in myofibrillar preparations from atrial and ventricular myocardium.

III. MATERIALS AND METHODS

A. ANIMALS AND BASIC MATERIALS

The experimental animals were 300-400 g male Sprague-Dawley rats (Biobreeding, Ottawa), and rabbits, guinea pigs, dogs and cats from stock. All animals were sacrified by a blow on the head, except cats and dogs which were anesthetized with Nembutal (i.p. 35mg/kg). Strips of left atria and posterior left ventricular papillary muscles of rat and various trabecular muscles from hearts of larger animals were rapidly excised. These strips of atria and ventricles were trimmed to visually similar widths and lengths. A modified Krebs-Ringer-bicarbonate solution (concentrations in mM: NaCl 117.4; $NaHCO_3$ 25.0; NaH_2PO4 1.2; MgSO4 1.2; KCl 3.6; $CaCl_2$ 2.5; glucose 11.1) was used for all experiments, unless otherwise stated. The pH was maintained near 7.4 by bubbling with 95% O_2, 5% CO_2. Temperature was controlled ($\pm$ 0.25°C) by a thermostat (Radiometer, Copenhagen) which circulated water through a jacket surrounding the bath solution.

All reagents were "Fisher Certified" or "Baker Analyzed", with the following exceptions: Norepinephrine (Levarterenol bitartrate-Winthrop); Isoproterenol-HCl (Winthrop); Reserpine (Serpasil-Ciba); Propranolol-HCl (Inderal-Ayerst); Creatine phosphokinase (Sigma); Creatine phosphate (Sigma); Adenosine 5'-diphosphate, disodium (Sigma); Calcium-free Adenosine 5'-triphosphate, disodium (Sigma); Deoxycholic acid (Schwarz-Mann); Bovine albumin (Fraction V - Baker). All chemicals were dissolved in glass distilled water.

B. ISOMETRIC CONTRACTIONS

1. Normal Myocardium

Atrial strips and papillary muscles or trabeculae from atria and ventricles of larger animals were attached to duplicate muscle stands and the experiments proceeded simultaneously on both atrial and ventricular muscles. The upper end of each muscle was attached to a lucite clip connected to a rigid rod of a Statham force transducer (G1 1.5-300). This strain gauge was bolted to an arm of a muscle stand (Palmer, England) and an adjustment screw controlled muscle length (Figure 5). Length (mm) was measured with a micrometer (Starrett, Mass., U.S.A.) attached in a fixed position relative to the adjustable arm of the Palmer stand. The lower muscle end was held by another lucite clip connected to a stationary L-shaped bar. The muscle, secured by the clips, was suspended in a modified Ringer solution. Two platinum plate electrodes (2.2 x 0.8cm) were arranged vertically on either side of the muscle and delivered square wave pulses, 5msec in duration, 10-20% above threshold voltage and usually at a rate of 6/min.

The output from the transducer was amplified in 2 successive stages (Lexington A-101 and Grass driver amplifier) or electronically differentiated prior to second stage amplification (Lexington A-111). Subsequently, these processed signals were recorded on tape (Hewlett-Packard 3960 FM tape recorder) and/or oscilloscope (Tektronix 564B with C-21 camera) in addition to a continuous record on a polygraph

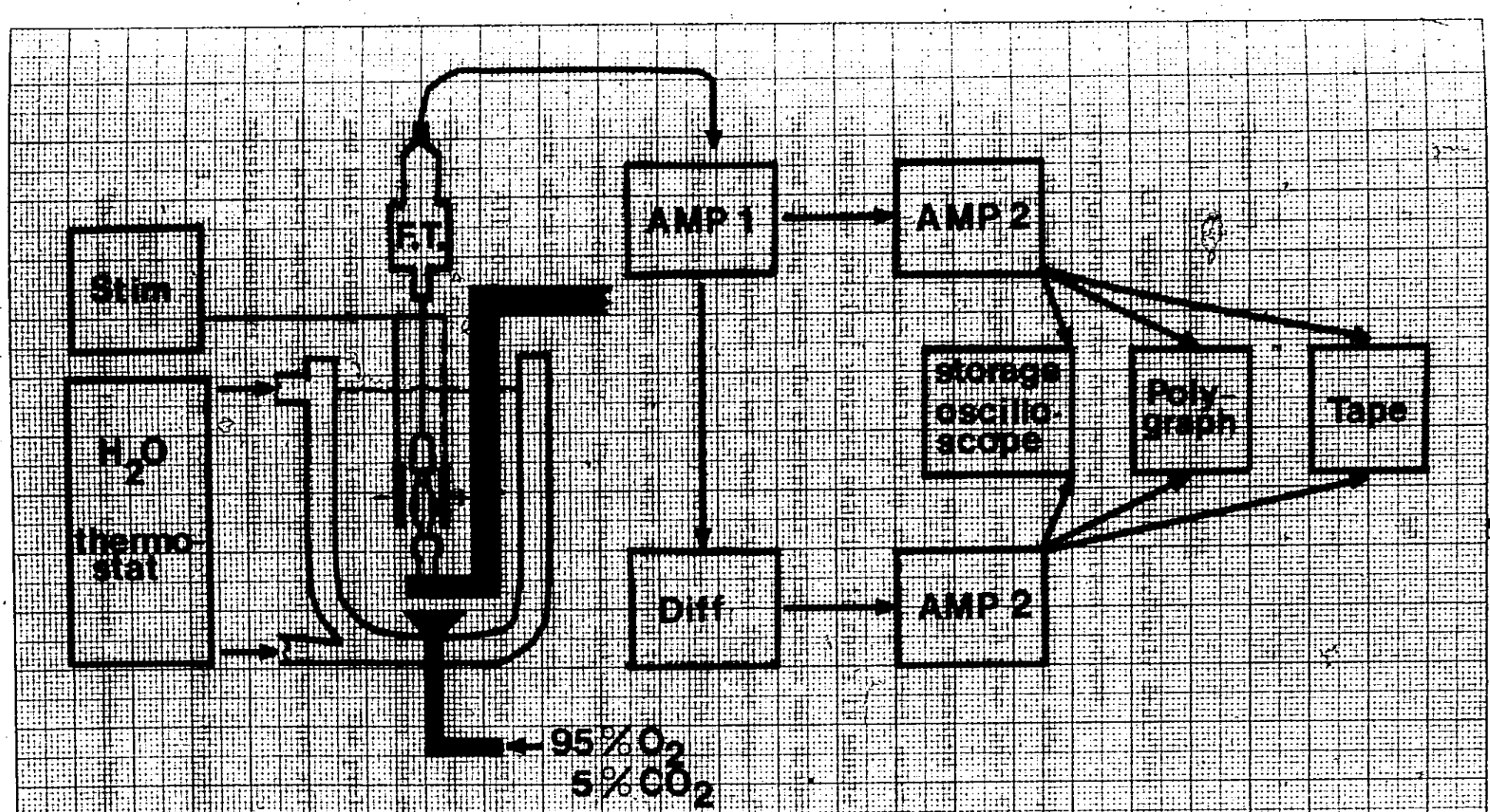


Figure 5 . Schematic Diagram of Apparatus for Monitoring Isometric Contractions
Stim = Grass S4 stimulator
F.T. = Statham GL-1.5-300, Force transducer
AMP 1 = Lexington A-10i, pressure amplifier
AMP 2 = Grass driver amplifier, Model 7DAC
Diff = Lexington A-111 electronic differentiator
Storage Oscilloscope = Tektronix type R564B
Polygraph = Grass 7P
Tape = Hewlett-Packard 3960 tape recorder

(Grass 7P).

2. Myocardium Treated With EDTA.

All experiments utilized the previously described apparatus with incorporation of the following additional scheme.

At the start of the experiment all muscles were equilibrated in normal Krebs-Ringer bicarbonate solution 1A (See Table 7 for composition) at 28°C for one hour while being stimulated at a rate of 6/min. After such stabilization at a set preload, solution 1B, containing no calcium, replaced solution 1A for a 15 minute presoak period during which the electrical stimulation was discontinued. The disruption solution II was then utilized for one hour after which contractures were elicited by solution III. (Table 7). The modified Krebs Ringer-bicarbonate solutions were adjusted to a pH of 7.4 by bubbling as mentioned previously. The disruption and contracture solutions were adjusted to pH of 7.0 with KOH before use.

All solution changes were accomplished with suction and required 10-15 seconds for a complete replacement. Preloads were selected from 0.25-2.0g and an arbitrary preload was chosen for each experiment and held constant by muscle length adjustment. The preload, before and after bath change, remained constant and was not affected by any physical maneuvers of the procedure.

3. Vce Derived From Isometric Contractions Incorporating Log Amplifier

The output from the force transducer (Statham Model UC-2), after

TABLE 7.

Composition of Solution Used prior to and during Contracture Development in EDTA Treatment Experiments

BATH SOLUTIONS

	I. MODIFIED RINGER		II. DISRUPTION	III. CONTRACTURE
	A	B		
NaCl	117.4	117.4	X	X
$NaHCO_3$	25	25	X	X
NaH_2PO_4	1.2	1.2	X	X
Glucose	5.5	5.5	X	X
$MgSO_4$	1.2	1.2	X	1.2
KCl	3.6	3.6	140	140
$CaCl_2$	2.5	X	X	2.5
EDTA	X	X	3	X
TRIS Buffer	X	X	10	10
Na_2ATP	X	X	5	5

Concentration of solutions in mM/L; (x) = not present

amplification (Lexington A101), was fed into both the logarithmic amplifier (#4116, Burr-Brown) and the differentiator (Lexington A-111) (Figure 6). The log amplifier output was then electronically differentiated (Lexington A-111). The primary force and the outputs from the differentiators and log amplifier were recorded as shown. Since a readout of dT/dt/T was desired, the electronic circuitry was based on differential calculus (Grossman, et al., 1971), where $(d/dy)\ \ln x = (dx/dy)x^{-1}$; Substituting for y = time (t) and x = tension (T), then $(d/dt)\ln T = (dT/dt)T^{-1}$. Furthermore $dL/dt\ CE = \frac{dT/dt\ CE}{dT/dL\ SE}$ (1) and also $dT/dL = KT + C$ (2). Therefore substituting (2) into (1) and assuming C to be small and K constant then $dL/dt\ CE = \frac{dT/dt}{T}$.

C. ISOTONIC AFTERLOADED CONTRACTIONS

This apparatus is similar to that described previously by Parmley and Sonnenblick (1967).

Upper and lower ends of the muscle were held by lucite clips welded to the ends of stainless steel wires (Figure 7). The lower wire was attached to the force transducer (Statham UC-2) after passing through a mercury-sealed opening in the bottom of the bath, while the upper wire was connected to one end of the isotonic lever. This isotonic lever (ratio of 17:1) was shaped from magnesium to obtain maximum stiffness with as little mass as possible and thereby obtain small inertia. Also, to produce further damping, preloads and afterloads were added to a weight pan suspended from the lever by a rubber band. Movement of the lever generated output from a Harvard rotary motion transducer (#2044). Electronic differentiation (Lexington

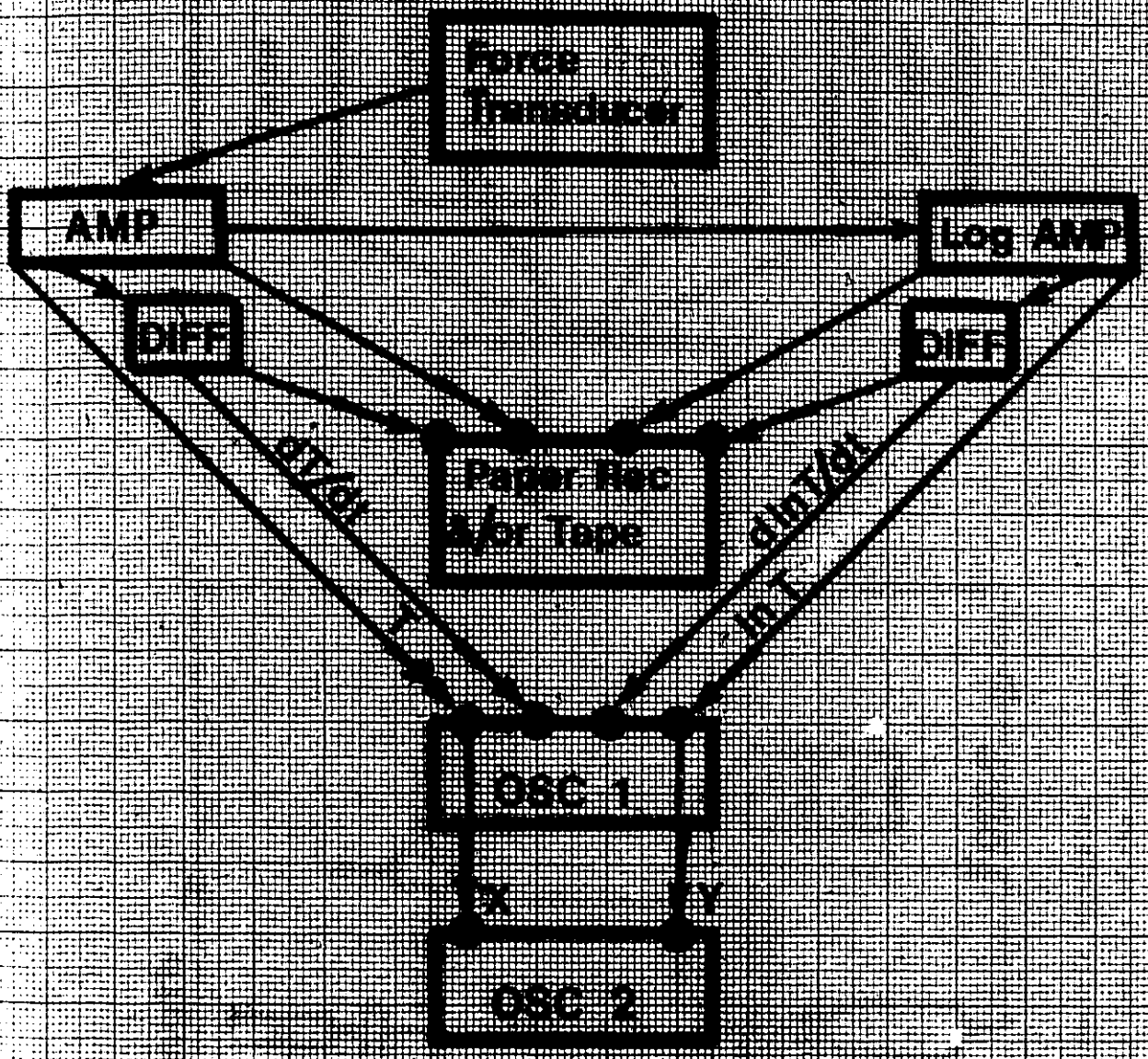


Figure 6. Scheme for recording vce and force-velocity curves. T = tension; dT/dt = first derivative of tension; lnT = natural logarithm of tension; dlnT/dt = first derivative of the log of tension; Force transducer = Statham type; AMP = Lexington A-101, pressure amplifier; DIFF = Lexington A-111, electronic differentiator; Log AMP = Kurz-Kasch, Model 4116, logarithmic amplifier; OSC 1 = Tektronix type K5048 oscilloscope with type 2B67 time base; OSC 2 = Tektronix type K5048 oscilloscope with type 2A63 dual amplifier; Paper Rec = Beckman, Type R-II Dynograph; Tape = Hewlett-Packard 3960 instrumentation tape recorder.

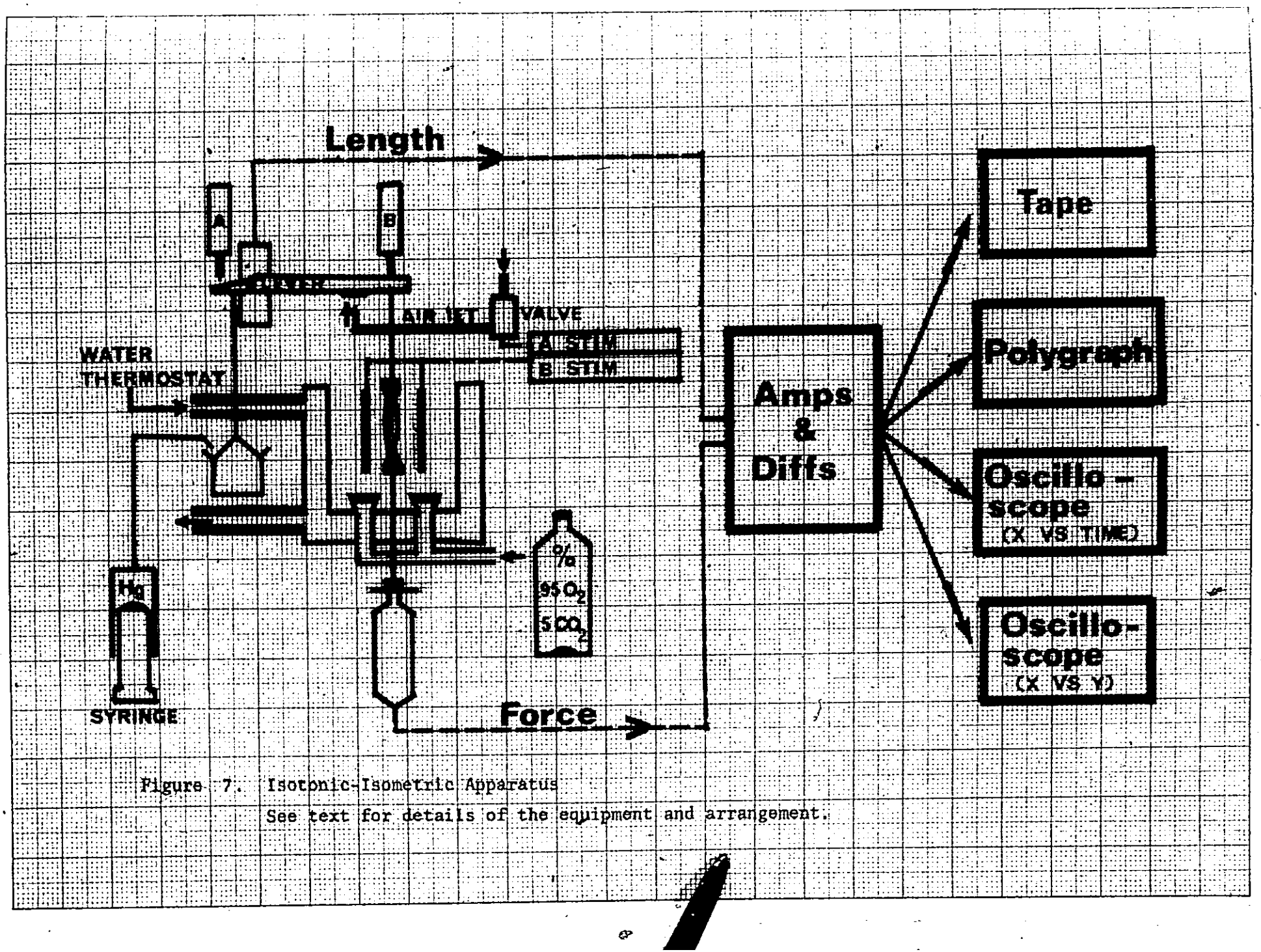


Figure 7. Isotonic-Isometric Apparatus
See text for details of the equipment and arrangement.

A-111) of the amplified (Lexington A-101) output signals from both force and length transducers measured the rates of change in these parameters. Static compliance of the system with the clips attached to a piece of solid plastic was 2μ/g.

Micrometer stops (A and B in Figure 7) were placed above the lever at each end. During isotonic shortening micrometer B was adjusted to just touch the lever and micrometer A was disengaged. This prevented further lengthening of the muscle when afterloads were added.

This apparatus was also used for quick-release experiments by incorporating an electric valve (Skinner, Conn., U.S.A.) which on command directed a jet of air onto a plate attached to the lever which forced the lever against micrometer stop B. At arbitrarily selected preloads and afterloads the muscle was stimulated (Grass S-88); however, it was initially prevented from shortening by the air jet. At the time of maximum tension, air flow was stopped by the valve which was controlled by a second stimulator (Grass S-88). This allowed muscle shortening with rapid lever movement and drop in tension. All transducer outputs were processed and recorded with instruments previously mentioned (Figure 6).

D. GLYCERINATED MYOCARDIUM

1. Isometric Contraction

Glycerol extraction of atrial trabeculae and papillary muscles was based on the method of glycerination of skeletal muscles developed by Szent-Gyorgyi (1949). Fibers were dissected to a diameter of 500-

1500u and immersed in 50% aqueous glycerin (0-2°C) containing 5mM NaH_2PO4, pH 7.0. Muscles were held by needles at "resting length" on cork plates to avoid curling; some were not "pinned" as a comparison. After 4 hours the muscles were placed in fresh extraction fluid and stored at -18°C for at least 2 weeks before use.

After the extraction period, fiber bundles were carefully trimmed to widths arbitrarily selected between 100 and 700 u diameter using a Zeiss dissecting microscope at 40 X magnification. Since cross-section of the fiber bundle presents an irregular circumference, sometimes elliptical or rectangular, measurement was made of the smallest diameter seen after rotating the fiber bundle one turn on its longitudinal axis.

After changing the fibers from a 50% glycerol extraction solution to 25% then to 10% glycerol, they were secured for isometric tension measurement on a modified Ranney-type myograph (1953). This consisted of a vertically-mounted Statham G1 strain gauge transducer to which a stainless steel rod and clip were attached; a second clip held the fiber bundle at its lower end. The bathing solution (5ml each), surrounding the muscle, were rapidly exchanged via a syringe attached to a polyethylene tubing which passed to the bottom of the muscle bath. Temperature was maintained at 26°C in a glass water jacket surrounding the muscle fiber bath. The recording apparatus was similar to that previously used (see Section B1.)

After attaching the muscle, a 25mg preload was added by ad-

justing muscle length. During this preload equilibration period, the muscles were immersed for 5 minutes in a 5mM EDTA-EGTA mixture (Wash No. 1, Table 8) in an attempt to reduce the magnesium and calcium to very low values. Another 5 minutes were allotted to Wash No. 2 in order to eliminate the excess EGTA and EDTA. Next, the fibers were stimulated to develop tension by the contracture solution (Table 8) which contained: 10mM ATP, 10mM $MgCl_2$ and 6 x 10^{-6}M calcium. The concentration of free calcium was calculated using the apparent association constant of the Ca-EGTA complex as 5 x 10^6 at pH 7.0 (Ruegg, 1971). Certain assumptions and chemical equations were used in the calculation of free calcium as a function of pH and EGTA concentration:

Where K = dissociation constant and $\frac{1}{K}$ = association or binding constant,

$$(EGTA)^{4-} \quad (Ca)^{2+} \quad = K \quad (EGTA\text{-}Ca)^{2-}$$

$$(Ca)^{2+} = K \quad (EGTA\text{-}Ca)^{2-} / (EGTA)^{4-}$$

$$pCa = pK + \log \quad \frac{(EGTA)4-}{(EGTA\text{-}Ca)^{2-}}$$

After accounting for competing effects of Mg^{2+} by formation of $MgEGTA^{2-}$;

at pH 7.0 $K = 2 \times 10^{-7}$ Nanninga and Kempen, 1971

$pK = 6.70$

at pH 6.0 $K = 1.9 \times 10^{-5}$

$pK = 4.72$

Table 8

Composition of Solutions Used prior to, during and after Contracture Development in Long-Term Glycerination Experiments.

BATH SOLUTIONS—GLYCERINATED MYOFIBERS

mM/L	WASH NO.1	WASH NO.2	CONTRACTURE	RELAXATION
KCL	100	100	100	100
NaH_2PO_4	20	20	20	20
NaN_3	10	10	10	10
$MgCl_2$	X	X	10	10
Na_2 ATP	X	X	10	10
EDTA	5	X	X	X
EGTA	5	X	X	10
CaEGTA	X	X	10	X

Concentration of solutions in mM/L;

(x) = not present

However, in an equimolar solution of Mg^{2+}, ATP and EGTA, it has been stated that nearly all Mg^{2+} is bound to ATP; also at pH 7.0 $MgATP^{2-}$ is the only stable complex.

2. Electron Microscopy

Two muscle pairs each containing an atrium and a papillary muscle were chosen for electron microscopic (E.M.) examination. The first pair was fixed prior to addition of contracture solution and the second one after a 10 minute contracture.

The muscles were fixed for 1 hour in 4% glutaraldehyde in phosphate buffer (pH 7.2), osmolarity 980 mOsmol/Kg H_2O (Sabatini, et al., 1963). Three samples of each muscle were removed from different regions, the size of each sample being approximately $1mm^3$. The samples were embedded in Araldite 502 (Ladd) as described by Coulter (1967). Sections $< 400\text{Å}$ thick were cut along both the longitudinal and transverse axes of the fiber with a glass knife on a Reichert ultramicrotome and mounted on carbon-coated grids. The sections were stained with uranyl acetate (saturated in 50% methanol, pH 4.4) for thirty minutes at room temperature, counter-stained with Reynold's lead citrate (pH 12.0) for 20 minutes at room temperature, and examined in a Siemens IA Elmiskop.

E. MYOFIBRILLAR ATPase

1. Normal Non-Glycerinated Myofibers

a. Preparation: Each assay used the myofibrils of both atria and ventricles from 2-5 male Sprague-Dawley rats (200-300g) isolated

according to the method of Perry and Grey (1956) with certain modifications (Seagren, 1971). The tissue was cleaned of excess blood, blotted dry on filter paper and minced with scissors for two minutes. Approximately 100mg of each tissue was homogenized using an adjustable speed stirrer (Fisher Stedi-Speed) with a ground glass mortar and pestle. All procedures were carried out at temperatures close to $0^{o}C$.

The homogenate was spun for 2 minutes at 50 x gravity using a fixed angle head in an International PR-2 centrifuge. The resultant supernatant, after being spun for 15 minutes at 100 x gravity, gave a double layered pellet. The upper layer, light yellow in color, contained disrupted muscle cells exposing sprays of myofibrils and many disconnected myofibrils as determined by phase contrast microscopic examination. This upper layer after resuspension in sucrose (1ml/100mg tissue), underwent three 15 minute washings at 1000 x gravity using Vortex mixing for each resuspension. The final precipitate was suspended in 3ml of a buffer containing 0.177M Tris, 5mM $MgCl_2$, 10^{-6}M $CaCl_2$ (Murphy and Hasselbach, 1968), 5mM NaN_3 (Fanburg, 1964; Aoki, 1971) at pH 7.40.

b. Assay: A portion of the Tris suspension, 2.5ml., was incubated at $37^{0}C$ for 5 minutes. ATP was added to a final concentration of 5mM. After 5 minutes the reaction was stopped with 1.0ml of 2.3M PCA (Wood, 1971) and plunged into ice.

Phosphate was determined by the method of Furchgott and de Gubareff (1956) employing a modification by Fiske and SubbaRow (1929).

Standards were prepared from 1mM KH_2PO4 and all color reactions developed after addition of 2.5% ammonium molybdate, ION H_2SO4, and Fiske-Subbarrow reagent. Readings were taken at 660mu on a spectrophotometer (Bausch and Lomb, Spectronic 88).

Protein was determined by a modification of the Lowry method (1951). Concentrations of the reagents were 4% Na_2CO_3, 4.9% NaK tartrate, 1% CuSO4 and Folin's Reagent. Readings were taken at 750mu on a spectrophotometer (Bausch and Lomb Spectronic 88). Gregory and Sajdera (1970) report that some buffers, e.g., Tris, prevent the normal amount of protein color formation. Therefore, the myofibrils were resuspended in 0.5N NaOH. Similarly the bovine albumin was prepared in 0.5N NaOH and used as the standard.

2. Glycerinated Myofibers

Refer to previous section E1 Normal Myofibers for both preparation and assay method with the following revision: Prior to myofibril isolation, atrial and ventricular myocardial samples were rapidly excised from the hearts, cut into small pieces and extracted in 50% glycerol for at least 30 days at $-18^{o}C$.

F: STATISTICAL ANALYSIS

1. Students "t-test"

Significant differences between the means were calculated for paired and unpaired data. No statistical significance was indicated when the p value was less than .05.

2. Linear Regression

This method involved least squares estimate of intercept and slope constants; also the correlation coefficients and standard errors of the estimate were calculated with additional program for comparing the slopes and slope errors between 2 sets of data. It was used to determine K in the following equation: $\frac{dT/dt}{MT} = K\left(\frac{1}{TMT}\right)$ and to determine the time to maximum Vce (Refer to sections IV B, 2 b and IV C, 2).

3. Orthogonal Polynomial Method

This was a procedure which fit least-square polynomials to bivariate data. The lowest degree polynomial (generally 3rd or 4th) which gave a good fit was chosen. It was used in an attempt to approximate the length-tension curves. (Section IV B, 1b)

IV. RESULTS

A. Normalization of Developed Tension and Its Velocity

An inherent problem in equating mechanical characteristics of two muscles is the choice of appropriate criteria which will allow expression of unique features of each muscle while still validly comparing them. The quantitative comparison of T and dT/dt from isometric contractions of A and V of the heart illustrates this type of fundamental problem. Developed tension is a product of force generating components of the muscle which are located between the thick and thin filaments of individual sarcomeres. Therefore, active tension is among other things dependent on the number of these force-generating sites in parallel. Thus, the arrangement of individual fibers in the tissue sample under investigation will affect the developed tension, which is usually measured across two points of the tissue sample; one being fixed, the other attached to the transducer. If all the fibers are situated parallel to the axis formed by the two points of attachment, then the developed tension would reflect the force generated by the sum of the muscle fibers. Developed tension of individual tissue samples could then be compared on the basis of their cross-sectional areas, which would reflect the number of fibers lying in parallel. The calculation is based on the cross-sectional area of a cylinder where volume = $\pi r^2 h$; and density of the muscle $\left(\frac{\text{mass}}{\text{volume}}\right)$ is taken as 1.065. Therefore, the final formula

becomes $\frac{\text{mass of muscle (mg)}}{1.065 \times \text{length of muscle (mm)}}$. This normalization of T and dT/dt per cross-sectional area is based on work by Ramsey and Street (1940) and Helander and Thulin (1962) and is a common practice. It is a meaningful and useful method if the following assumptions are accepted: 1) cylindrical geometry of the muscle bundle, 2) parallel arrangement of the fibers, and 3) equal number of myofibrils per unit of cross-section of the fiber.

Normalization by using circumference of the muscle has been suggested. Isometric tension of glycerinated rabbit and crab muscle was linearly related to the circumference of the fiber bundle rather than to the cross-sectional area (Stanley and Villafranca, 1970). The underlying basis suggested for employing circumference was that the differences in areas between concentric circles was directly proportional to the circumference of the larger circle.

The process of normalizing values by cross-sectional area can be justified in most cases for papillary muscles but not in ventricular and atrial strips where the above-mentioned criteria are not satisfied. In light of this, indices were sought which would allow a reasonable comparison of these muscles. One such measurement, the time from stimulation to maximum tension (TMT) is independent of cross-sectional area and therefore allows a direct quantitative comparison of one feature of A and V twitch contractions.

B. Normal Myocardium - Isometric Twitch Contractions

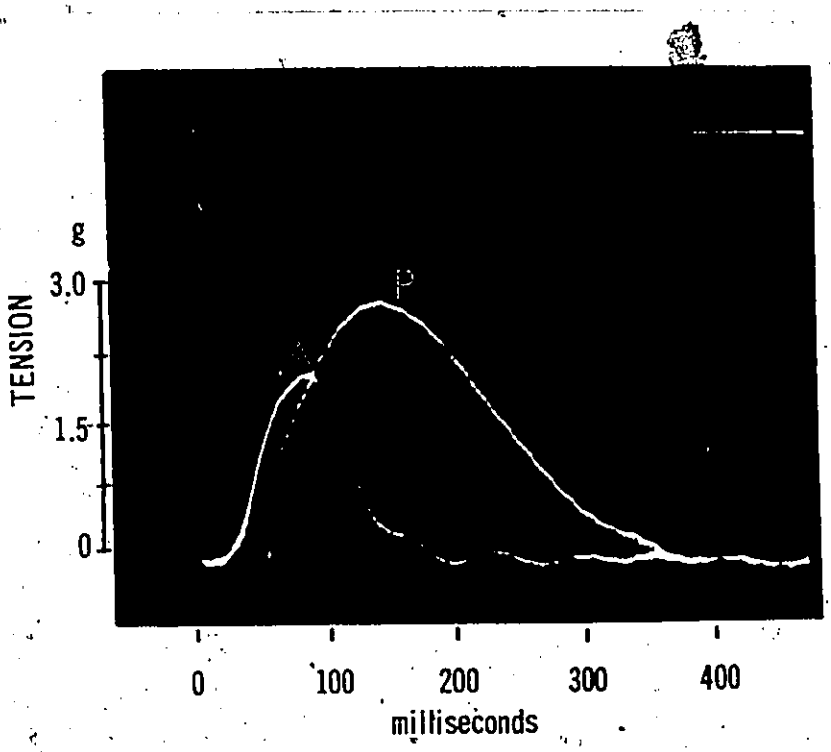
The difference in TMT between A and P has been emphasized in rat as presented by example in the Introduction. It remains to be seen if this phenomenon may be present in other animal species and under a variety of experimental conditions.

Experiments were performed after the muscles were stretched to, and equilibrated at, equivalent points on the basic curve of length versus tension. Specifically, these points were at L max (the length at which maximum developed tension occurred) or at 75% L max.

1. Shapes of the Contraction Curves of A and P and the Influence of:

a) Age of the Animal

Evidence from microscopy indicates that rat ventricular cells during the first 2 weeks of post-natal life are very similar to atrial cells of adult rat (Schiebler and Wolff, 1966). There is no evidence of a T system and since this subcellular structure may be implicated as a possible determiner of the twitch time course, a comparison of A and P twitch time courses in very young animals was suggested. The experimental records (Figures 8 a and b) indicate that the difference in TMTs between A and P is still present in muscles removed from young post-natal rat and dog.



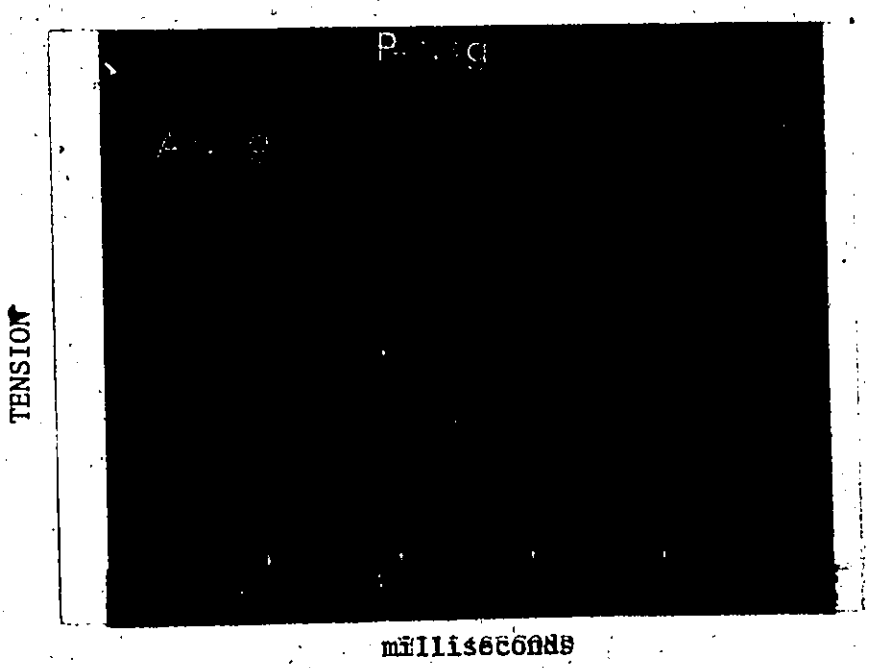


Figure 8. Isometric contractions of A and P of young animals.

(a.) rat, 8 day old
(b.) dog, 24 hr old

Stimulation rate was 5/minute and temperature was 28°C.

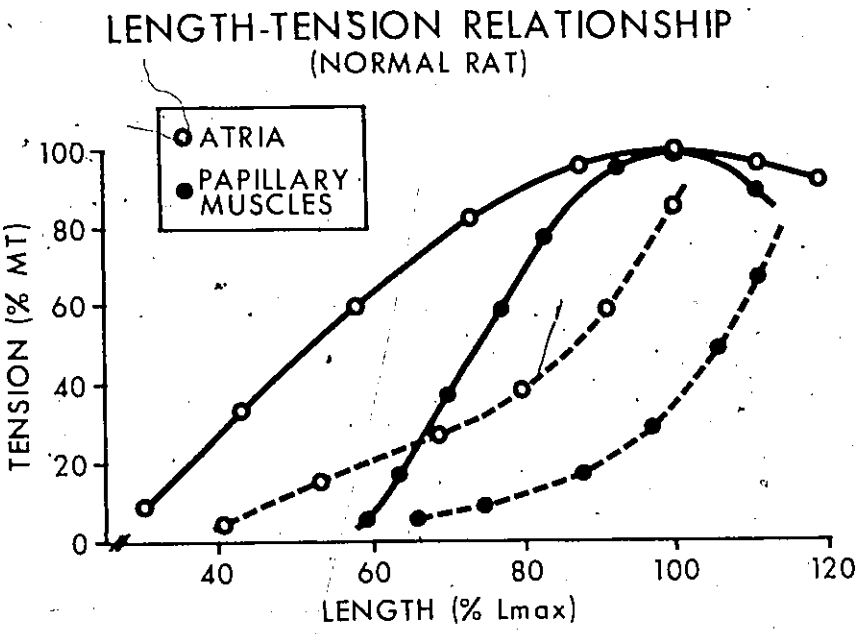
b) Muscle Length

Active and passive length-tension curves for A and P at a stimulation rate of 5/minute and temperature 28°C were generated. Curves have been plotted after using a computer program which undertook a least-square polynomial analysis of any number (<100) of length-tension (x-y) values (Figures 9 a and b) for 8 atria and 8 papillary muscles.

Active tension, as a function of length, generated a length-tension curve which intersected the length axis between 50 and 60% in P (Figures 9 a and b). In other words, active tension approached zero in this length range as % of L max. In A the active tension crossed the length axis at 20-30% of L maximum. Length increments beyond L max were avoided in experiments where the muscles were to be used further. However in certain experiments muscles were stretched to as much as 120% L max. At 95-105% of L max there was a plateau region followed by a sharper fall in active tension as length increased above 105% in both A and P. At L max the resting tension constituted 25-35% of MT in P but 80-90% of MT in A. Beyond L max the resting tension rose more sharply in P but this sharper inflection in the curve was noticed at 70-80% L max in A. Reserpinized animals showed similar results.

The addition of increasing amounts of load (by stretching the muscle) does not significantly alter the TMT (Figure 10). Even pre-loads, beyond those which will cause progressively greater actively developed tension, still do not significantly change TMT (see papillary muscle - Figure 10, curves numbered 4, 5).

a.



b.

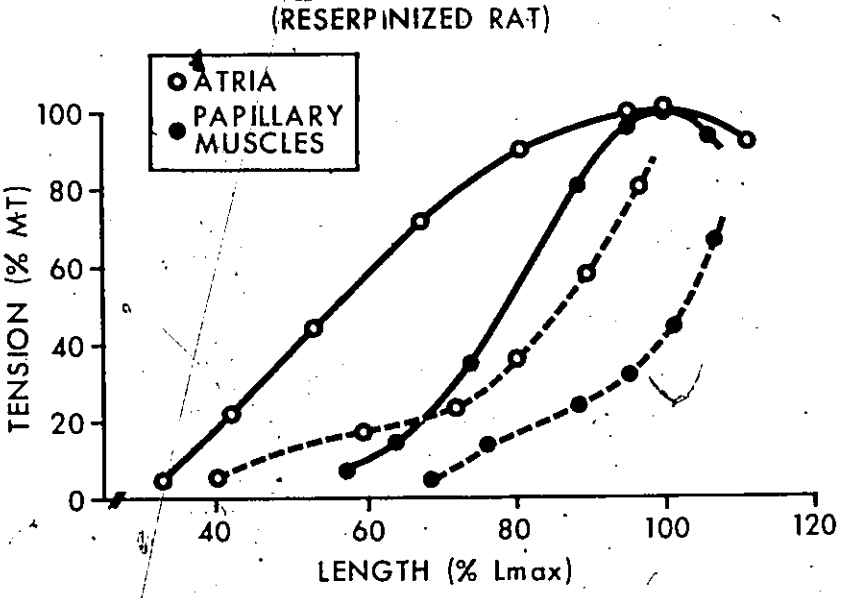
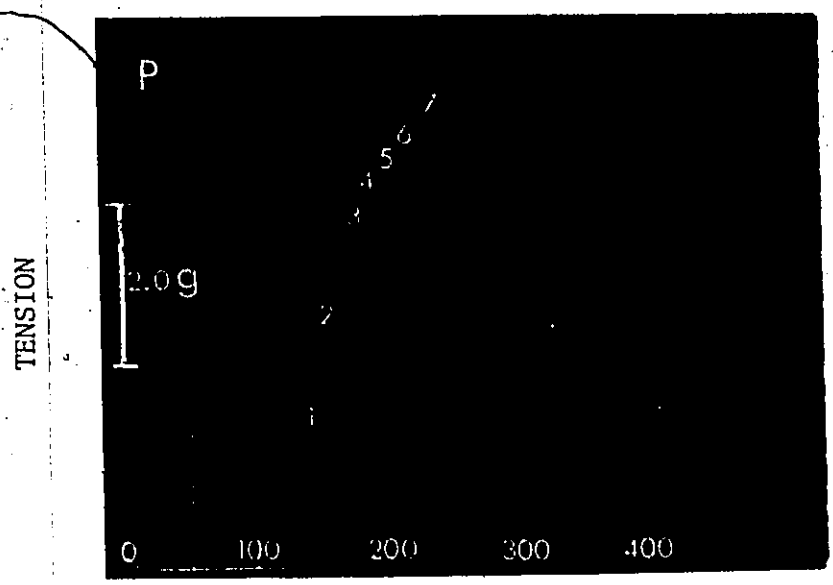


Figure 9. Length-tension relationship in normal (a) and reserpinized (b) rat comparing A and P. Optimal length (L max = 100%) was the length at which maximal developed tension occurred (MT = 100%). Temperature was 28°C. Dashed lines represent RT.



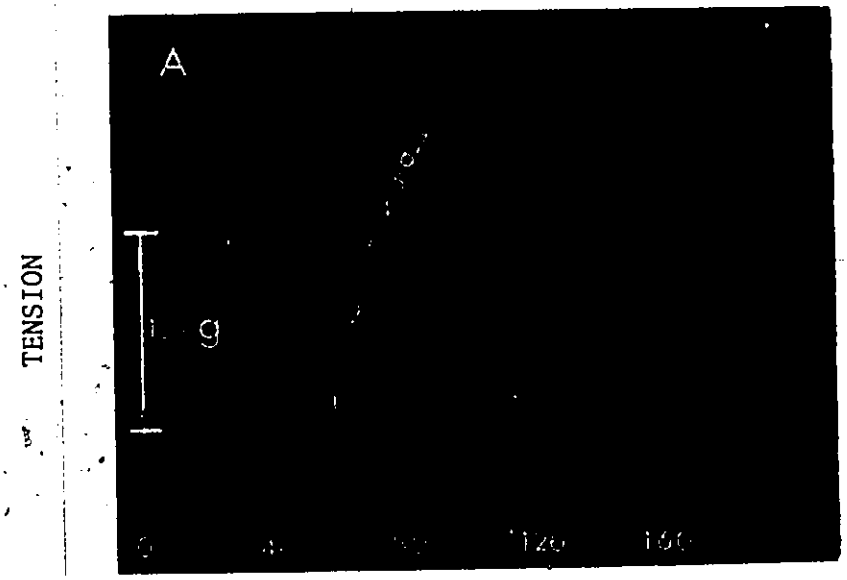


Figure 10. Twitch contractions of normal rat papillary muscle and atrial strip with preload variation. Preload (g) was as follows:
P: 1) .25, 2) .50, 3) 1.00, 4) 5.50, 5) 4.50, 6) 2.00, 7) 3.00
A: 1) .25, 2) .50, 3) .75, 4) 1.00, 5) 1.25, 6) 1.75, 7) 2.25
with the appropriate contractions numbered. Stimulation rate was 5/minute at 28°C.

c) Stimulation Rate

The negative inotropic response (decrease in T) in rat observed on increasing the stimulation rate was seen in both A and P and was similar to previous observations (Blinks and Koch-Weser, 1963). The % decrease in T is similar in both muscles compared at the same rate (Figure 11). Notice, however, that the atrial TMT does not change significantly whereas there is a significant TMT decrease in papillary muscles as driving rate is increased. Conversely, in atria the dT/dt decreases more than in papillary muscles as rate is increased.

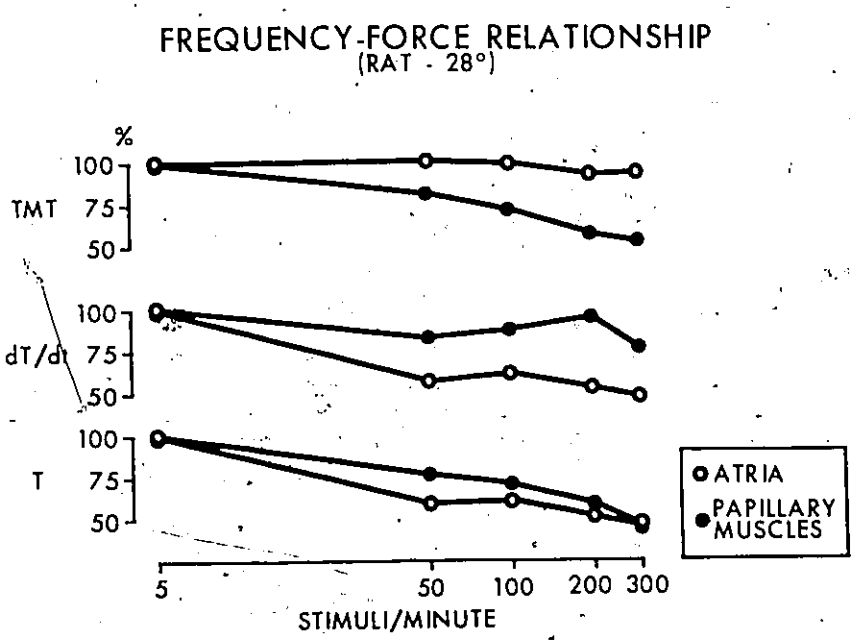


Figure 11. Effect of rate of stimulation on TMT, dT/dt and TMT in rat atria and papillary muscles. Values at 5 stimuli/minute were chosen as 100%. Each point is the mean value of 8-12 A or P.

d) Bath Temperature

Temperature variation also alters the rate and duration of T development as well as the absolute amount of actively developed T. In both A and P, the MT in most cases occurs at a temperature of 28°C, but there was no major decrease at 23°C (Figure 12 a and b).

A summary of data from isometric twitch contractions at a stimulation rate of 5/minute is presented in Table 9 . For a complete examination of the same parameters at a number of stimulation frequencies, refer to the two unabridged Tables A and B in the appendix. At temperatures 18 through 38°C, the maximum dT/dt is higher in A than in the P compared at the same temperature. This is particularly significant when one considers that active tension is significantly less in atria at all temperatures.

i) Temperature Coefficients

For meaningful comparisons of temperature effects on the rates of various biological processes, the rates of reaction must be compared for the same temperature interval. The ratio of the rate of activity at a given temperature to rate at a given temperature 10°C lower is called the temperature coefficient (Q_{10}) and quantitatively compares the effect of temperature upon chemical reactions, i.e. it is a factor by which a reaction velocity is increased for a 10°C temperature interval where data may be gathered for larger or smaller intervals. The Van'tHoff equation for calculating temperature coefficients follows:

$$Q_{10} = \frac{K_2}{K_1}^{\left(\frac{10}{t_2-t_1}\right)}$$

or $$\log Q_{10} = \left(\frac{10}{t_2-t_1}\right) \log\left(\frac{K_2}{K_1}\right)$$

K_1 is reaction rate at temperature t_1

K_2 is reaction rate at temperature t_2

In general, the temperature coefficients of various parameters for A are not significantly different than for P. This can be seen in Figure13 for Q_{10}'s of dT/dt/MT and in Figure14 showing Q_{10}'s for TMT, dT/dt and tension.

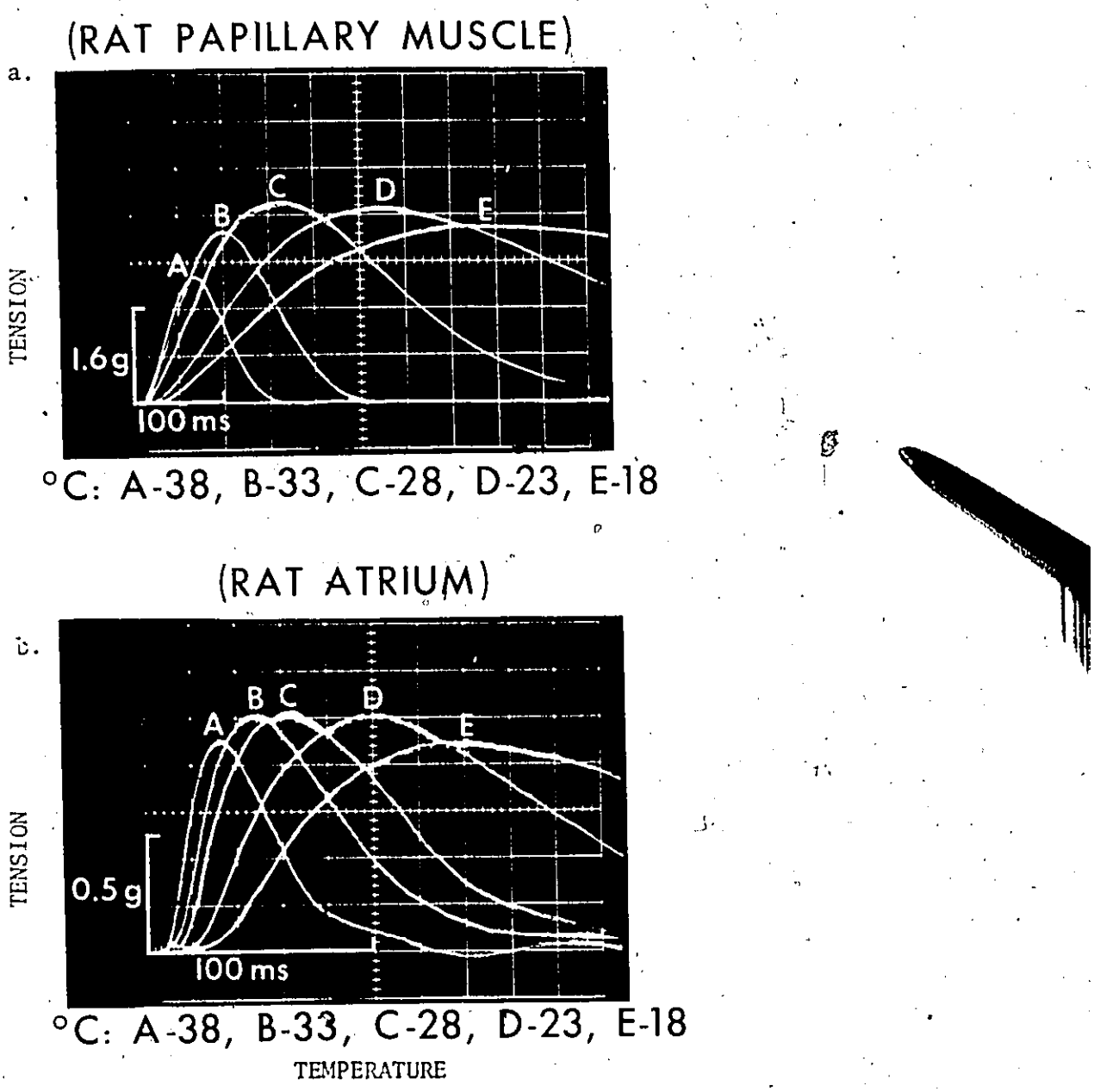


Figure 12. Isometric contractions of rat papillary muscle (a) and atrium (b) at different temperatures (°C). Stimulation rate was 6/minute.

TABLE 9

Isometric Contractions of Atria and Papillary Muscles (Rat)

Muscle	Temperature (°C)	Resting Length (mm)	Maximum Tension (g)	Time to Maximum Tension (msec)	Maximum Rate dT/dt (g/sec)
A	18	5.21±.222	2.00±.179	147±8.0	25.3±3.38
P	18	3.82±.319*	2.48±.378	359±16.6*	12.0±1.81*
A	23	5.00±.221	2.26±.150	88±4.9	46.6±3.61
P	23	3.79±.282*	3.20±.352	248±8.0*	22.4±2.29*
A	28	4.80±.260	2.07±.143	54±2.6	66.4±4.88
P	28	3.62±.303+	3.33±.347*	160±3.8*	37.7±4.16*
A	33	4.82±.300	1.68±.135	36±2.1	86.9±8.61
P	33	3.63±.299+	2.60±.366	81±4.2*	47.8±6.12*
A	38	4.80±.260	1.60±.112	26±1.6	100.0±7.21
P	38	3.64±.296+	2.25±.248	62±2.0*	57.0±5.80*

Mean values ± SEM. A-atria, P-papillary muscles. Stimulation rate 5/minute. Statistical significance between A and P at same temperature, $p < 0.05$+, $p < 0.01$*. Values are means ± SEM of 8-12 A or P.

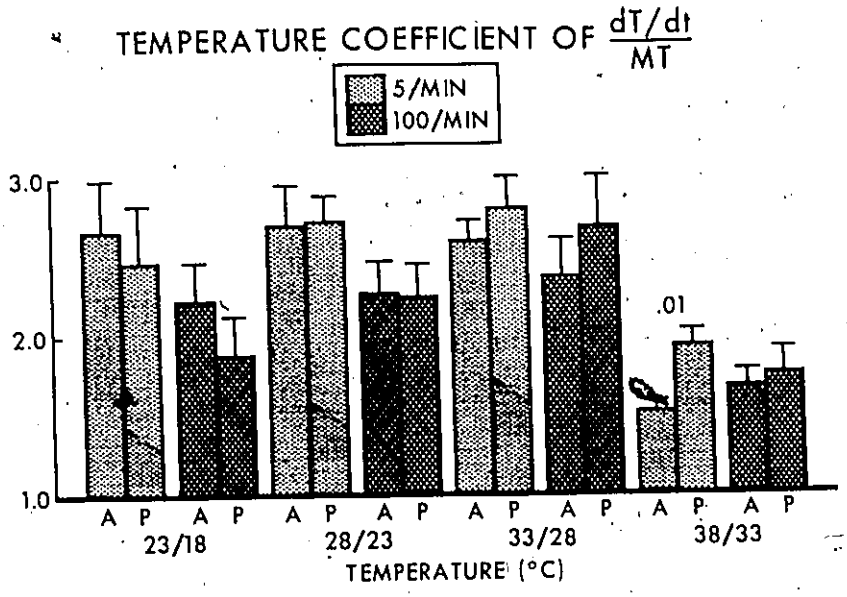


Figure 13. Q_{10} values of $\frac{dT/dt}{MT}$.

Mean ± SEM of $\frac{dT/dt}{MT}$ comparing rat atria to papillary muscles at 5 and 100 stimuli/min and through four temperature ranges. Statistically significant differences are indicated by the p value. n= 8-12 A or P.

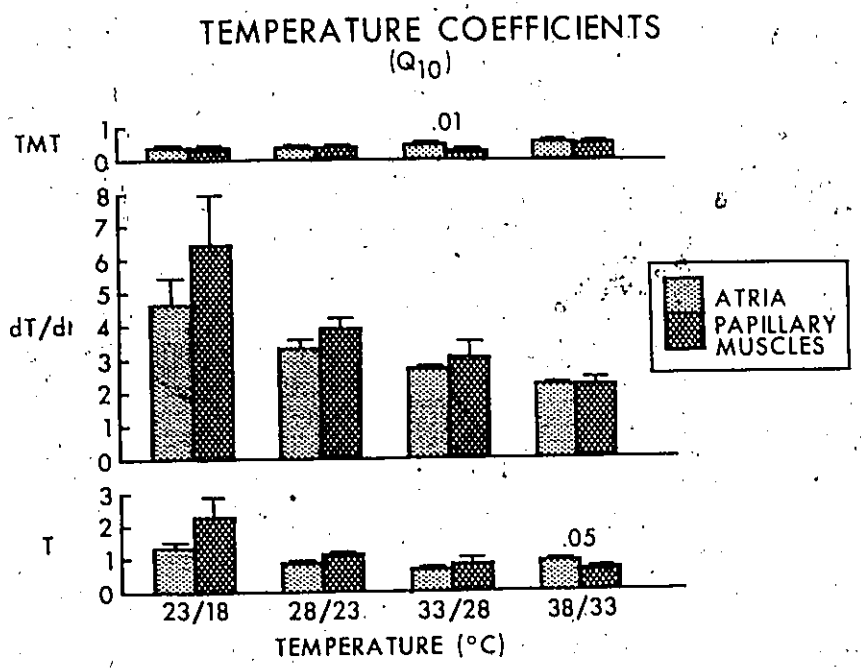


Figure 14. Q_{10} values of TMT, dT/dt and MT. Mean±SEM of TMT, dT/dt and MT comparing rat atria to papillary muscles through four temperature ranges. Statistically significant differences are indicated by the p value. n = 8-12 A or P. Q10 < 1 for TMT because TMT decreases as temperature is elevated.

ii) TMT of A and P

The intent of this experiment was to alter the shape of the twitch curves in order to change the A contraction to that of the V. Since temperature alteration produces substantial changes in the mechanical parameters of the myocardium, it was used to prolong the duration of TMT in A and shorten the duration of TMT in P. When the oscilloscope tracings of an A twitch at 22°C and a P at 30°C were superimposed (Figure 15), then only the very onset of contraction and last 1/3 of relaxation deviates from each other. Thus the shapes of contraction and relaxation in these two muscles may be made almost identical by appropriate temperature adjustment.

iii) Interrelationship of TMT, TM dT/dt, and TM Vce

Since TMT is significantly different between A and P, it was of interest to compare the time relationship of other mechanical parameters. The velocity of the contractile element, Vce, may be followed through time relative to T and dT/dt after incorporation of a logarithmic amplifier into the circuitry used for recording isometric contractions. Representative contractions with the electronically derived dT/dt and Vce are shown in Figure 16. Mean values for various parameters recorded from A and P are shown in Table 10. Average weights and lengths as well as the respective preloads placed on the muscles, were not significantly different between A and P. As expected, the TMT and time to maximum dT/dt values were definitely much less in the A; however, the time to maximum

Vce (TMVce) values were not statistically different from the P at 3 of the 5 temperatures. Consequently, when the TMVce values were related to the TMT and expressed as %, the values for A and P were widely separated (Figure 17). The actual % for A ranged from 24 to 42 and for P from 7 to 19 through the temperature range 18-38°, respectively. This information indicates that the peak Vce occurs later in the twitch contraction of A than it does in P. Although not as widely separated the $\frac{TMdT/dt}{TMT}$ values were also significantly different with the maximal dT/dt occurring at a later time in the atrial twitch (31-50%) as opposed to the papillary muscle twitch (25-42%) through the 18-38°C temperature range (Figure 17).

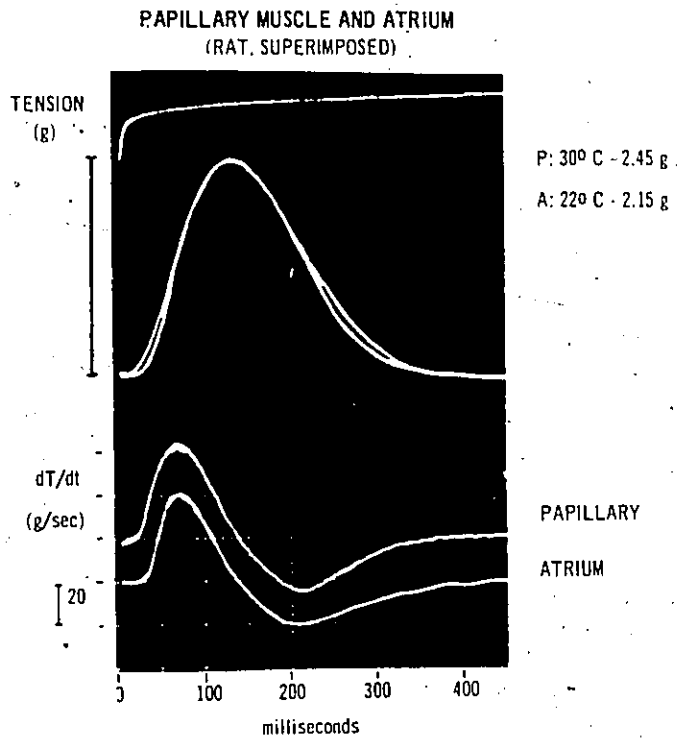


Figure 15. Isometric contractions from an A at 22° and a P at 30°. Absolute values of tension (T) are shown in the upper right; slight adjustment of the vertical gain on the oscilloscope produced the same amplitude. Maximum rates of tension development (dT/dt) for each muscle are the bottom records. The time axis is identical for T and dT/dt.

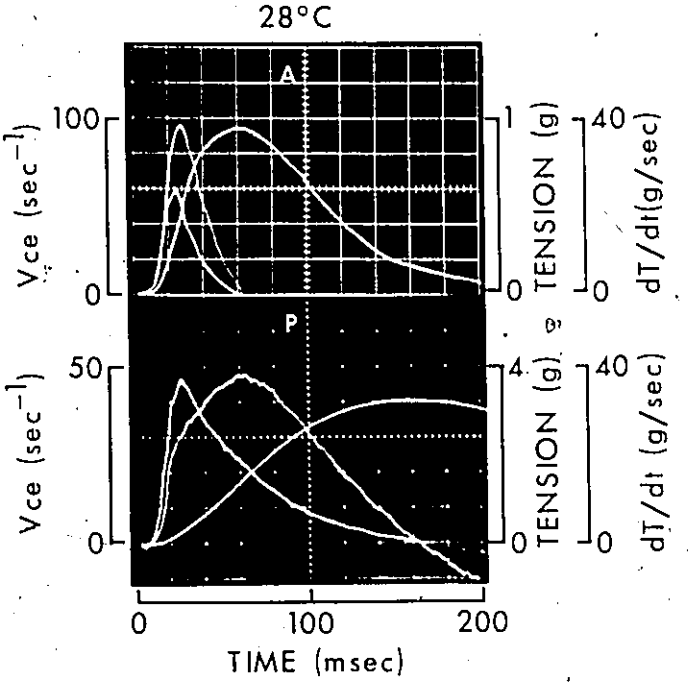


Figure 16. Isometric twitch contractions: T, dT/dt and Vce. One representative A and P and the appropriate calibrations are shown.

TABLE 10

Isometric Contractions: Time Relationship of T, dT/dt and Vce

Temperature (°C)	Muscle (AorP)	Preload (g)	Maximum Tension (g)	Time to Max. Tension (TMT-msec)	Time to Max. dT/dt (msec)	Time to Max. Vce (TMVce-msec)	Latent Period (msec)	TM Vce / TMT X 100 %
18°	A	.86±.092	.90±.112	128±5.3	39±1.0	31±2.3	26.7±0.56	24±1.8
	P	.91±.084	2.18±.191*	357±16.0*	88±4.2*	26±2.1	26.8±0.40	7±0.7*
23°	A	.88±.086	1.01±.132	84±3.1	29±2.4	21±1.4	17.8±0.17	25±0.8
	P	.83±.091	2.70±.203*	254±12.4*	76±2.1*	21±1.3	17.5±0.22	8±0.3*
28°	A	.94±.082	1.07±.131	57±3.6	22±0.6	18±0.5	12.5±0.34	32±2.1
	P	1.05±.091	2.66±.192*	154±6.4*	50±2.6*	14±0.7+	12.8±0.31	10±0.6*
33°	A	.86±.083	1.03±.088	39±1.1	20±0.7	17±0.5	9.3±0.25	42±1.6
	P	.81±.093	2.27±.232*	95±4.1*	38±2.0*	13±1.0+	9.3±0.21	14±0.8*
38°	A	.83±.082	1.03±.103	31±1.7	15.3±0.5	13±0.6	7.2±0.11	42±2.2
	P	.83±.101	1.84±.211*	65±1.4*	28.3±1.2*	12±0.6	7.7±0.33	19±0.6*

Mean values ± SEM. A = atria, P = papillary muscles. Stimulation rate 6/minute.
A: weight = 3.00 ± .331, length = 7.96 ± .552; P: weight = 3.01 ± .332, length = 7.88 ± .511. Statistically significant difference between A and P at same temperature - $p < 0.05^{+}$, $p < 0.01^{*}$

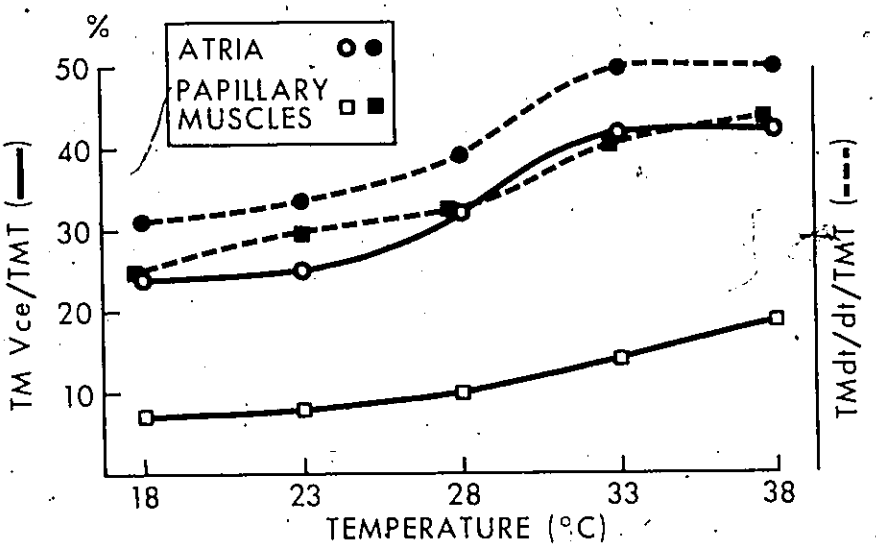


Figure 17. The ratio of time to maximum Vce to TMT (left) and the ratio time to maximum dT/dt to TMT (right) as a function of bath temperature. Each point represents mean ± standard error of 6 rat atria and 6 papillary muscles. SEM is smaller than the symbols shown.

e) Ionic Changes in Bathing Solution

In another attempt to modify the shapes of atrial and ventricular twitch curves so that one approximates the other, the ionic composition of the bath was altered.

i) Calcium and Sodium

When either NaCl (117.4 mM) or $CaCl_2$ (2.5 mM) was reduced to 3/4 and then to 1/2 of the normal concentrations, the duration of TMT was not changed. When the normal bathing solution was replaced by solutions with no $CaCl_2$ or no NaCl then the mechanical output response changed faster in the A than in the P. For instance, MT decreases 89% in A and only 45% in P after the first 3 minutes. In the A the reduction of MT is brought about by the decreased rate of tension development as indicated by dT/dt, while TMT remains either unchanged or is slightly prolonged (Table 11). On the other hand in the P the reduction of T is brought about by decrease in dT/dt as well as in the TMT. The results of these few experiments indicate that in calcium-free Ringer solution the intracellular Ca^{2+} concentration may be decreased faster in the isolated A than in the P.

One very interesting observation is that when muscle lengths were varied at low bath calcium concentrations the TMT was also changed. Figures 18 and 19, (a) show a P and A in 1.5 mM $CaCl_2$ at various lengths whereas Figures 18 and 19(b) show the same muscles in .75 mM $CaCl_2$ at various lengths. The TMTs are significantly increased as the muscles

are stretched in low calcium (.75 mM). This increase in TMT is less at a higher calcium concentration (1.5mM) and no significant TMT increase was seen in normal calcium (2.5 mM).

(ii) Strontium

Buccino, et al. (1967) indicated that strontium (Sr^{2+}) differed from all other inotropic agents which elevated T and $\Delta T/\Delta t$ because it also augmented the TMT in cat heart strips. In addition, total tension developed in potassium chloride contractures of rabbit auricles was up to 20 times larger in a solution containing Sr^{2+} instead of Ca^{2+} (Busselen, 1971).

In experiments on rat atria and papillary muscles, the bath solution which contained calcium was replaced by one which contained Sr^{2+}. Figure 20 shows twitch contractions before and at various times after the replacement. Records of T, dT/dt and V_{ce} in normal Ca^{2+} and after 40 minutes in Sr^{2+} may be seen in Figure 21. Both T and TMT were significantly increased in the A. The total duration of the contraction increased about 4.5 x in the A and 2.5 x in the P whereas the TMTs increased approximately 2 x in both A and P. The atrial relaxation time appears lengthened by about twice as much as the papillary muscle relaxation period after 40 minutes in Sr^{2+}.

TABLE 11

Contraction Parameters After Altering Bath Solution

	Normal to No $CaCl_2$		Normal to No NaCl	
	ATRIUM	PAPILLARY	ATRIUM	PAPILLARY
Tension Development (T)	-89	-45	-83	-65
Time to Maximum Tension Development (TMT)	20	-15	0	-25
Maximum Rate of Tension Change (dT/dt)	-95	-49	-92	-60
Total Contraction Duration (TTT)	-28	-11	-28	-34

Calculated per cent changes from normal values just prior bath alteration to values recorded 3 minutes after bath adjustment. Rat left atrial strips and ventricular papillary muscles at 28°C., stimulation rate 5/minute. No preload or length alteration; muscles at 75% L max.

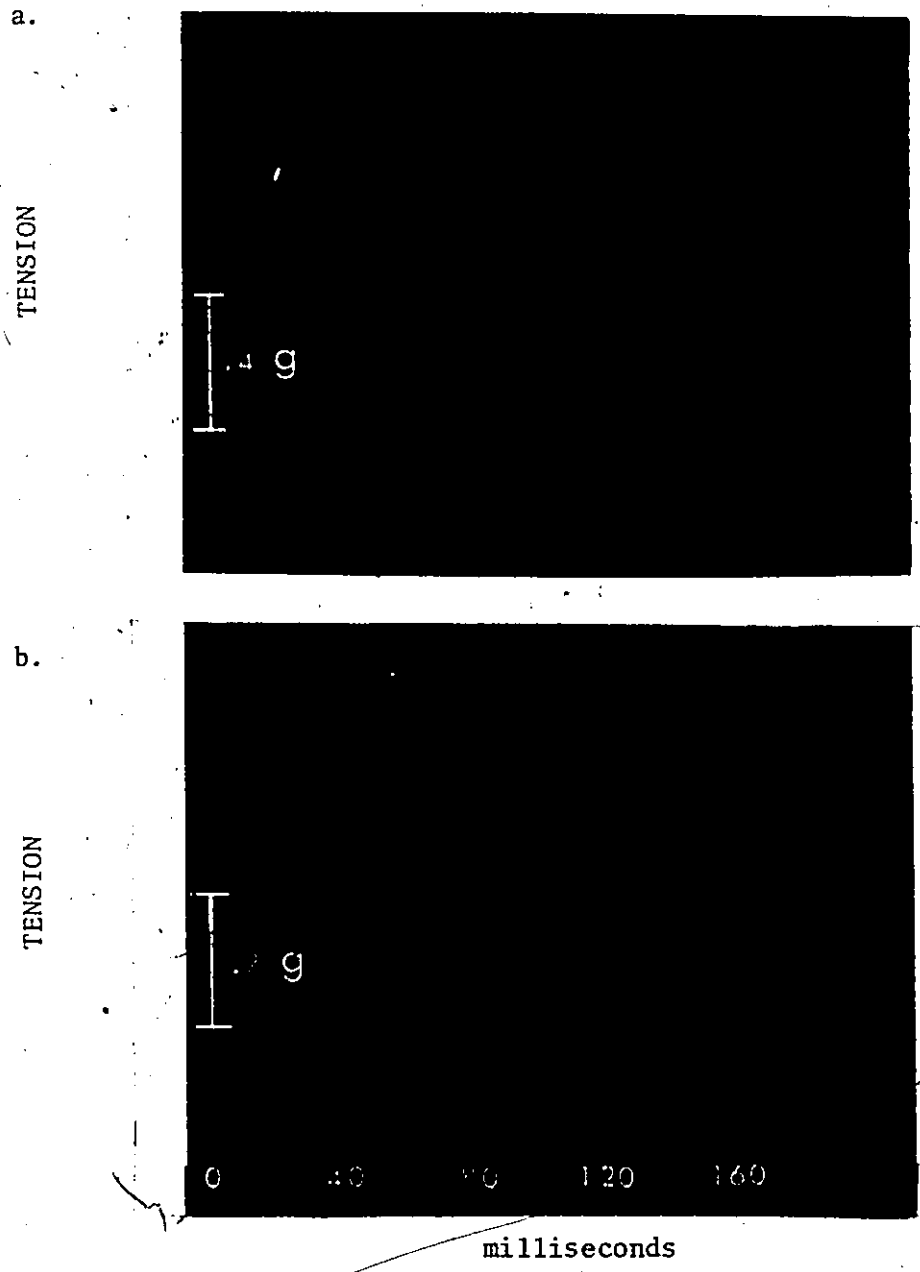


Figure 18. Isometric contraction of an A at two different bath concentrations of calcium followed by preload change in (a.) 1.5 mM $CaCl_2$ (b.) .75 mM $CaCl_2$. Muscle lengths were decreased to a length which gave near 0 developed tension and then increased to a length which included the former L max. The time axis refers to all recordings. 5/ minute stimulation rate was used at 28°C.

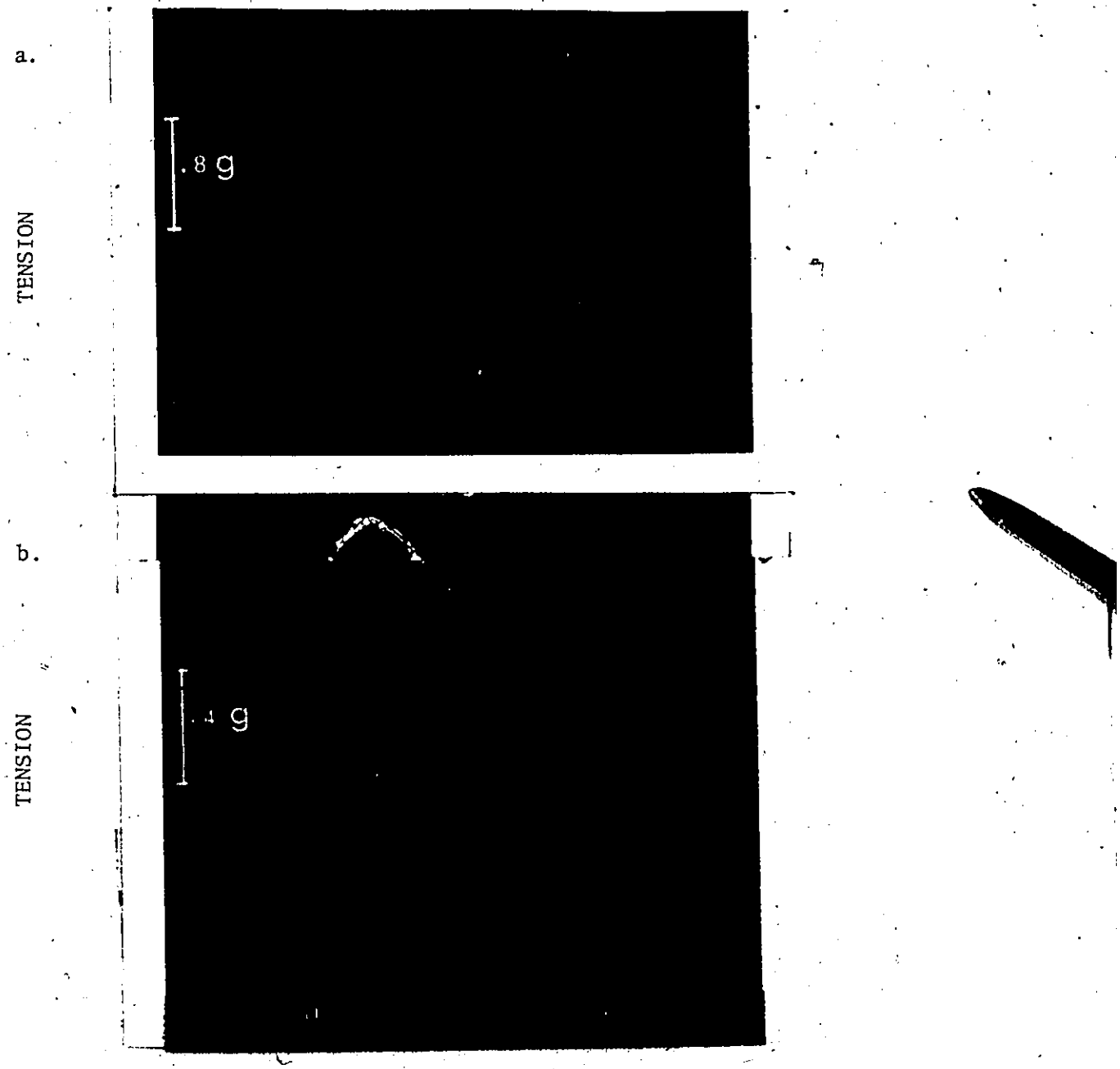


Figure 19. Records of a P at two different bath concentrations of calcium followed by preload change.

(a.) 1.5 mM $CaCl_2$ (b.) .75 mM $CaCl_2$
Muscle lengths were decreased to a length which gave near 0 developed tension and then increased to a length which included the former L max. The time axis refers to all recordings. 5/minute stimulation rate was used at 28°C.

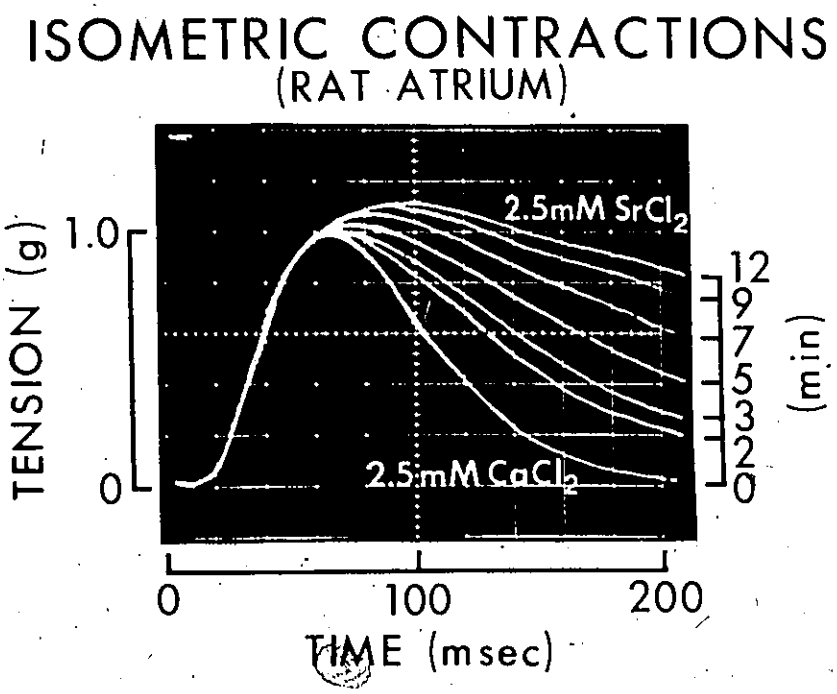


Figure 20. Comparison of isometric contractions of A in 2.5 mM $CaCl_2$ followed by change to a bath solution containing 2.5 mM $SrCl_2$ instead of $CaCl_2$. Minutes (0-12) at right indicate time after change to $Sr\ Cl_2$.

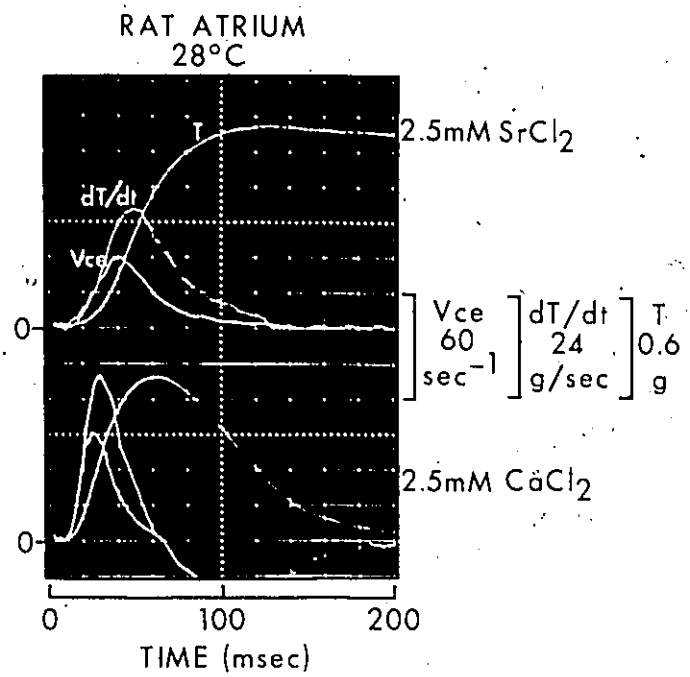


Figure 21. Comparison of isometric contractions of an A in 2.5 mM $CaCl_2$ and then after 40 min. in 2.5 mM $SrCl_2$. The tension (T), maximum rate (dT/dt) and dlnT/dt (Vce) scales are indicated at the right for both the $SrCl_2$ and $CaCl_2$ contractions.

f) Reserpine and/or Propranolol Treatment

Electrically stimulating isolated strips of myocardium by suprathreshold stimuli from field electrodes may excite intracardiac nerves and possibly liberate chemical mediators. Since there are higher concentrations of acetylcholine and norepinephrine in the A than in the V, a proportionately greater amount of these compounds may be released in the A with consequent reflection in the contraction curves. Blinks (1966) found that pretreating cats with reserpine (5mg/Kg) eliminated the sympathomimetic effect of field stimulation. Koch-Weser (1965) showed similar effect in kitten. However, in reserpine-treated guinea pigs the sympathomimetic action was eliminated only after addition of propranolol (10^{-6}M). In the present experiments rats were injected intraperitoneally with reserpine on 3 consecutive days prior to killing. The total dosage of reserpine was 5mg/Kg. These treated animals noticeably responded to this dose (diarrhea, lethargic). In a few experiments propranolol(2×10^{-5}M) was added to the bath. No significant differences in MT, dT/dt, and TMT was found either in A or in P after reserpine or propranolol or a combination of both (Table 12).

TABLE 12

Effect of Reserpine and Propranolol on Atria and Papillary Muscles of Rat

	Number of Animals	Resting Length (mm)	Maximum Tension-T (g)	Time to Maximum T (msec)	Maximum Rate dT/dt (g/s)	$\frac{dT/dt}{MT}$ ($sec.^{-1}$)
ATRIA						
Normal	5	8.00±1.34	2.25±.49	62.2±1.96	73.6±20.1	32.0±2.7
Propranolol	1		-8.8%	0.0%	-3.3%	5.7%
Reserpine	6	7.53±0.89	2.42±.20	59.2±3.96	85.3±6.5	35.4±0.9
Propranolol	3		-0.7%	-0.5%	-1.5%	-0.7%
PAPILLARY MUSCLES						
Normal	8	7.94±0.40	6.44±.57	155.9±5.66	78.5±6.7	12.3±0.4
Propranolol	3		-1.3%	-6.4%	0.1%	1.5%
Reserpine	3	10.38±1.36	6.15±.65	158.2±11.2	77.1±7.9	12.6±0.5
Propranolol	3		-4.2%	-1.5%	-5.1%	-1.4%

Mean values ± SEM in isometric twitches at L max. Reserpinized animals received a total 5 mg/kg intraperitoneally in 3 doses on 3 consecutive days prior to experiment

Propranolol, 2×10^{-5}M, was added to some animals in each group, values given are % changes after 20-40 minutes. Bath temperature 28°C, stimulation rate 5 per minute. No statistical significance was observed comparing normal to reserpinized animals for either A or P.

2. Analysis of the Atrial and Ventricular Contraction Curves

a) TMT Comparisons

Certain experiments were designed to investigate the possibility that there were temperature ranges or stimulation rates at which the TMTs of A and V were similar. The resultant effect of temperature and stimulation rate on TMT is presented in Figure 22. The TMT of A is insensitive to stimulation rate change, whereas the TMT of V is significantly increased by an increase in stimulation rate throughout the range of temperature 33 to 18°C.

i) Species Differences

The differences between atrial and ventricular TMTs are expressed as ratios by dividing ventricular TMT by the atrial TMT. Since the TMT of V is always greater, the ratio is always greater than 1. The ratios are presented in Figure 23 for guinea pig and cat as well as the rat at various temperatures. In general, it appears that the TMT differences are more striking in animals where the heart rate is slower (cat vs. rat) or in one species when the stimulation rate is lowest (rat) (Table C in the Appendix).

ii) At Similar Tensions

It is important to note that in several specifically chosen A and P, where the maximum developed tensions were similar, the TMTs were still very much different. Also, in these specific cases the dT/dts were higher in A than in P (Table 13).

iii) As a % of Total Contraction Time

The time taken to achieve peak tension was a fairly constant % of

the total contraction time measured to end of relaxation at various stimulation rates or temperatures (Table 14). In rat and guinea pig the TMT was approximately 33% of the total contraction time with no significant difference between A and V.

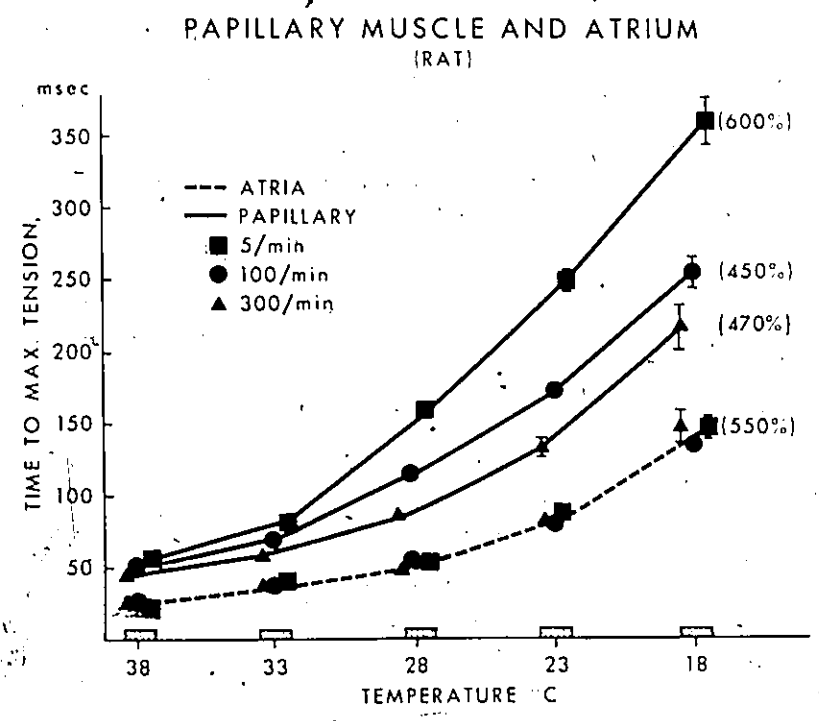


Figure 22. Effect of changing temperature on TMT at various stimulation rates. Mean values ± SEM; the vertical bars are omitted when the SEM is smaller than the symbols. %'s listed at right side of each frequency curve represent increase from lowest to highest values in that respective curve. n = 8-12 A or P.

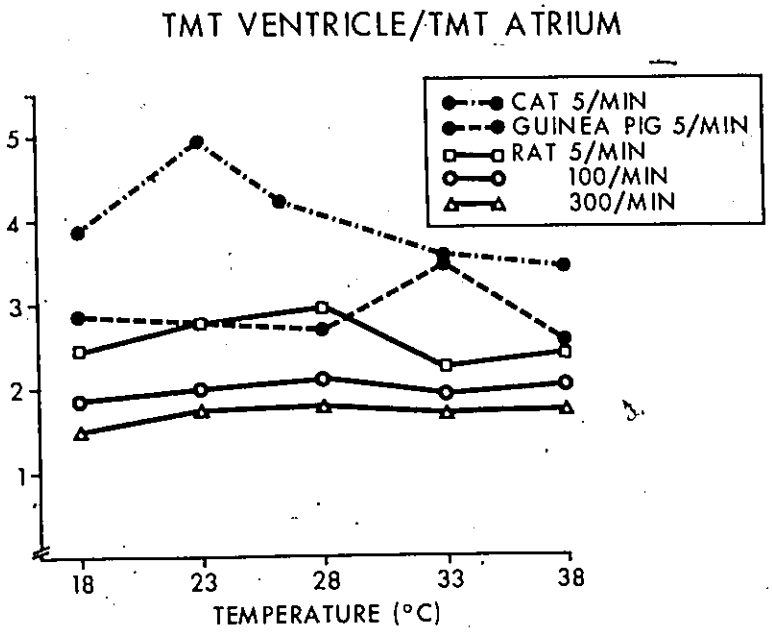


Figure 23. Effects of change in temperature and stimulation rate on the ratio of TMT of V divided by the TMT of A. Three species: cat, guinea pig and rat are represented.

TABLE 13

Isometric Contractions of Atria and Papillary Muscles at Similar Tension

Temperature	Stimulation Rate	Muscle Number	Maximum Tension		Time to Maximum Tension		Maximum Rate	
			A	P	A	P	A	P
°C	/min		g		msec		g/sec	
18	5	1	1.50	1.50	130	350	20	7
	100	2	2.40	2.40	130	220	40	18
23	5	3	2.05	2.15	70	230	43	16
	100	4	2.30	2.30	75	185	59	20
28	5	5	2.25	2.25	40	170	72	23
	100	6	2.30	2.40	55	125	77	31
33	5	7	1.55	1.60	40	80	83	32
	100	8	1.55	1.60	40	55	64	36
38	5	9	1.20	1.20	25	60	80	37
	100	10	0.90	0.90	25	70	107	21

A and P were chosen at similar MT to compare TMT and dT/dt values. Values are given at temperatures of 18-38°C and 5 and 100/minute stimulation rate.

Table 14.

Time to Maximum Tension as a % of Total Contraction Time

Animal	Temperature (°C)	Muscle (A or P)	Time to Maximum Tension (%)		
Guinea pig	18	A	36.7	A Mean = 33.0±.59, n = 40	
		P	21.3		p - N.S.
	23	A	33.7		
		P	20.6	P Mean = 32.8± 1.34 n= 29	
	28	A	30.9		
		P	30.9		
	33	A	34.1		
		P	38.4		
	38	A	32.2		
		P	34.5		
Cat	18	A	32.7	A Mean = 31.0±.52, n=41	
		P	36.3		p <.001
	23	A	28.8	P Mean = 37.6± .55 n=30	
		P	35.1		
	28	A	29.3		
		P	35.7		
	33	A	32.2		
		P	38.8		
	38	A	30.6		
		P	39.3		
Rat	18	A	31.7	A Mean = 32.4± .66, n=46	
		P	28.2		p - N.S.
	23	A	32.8	P Mean = 33.9± .66, n=39	
		P	29.8		
	28	A	34.8		
		P	34.6		
	33	A	32.5		
		P	36.7		
	38	A	30.6		
		P	35.4		

b) dT/dt/MT Comparisons

In characterizing an isometric twitch, the following basic equation has been formulated (Buccino et al., 1967)

Maximum tension = mean rate of tension development multiplied by the time to maximum tension

or

$$MT = \left(\frac{\Delta T}{\Delta t}\right)(TMT) \qquad (1)$$

In order to compare skeletal muscles of various sizes and geometry, Hartree and Hill (1921) used an index which incorporated both rate of tension development and maximal tension, that is $\frac{dT/dt}{MT}$ (2). Described verbally, it is "the maximum rate of proportional rise of tension" expressed in units of time^{-1}. The index seems logically suited for comparing A and P contractions where there is a difference in tension produced.

In order to gain a better understanding of the index, equation (1) may be substituted for the denominator MT of the index (2) so that

$$\frac{dT/dt}{MT} = \frac{dT/dt}{(\Delta T/\Delta t)(TMT)}$$

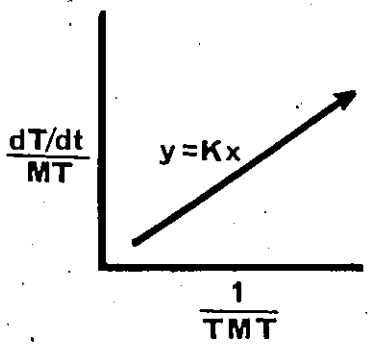
or

$$\frac{dT/dt}{MT} = \left(\frac{dT/dt}{\Delta T/\Delta t}\right)\left(\frac{1}{TMT}\right); \text{ allow } K = \frac{dT/dt}{\Delta T/\Delta t}$$

therefore

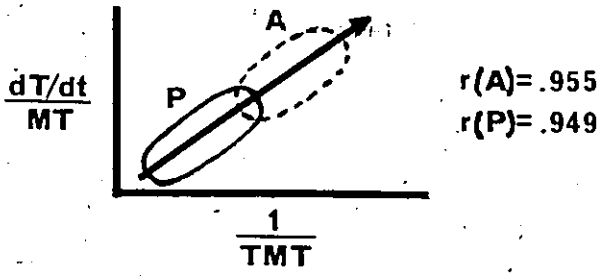
$$\frac{dT/dt}{MT} = K\left(\frac{1}{TMT}\right) = K\ (sec^{-1})$$

furthermore, graphically



i) Relationship to TMT

With temperature alteration from 18-38° both $\frac{dT/dt}{MT}$ (Figure 24) and TMT are changed (Figure 22). At all temperatures and stimulation rates the $\frac{dT/dt}{MT}$ is significantly higher in the A than in P. (Figure 24 and Table D in Appendix). By taking the inverse of TMT and plotting, as done above, a linear relationship (slope K) was attained for rat atrial strips and papillary muscles as shown below:



The K values for A and P from a variety of species and at various stimulation rates were compared. The results shown in Table 15 include slope (K), intercept, standard error and statistical significance between A and V. There was no statistically significant difference in this slope for A compared to V within the species cat, guinea pig and rat at various temperatures or stimulation rates.

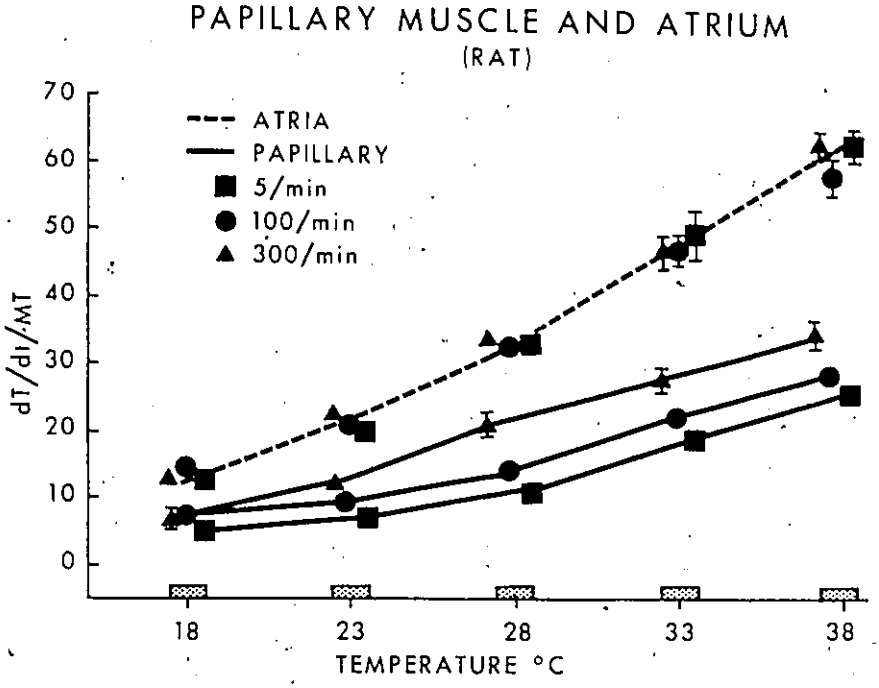


Figure 24. Effect of changing temperature on maximum rate of tension development normalized per maximum developed tension ($\frac{dT/dt}{MT}$). A and P are compared at various stimulation rates. Mean values ± SEM; vertical bars are omitted when SEM is smaller than the symbols. n= 8-12 A and P.

TABLE 15

Relationship Between $dT/dt/MT$ and $\frac{1}{TMT}$

Animal	Muscle	Stimuli per Minute	Slope K	Slope ± SEM	Variance ± SEE	Intercept A	Fit r	Difference d	Difference ± SEM	t
rat	A	5	1.618	.0011	4.94	2.70	.963	.225	.112	2.01
	V		1.393	.0012	1.63	1.74	.976			
	A	100	1.383	.0012	6.07	6.31	.933	.030	.130	.229
	V		1.413	.0015	2.25	1.73	.960			
	A	300	1.589	.0010	4.52	2.25	.970	.075	.066	1.13
	V		1.514	.0023	4.38	1.42	.911			
guinea	A	5	1.464	.0024	3.77	2.33	.956	.231	.232	.993
pig	V		1.694	.0101	2.16	- 0.40	.932			
	A	100	1.420	.0026	4.10	6.48	.946	.284	.305	.932
	V		1.704	.0142	1.92	- 0.53	.928			
	A	300	1.414	.0026	4.50	8.76	.949	.280	.396	.707
	V		1.694	.0158	1.58	0.89	.963			
cat	A	5	1.494	.0018	2.15	3.09	.980	.029	.201	.146
	V		1.524	.0076	0.60	0.73	.984			
	A	100	1.474	.0029	3.34	4.74	.950	.122	.583	.209
	V		1.353	.0556	1.32	2.99	.822			
	A	300	1.410	.0060	3.68	5.24	.922	.380	1.02	.372
	V		1.030	.2170	2.43	6.44	.475			

Relationship between the ratio of maximum rate of tension development and maximum developed tension $\frac{(dT/dt)}{MT}$ and the inverse of time to maximum tension ($\frac{1}{TMT}$) at temperatures 18-38°C and expressed as linear regression: $Y = K \pm SEM \times X + A \pm SEE$. r represents the goodness of fit; differences between the regression lines of atria and ventricles at the same frequency of stimulation were evaluated by standard t-test.

ii) Effect of Temperature and Stimulation Rate

It is interesting to note that, compared at the same temperature, the atria always reside farther away from 0 (x-y intercept) than the papillary muscles (Figure 25). Furthermore, as temperature is increased the papillary muscles are moved along a common slope K to the region of the atrial muscles and the atrial muscles are moved farther up the linear line. However, when stimulation rate is increased, the A are not shifted upward, as is the case with P (Figure 25).

iii) Muscle Length

The index $\frac{dT/dt}{MT}$ remained constant over the entire range of fiber lengths studied. This observation along with the insensitivity of TMT to length change is shown relative to the active and passive length-tension curves for a representative papillary muscle (a) and atrium (b) (Figure 26).

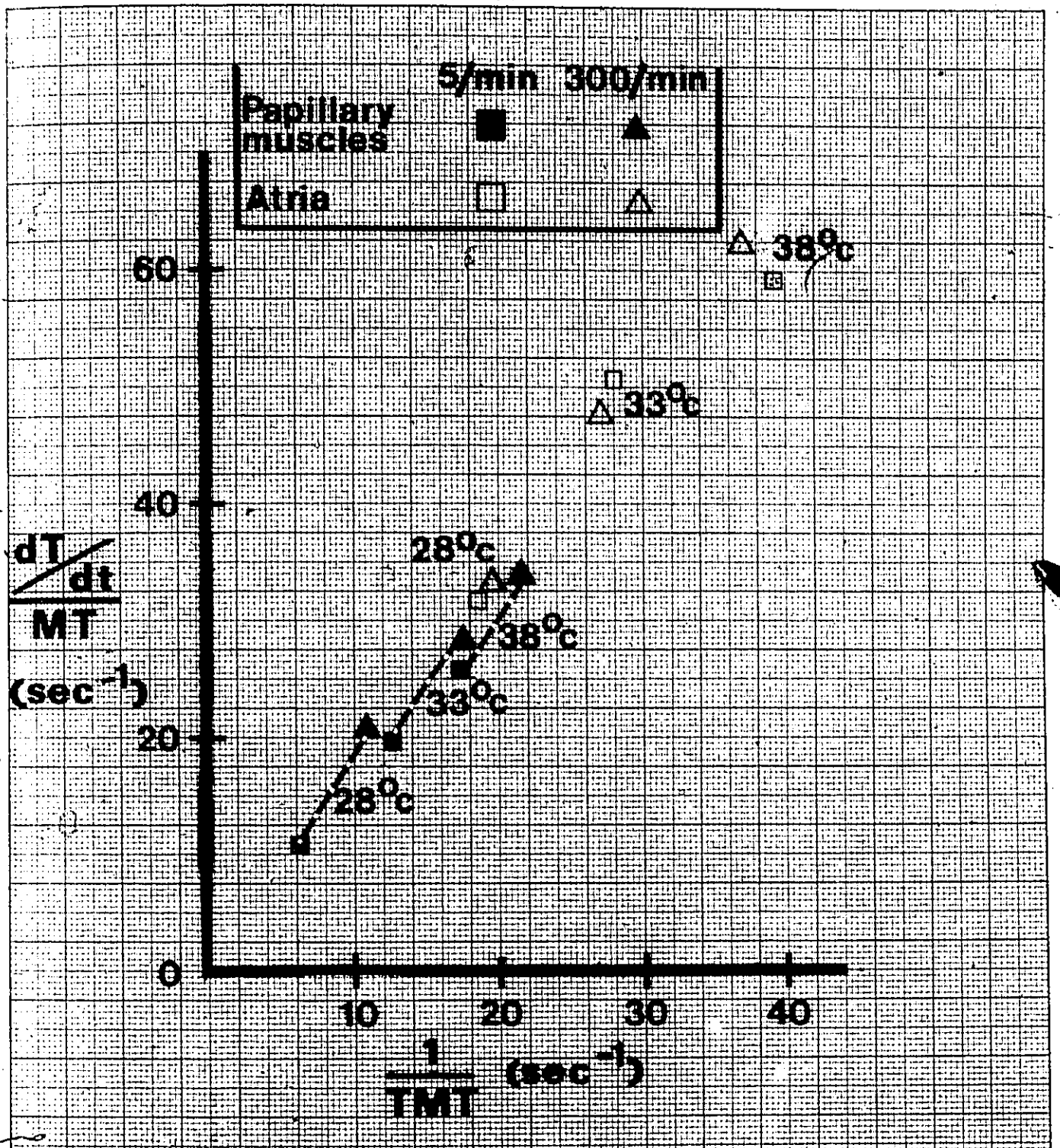


Figure 25. Relationship of $dT/dt/MT$ and $\frac{1}{TMT}$ at three different temperatures. Stimulation rate was varied from 5 to 300/minute at each temperature for A and P.

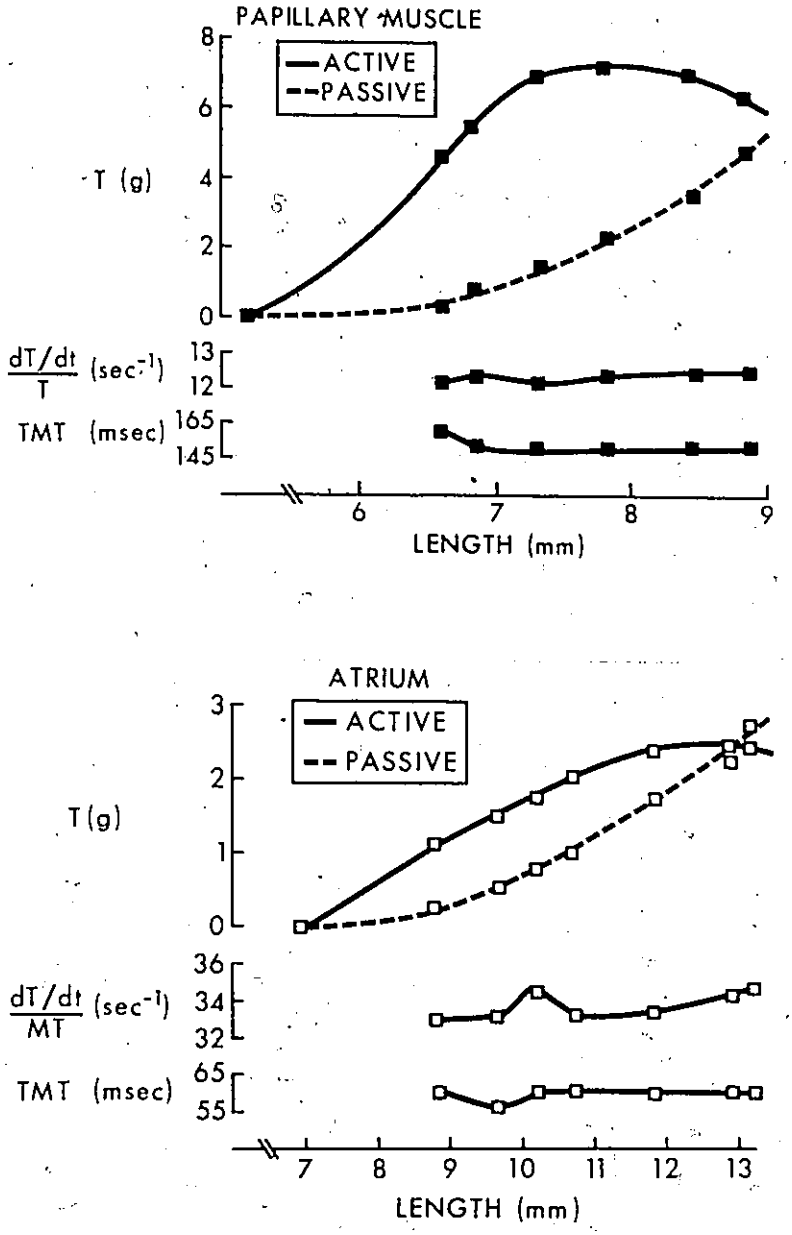


Figure 26. Relationship of T, $\frac{dT/dt}{MT}$, and TMT to increasing muscle length. Both active and passive T are shown for a representative rat A and P.

c) Force-Velocity Curves

Using a recent method for monitoring the force-velocity relationship, the vector loop formed by the dT/dt (T^{-1}) against T was monitored from twitch to twitch and gave instantaneous plots of force versus velocity. The force-velocity (F-V) curves for rat papillary muscle at several temperatures and also low Ca^{2+} (Figure 27) may be qualitatively compared to the atrial strip (Figure 28). Although the papillary muscle F-V curves may seem typically hyperbolic compared to other studies on ventricular muscle and to skeletal muscle, the atrial F-V curves are at best linear and certainly do not appear hyperbolic. The F-V curves under the influence of an increase in stimulation rate and/or isoproterenol are qualitatively affected to the same extent in these 2 muscles (Figures 29 and 30). In summary the F-V curves determined by this method are responsive to known inotropic agents such as calcium, isoproterenol and stimulation rate in both A and V.

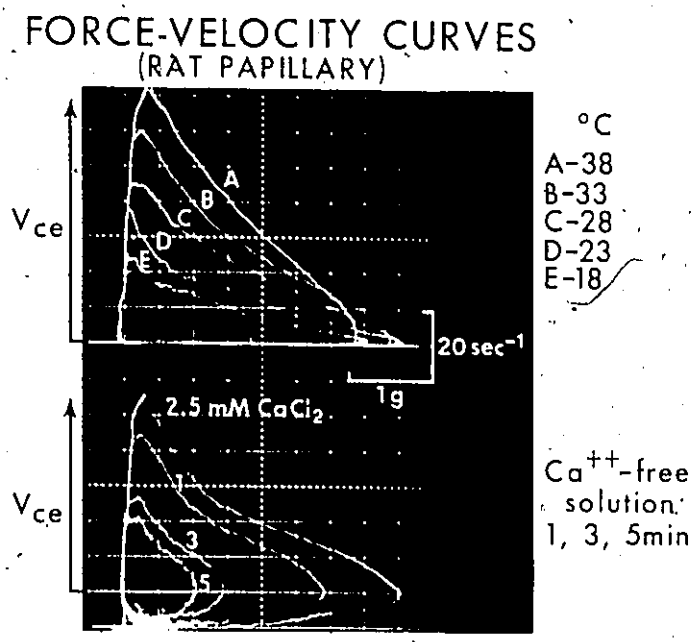


Figure 27. Force-velocity vector loops of rat papillary muscle at various temperatures (top) and following change to a $CaCl_2$-free bath solution at 28°C (bottom). The scales per axes for all curves are inset. Temperature is shown on the upper right and the minutes after change to $CaCl_2$-free bath solution are shown in lower right.

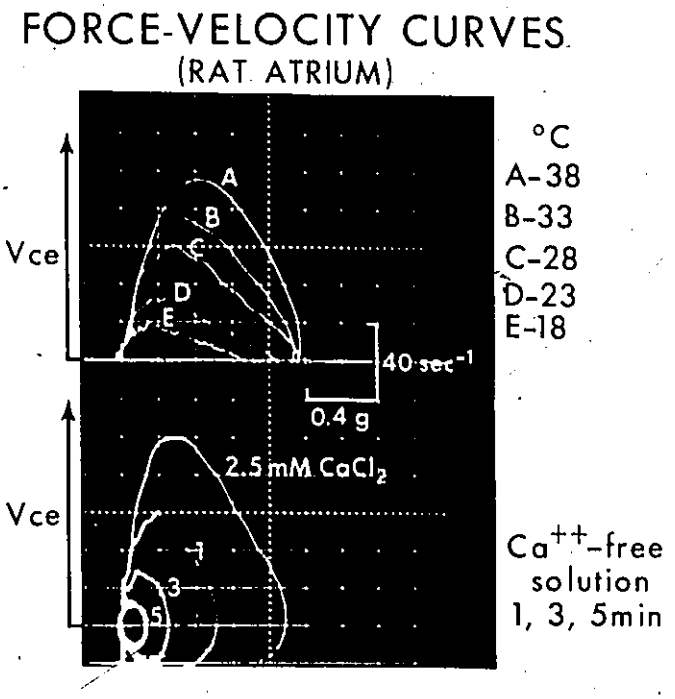


Figure 28. Force-velocity vector loops of rat atrium at various temperatures (top) and following change to a $CaCl_2$ bath solution at 28° (bottom). The scales per axes for all curves are inset. Temperature is shown on the upper right and the minutes after change to a $CaCl_2$-free bath solution are shown in lower right.

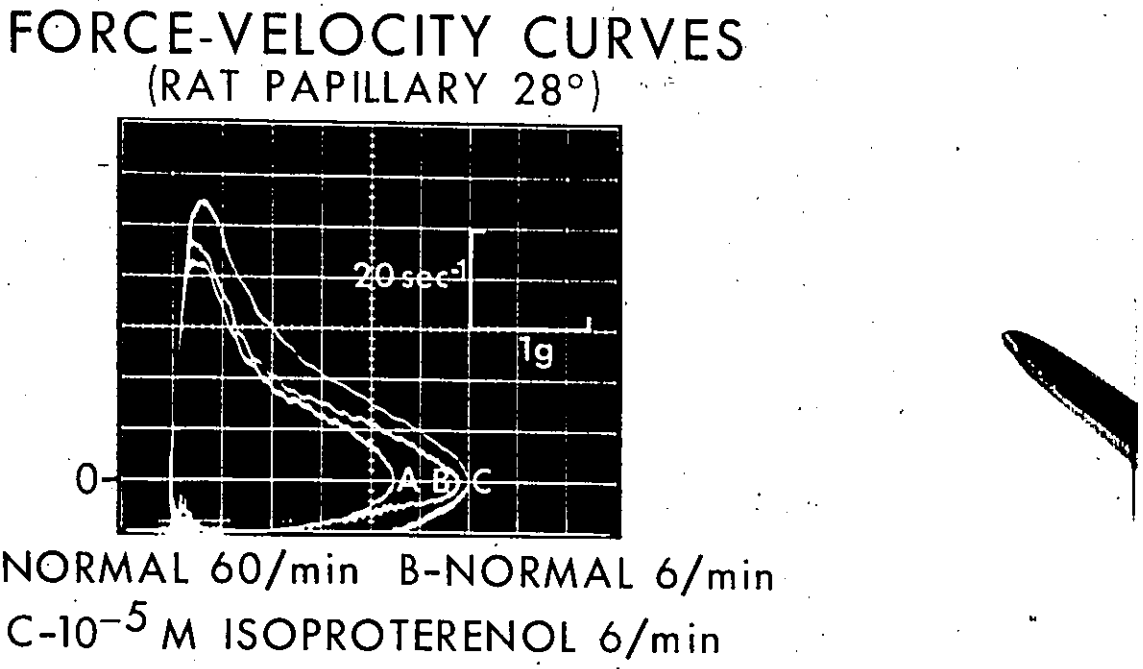


Figure 29. Effect of isoproterenol and stimulation rate on force-velocity vector loops of normal rat papillary muscle. The 6 and 60/minute stimulation rate is followed by addition of isoproterenol and subsequent 6/minute stimulation rate.

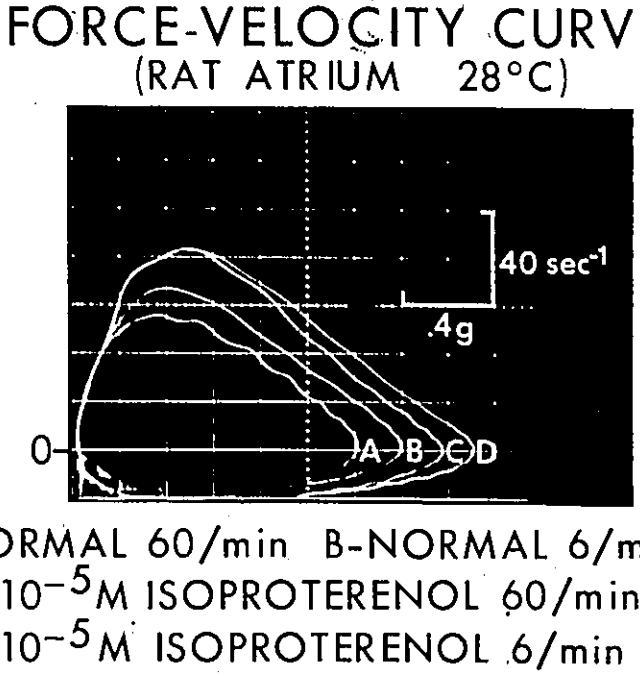


Figure 30. Effect of isoproterenol and stimulation rate on force-velocity vector loops of normal rat atrium. The 6 and 60/minute stimulation rate is followed by addition of isoproterenol and subsequent 6 and 60/minute stimulation rate.

C. Normal Myocardium-Isotonic Twitch Contractions

The data from isometric contractions show that maximum dT/dt is higher in A than V. Since this variable is an indicator of the velocity of shortening of the contractile element (V_{ce}), it was of interest to assess the V_{ce} under isotonic conditions when the SE for each muscle is constant.

1. Maximal Shortening Velocity

Strips of A and P were utilized and shortening velocity (dL/dt) was determined at different preloads and 0 afterloads. Figure 31 is illustrative of the type of recordings on which the analysis was based, where muscle length change (shortening) and the first derivative are shown for an A and P at various afterloads. Table16 summarizes these data including resting lengths; and Figure32 is a graphical presentation of shortening velocity at various preloads. The results clearly indicate that at any preload the maximum dL/dt was significantly higher in A than in the V whether the velocity values were expressed as mm/sec or muscle length per unit of time (ML/sec).

2. Time to Maximal Shortening Velocity

The time at which the contractile elements are capable of shortening with maximal velocity ($V_{ce}max$) at zero load was derived from isotonic contractions at increasing afterloads in the rat atria and papillary muscles. Initially the experiment was performed on cat papillary muscles to determine if the results would resemble those found by Brutsaert (1971). He found that the time from stimulation

to peak velocity of shortening in isotonic contractions was a linear function of increasing afterload. Therefore, the intercept of this function with the time axis at zero gave an estimate of the time to onset of maximal V_{ce}. Furthermore this time to maximal V_{ce} was 19-21% of the time to maximal tension development in an isometric contraction. Essentially my results with the cat duplicate those of Brutsaert. At preloads 0.22g/mm^2 and 0.99 g/mm^2 the time to V_{ce} max was 17 and 20%, respectively, of the TMT. However, in the rat papillary muscles the %'s are 36 and 29 for preloads of 0.46 g/mm^2 and 0.92 g/mm^2, respectively. In comparing rat atria at 0.25 and 0.50 g preload, the surprising finding was that the extrapolated times to V_{ce} max were not statistically different from those in P (Figure 33). Consequently, the expression of time to V_{ce} max as a % of TMT was over 60% in A. Although the extrapolated time to V_{ce} max as a % of TMT was not significantly affected by preload (0.2 - 0.9 g/mm^2) in the cat, the results presented here suggest that preload does influence this time to V_{ce} max in rat.

3. Quick Release for Measuring the Extension of the SE.

As mentioned previously, the sudden release of a muscle to zero tension may give an indication of the % of muscle length change which is necessary to re-shorten the SE from an extended position. In one series of experiments A and P were released from the peak of isometric twitch contractions and allowed to shorten under various afterloads including 0 afterload (Figure 34). The preloads were

0.25 g and mean values ± SEM for the amount of shortening as a % of muscle length were:

A - 8.02 ± .580 and P - 6.46 ± .629 for seven A and seven P. There was no statistically significant differences between these values, indicating that the SE was stretched to a similar amount as a % of total muscle length.

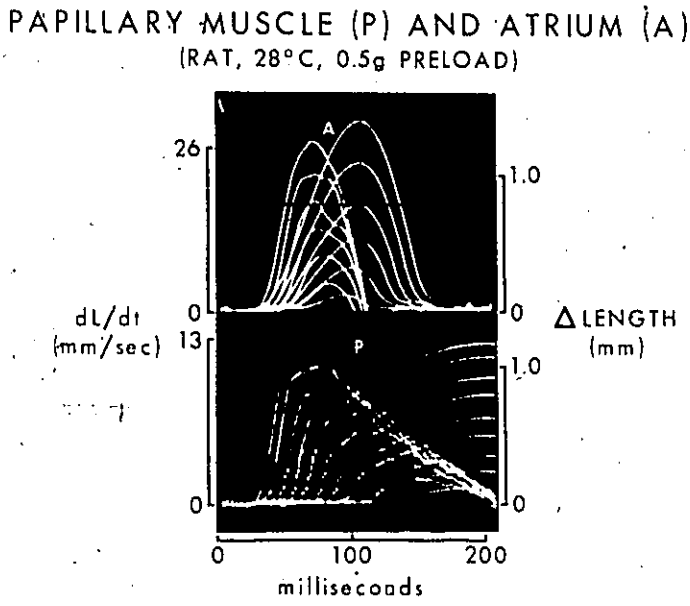


Figure 31. Isotonic after-loaded contractions of a representative A and P. Afterloads were increased by 0.25 g increments. Both length change (L) and the maximum rate of length change (dL/dt) are shown.

TABLE 16

Isotonic Contractions of Atria and Papillary Muscles of Rats

Muscle	No	Preload (g)	Resting Length (mm)	Maximum Shortening (mm)	Peak Shortening (mm/sec)	Velocity (ML/sec)	$\frac{dL/dt}{\Delta L/\Delta t}$ (Sec^{-1})
A	8	0.0	4.55±.39	0.46±.09	9.69±3.66	2.17±.31	1.38±.06
P	8	0.0	4.66±.35	0.30±.06	3.99±0.79	0.88±.19*	2.18±.52*
A	7	0.25	5.93±.52	0.97±.07	22.05±1.27	3.96±.49	1.53±.04
P	8	0.25	5.45±.31	0.67±.09*	8.26±1.47+	1.48±.23*	2.08±.10*
A	8	0.5	7.32±.60	1.09±.10	24.56±1.39	3.47±.26	1.51±.03
P	8	0.5	6.16±.40	0.78±.13	8.14±1.39*	1.28±.17*	1.76±.05*
A	8	1.0	8.32±.69	0.99±.09	21.49±1.75	2.69±.27	1.47±.03
P	8	1.0	7.19±1.34	0.82±.11	7.22±0.99*	0.99±.33*	1.58±.07
A	6	1.5	9.94±.46	0.83±.14	16.92±2.09	1.74±.26	1.45±.06
P	7	1.5	7.58±.57*	0.72±.10	6.07±0.93*	0.80±.11*	1.52±.08

Mean values ± SEM. A - atria, P - papillary muscles. Measurements were made at 0 afterload. Statistically significant difference between A and P at same preload, $p < 0.05$+, $p < 0.01$*. Bath temperature 28°C, stimulation rate 12 per minute.

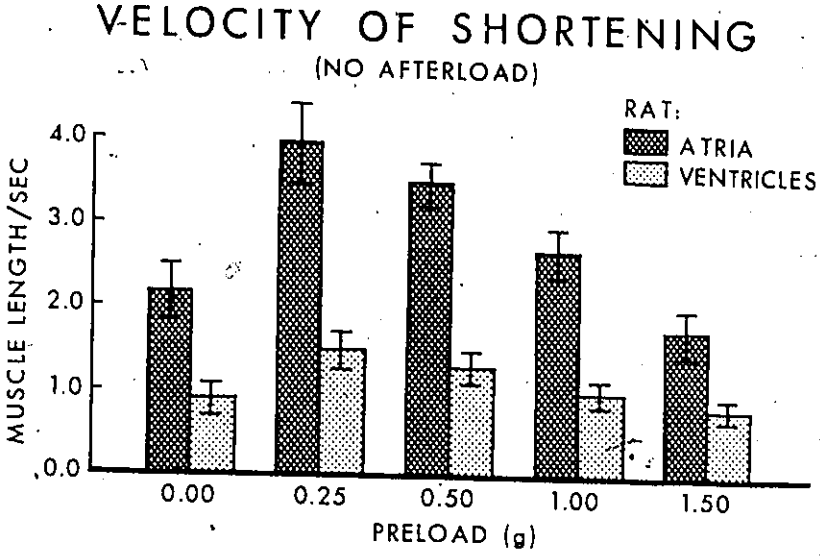


Figure 32. Isotonic shortening velocity (ML/sec) at several preloads for both A and P. No afterload was added. Mean ± SEM of 6-8 muscles.

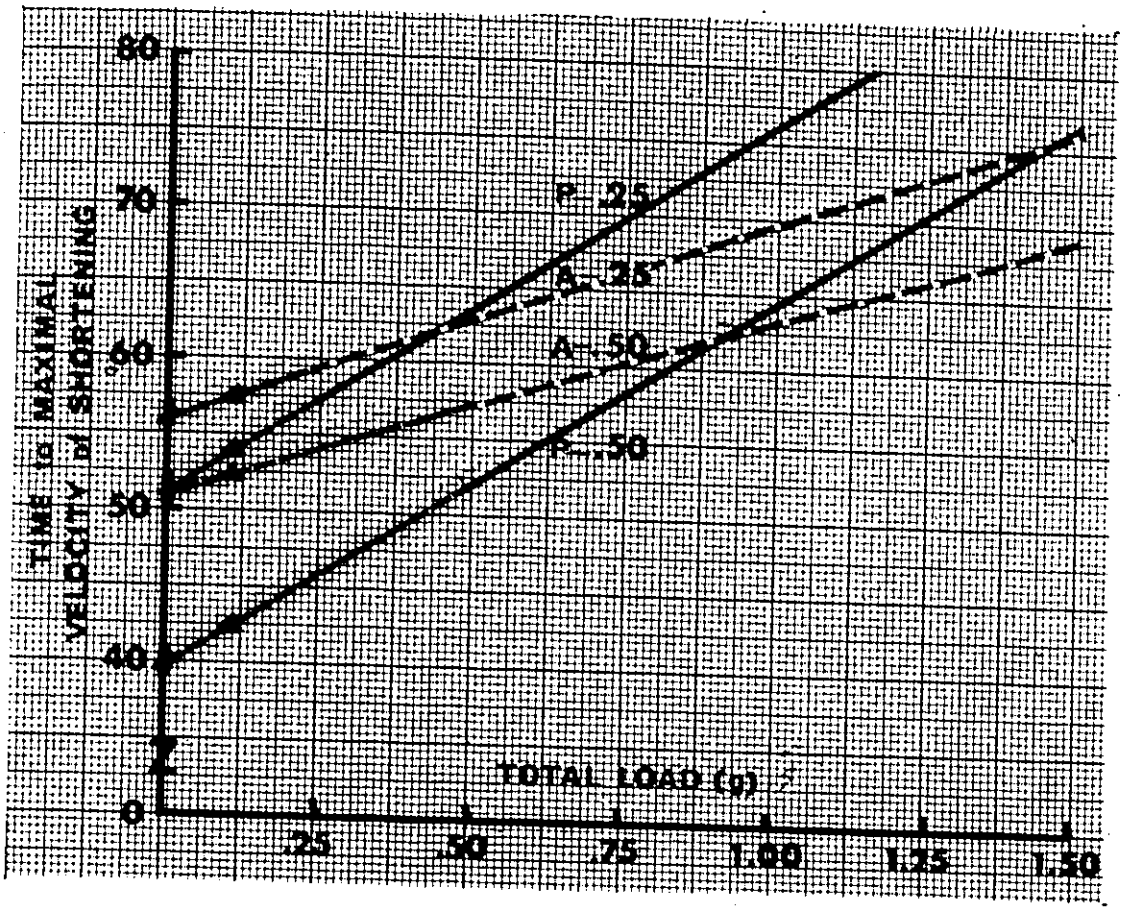


Figure 33. The time from stimulation to peak velocity of shortening as a function of total load. Papillary muscles and atria of rat at 28°C; preloads are indicated next to line. The mean regression lines for all curves plotted above and the equation of the form $Y = ax + b$ for each line is:

P	(0.25)	Y	=	25.2 (±1.25) X + 52.2 (±20.00)
P	(0.50)	Y	=	25.2 (±.80) X + 39.5(±15.77)
A	(.25)	Y	=	11.4 (±1.27) X + 57.5(±4.95)
A	(.50)	Y	=	11.1 (±.72) X + 52.0 (±4.17)

Each regression line is derived from 37-45 data pairs from 8 animals. Although the goodness of fit (r) values range from .61 to .75, a statistical test for a linear relationship of the data $\left(\sqrt{\frac{r}{\frac{1-r^2}{n-2}}}\right)$ suggests that all samples are significantly different from a population in which there is no linear relationship.

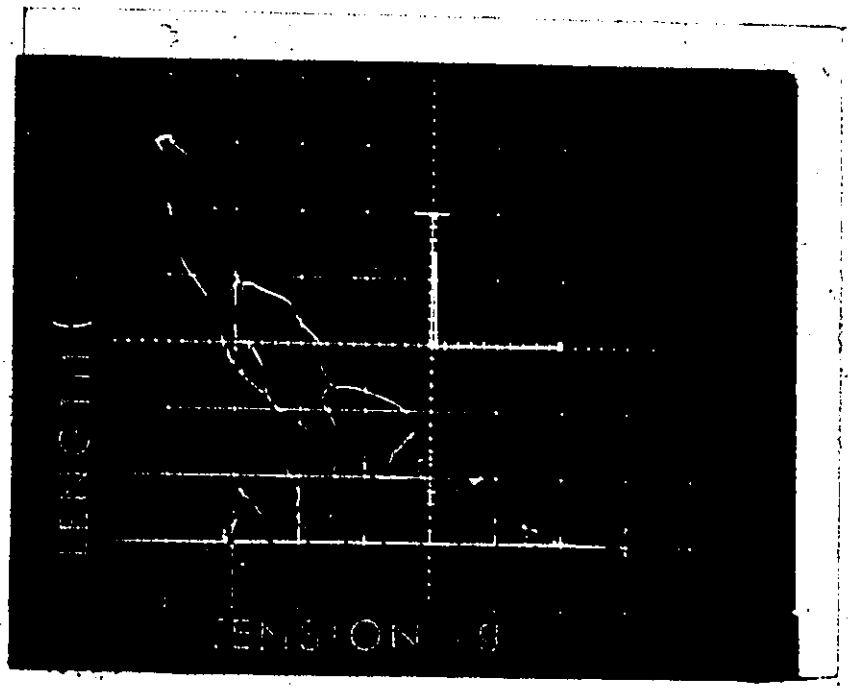


Figure 34. Phase-plane plot of tension development before and shortening after quick release. This representative P develops tension isometrically from A to maximum tension at C. At MT it is released to freely shorten against a selected afterload. This muscle was released to .75 g, .50 g, .25 g and 0 afterload, respectively in 4 twitches. Shortening and tension decrease describe the curve from C to B in a release to 0 afterload. The amount of muscle shortening (A-B) was measured at 0 developed tension after the release.

D. Isolated Contractile Systems

1. EDTA-Treated Myofibers

EDTA treatment was a procedure selected to evoke high sarcolemmal permeability and thereby eliminate the action potential generation. Records of the twitches before EDTA as well as the contractures initiated with calcium after EDTA were stored on tape (Figure 35 for a replay of one A and one P experiment). Data from these experiments are compiled in Table 17. In particular, note the significantly shorter time to peak contracture tension in A than in P; also that the initial rate for the first 30 seconds and the mean rate to peak tension are higher in A. Since normalizing the rate per cross-sectional area in atria may not be acceptable, it seemed reasonable to compare the rates per unit of maximal developed tension. Initial rates/maximal tensions yielded values which were all significantly higher in A than P. The curves in Figure 36 support this observation that the A are faster. The tension at each minute during contracture development is expressed as a % of the maximum tension during the first 10 minutes and indicates that the average time to peak contracture tension in atria is significantly less than in the P.

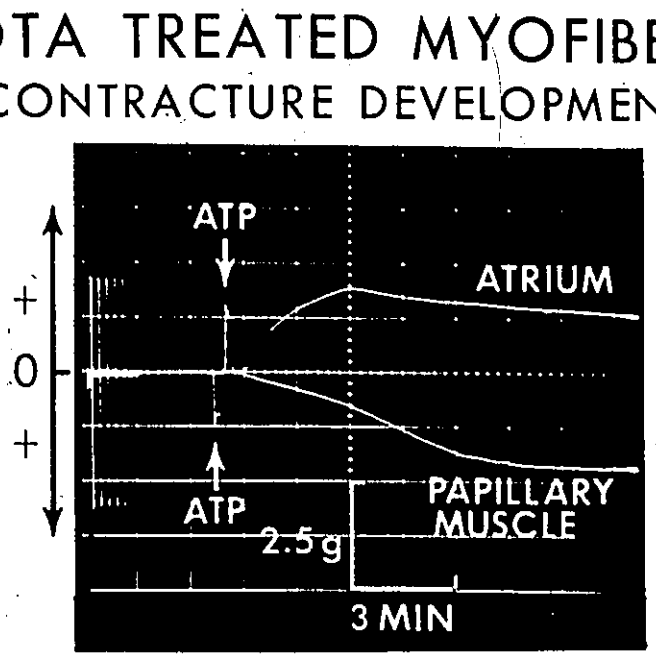


Figure 35. Isometric tension in a representative A and P from an EDTA experiment. From the left, normal twitch contractions are shown in a + upward direction for A and + direction downward for P. The addition of Ca^{2+}, Mg^{2+}, and ATP stimulate the contractures shown.

TABLE 17

Summarized Data from Experiments with EDTA-Treated Rat Heart Fibers

		ATRIA	PAPILLARY MUSCLES	
Weight	(mg)	5.83 ± .654	4.12 ± .360	p<.05
Length	(mm)	7.62 ± .726	5.81 ± .379	N.S
Twitch tension	(g)	1.40 ± .347	2.52 ± .475	N.S
Contracture:				
Maximum tension	(g)	.86 ± .121	1.067 ± .167	N.S
Time to max. tension	(min)	2.92 ± .196	7.17 ± .715	p<.001
Initial rate	(g/min)	.31 ± .051	.15 ± .019	p<.02
Mean rate	(g/min)	.80 ± .139	.30 ± .055	p<.01
$\frac{\text{Initial rate}}{\text{maximum tension}}$	(min^{-1})	.96 ± .077	.28 ± .034	p<.001

MEAN ± S.E.M., n = 6. N.S. = Not significant

The initial rate of tension development is calculated through the first 30 seconds and mean rate is calculated through maximum tension.

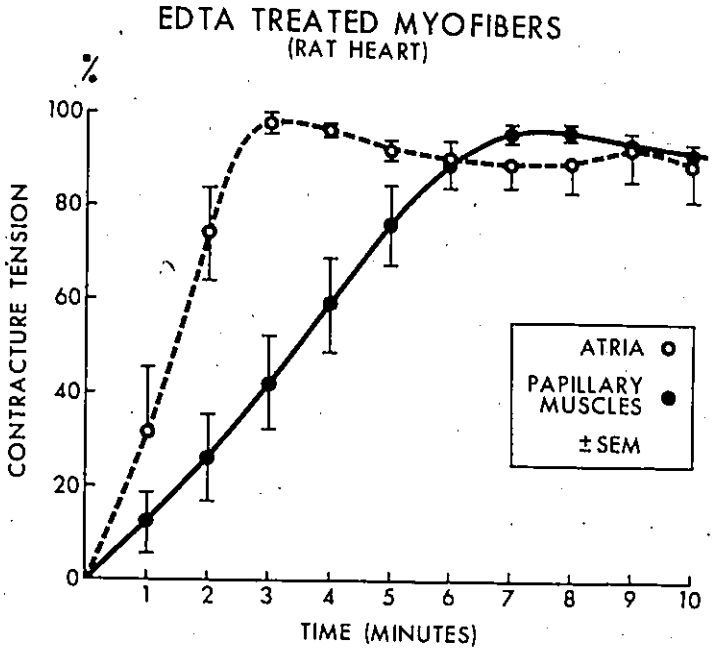


Figure 36. Time course of contracture development in EDTA-treated myofibers.
Tension is calculated as a % of peak tension during 10 minutes for A and P at each minute following the start of contracture. Mean values ±S.E.M. are shown for 6 atria and 6 papillary muscles.

2. Glycerinated Myofibers

a) Brief Glycerination

It has been shown that treatment of muscle with glycerol and subsequent removal causes a temporary loss of contractile response in A and not in V of guinea pig (Strossberg et al., 1972). In a similar experiment at the beginning of my studies on glycerol treatment, strips of rat heart were exposed to a 400 mM glycerol solution. An atrial strip and a papillary muscle were equilibrated for 1 hour until a steady twitch tension in normal Krebs-Ringer bicarbonate was observed; MT of A = 1.0g, P = 4.0g. Next, the muscles were immersed in 400 mM glycerol solution for 1 hour; MT of A = 0.6g, P = 2.7g and was constant. The muscles were subsequently reimmersed in the normal Krebs. After 2 minutes no twitches could be elicited in the A; however twitch contractions in P were still developing MT of 1.4g. The resting tension rose in both muscles. The stimulation rate was continued at 5/minute and after 10 minutes the atria began twitching once again. At 30 minutes the MT of A was 1.0g, and P was 3.20g. After return to normal Krebs from a glycerol solution the loss of tension development is complete for a certain period of time in atrial muscle. However, the ventricular muscle never looses its ability to develop twitch tension. There is no major change in the TMT of the A or P before or after glycerol treatment.

b) Long-Term Treatment

The basic idea in treating muscle fibers with glycerol for several weeks or months was to disrupt or extract cell components other than the contractile proteins.

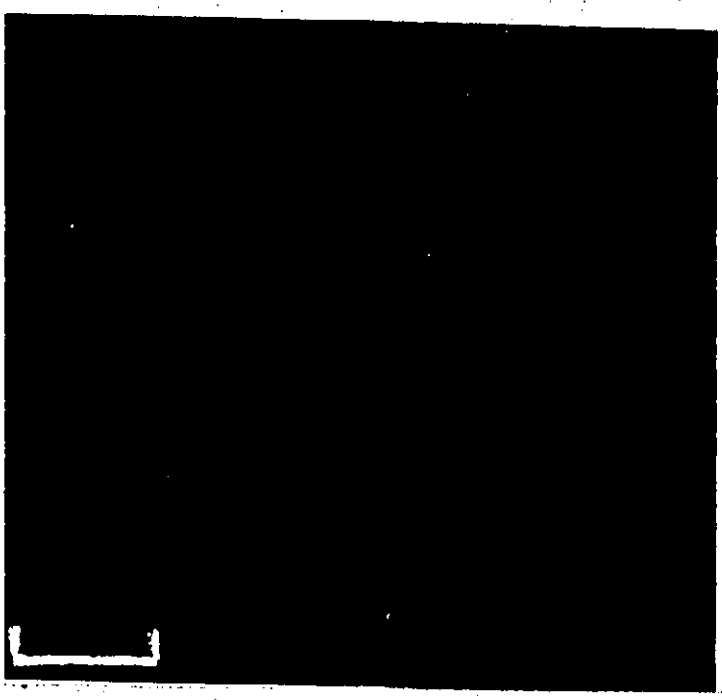
i) Electron Microscopy

Electron microscopy of sections from these glycerinated muscles indicated that a major % of the mitochondria were swollen and/or disrupted with the internal cristae disarranged. The sarcomeres were normal in appearance and near 2μ in length (See Figures 37 and 38 for transverse and longitudinal sections from a P and A). It is worth noting that on transverse section the atrial sample was indeed cross-sectioned, that is, no longitudinally oriented myofibrils were observed; furthermore the longitudinal section does not contain transversely sectioned myofibrils. This statement is made, accepting the limited sampling procedure of E.M. and the extent of this study.

ii) Dose-Response Curves for ATP and Calcium

With the contractile proteins and limited other subcellular structures intact, the contractures were initiated after addition of Ca^{2+}, and Mg^{2+} and ATP. Calcium and ATP dose-response curves are shown in Figure 39 and optimal concentrations (10 mM ATP and 6×10^{-6} M Ca^{2+}) to produce MT are similar in A and P. Magnesium concentration paralleled the ATP concentration with the assumption that Mg^{2+} and ATP combined in equimolar amounts.

a.



b.

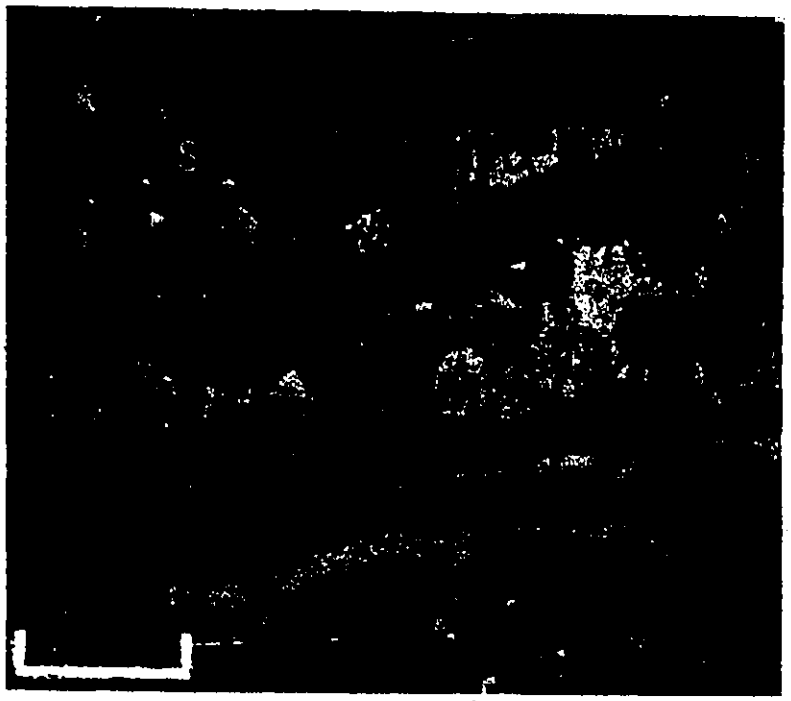


Figure 37. Electron micrographs from a glycerinated (50 days) papillary muscle fiber. M = mitochondrion, S = sarcomere

a.) transverse section, X4250
b.) longitudinal section, X5750

a.

b.

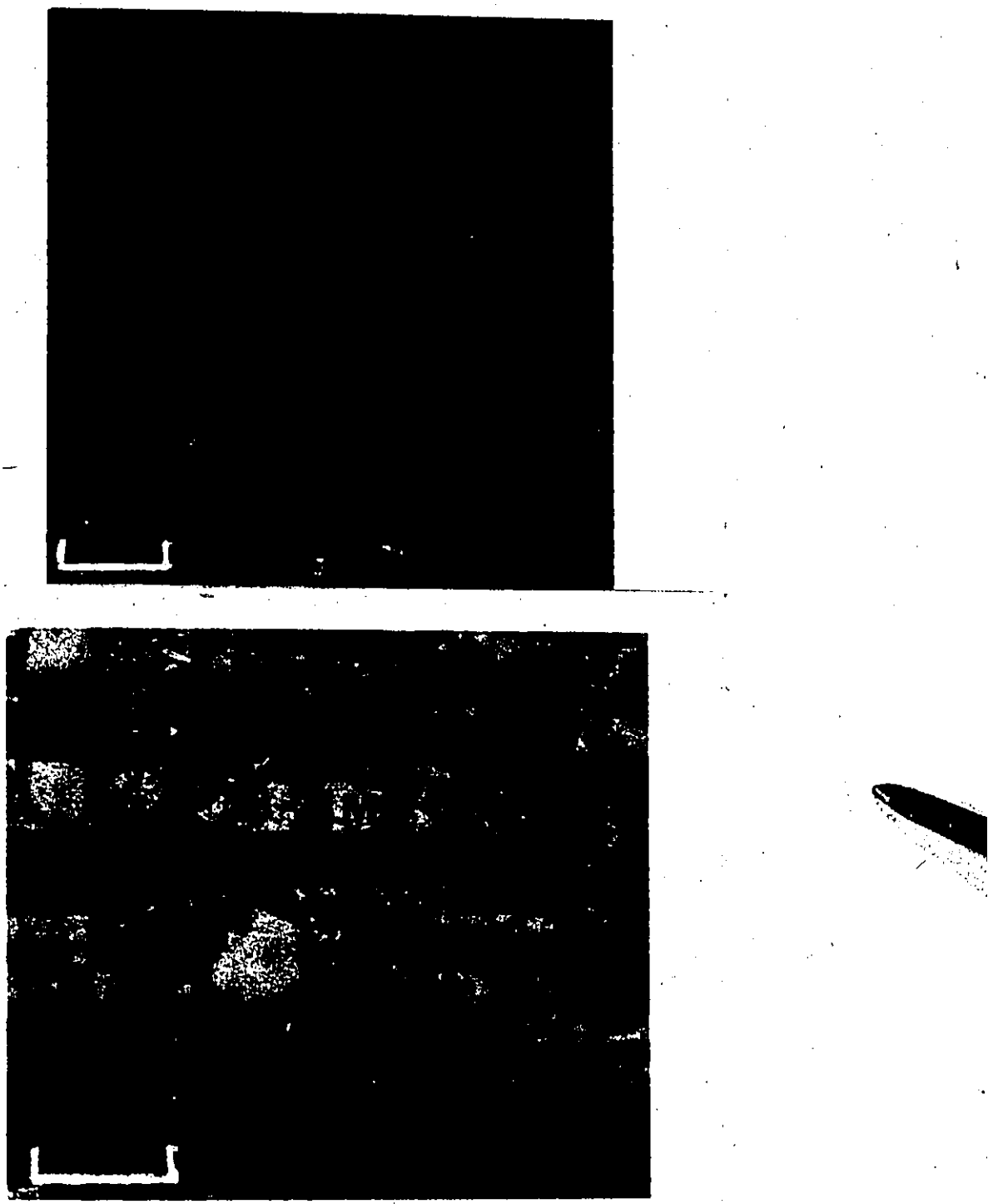
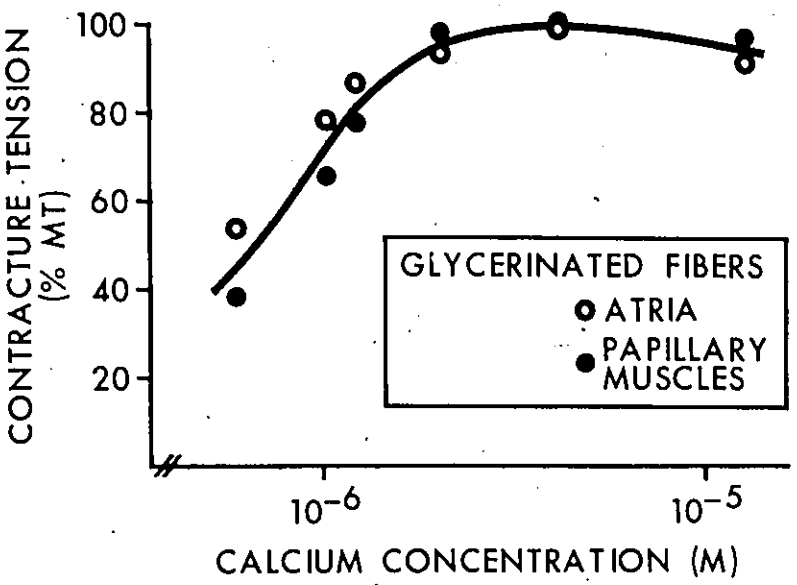


Figure 38: Electron micrographs from a glycerinated (50 days) atrial muscle fiber. M = mitochondrion, S = sarcomere.

a.) transverse section, X4000
b.) longitudinal section, X5625



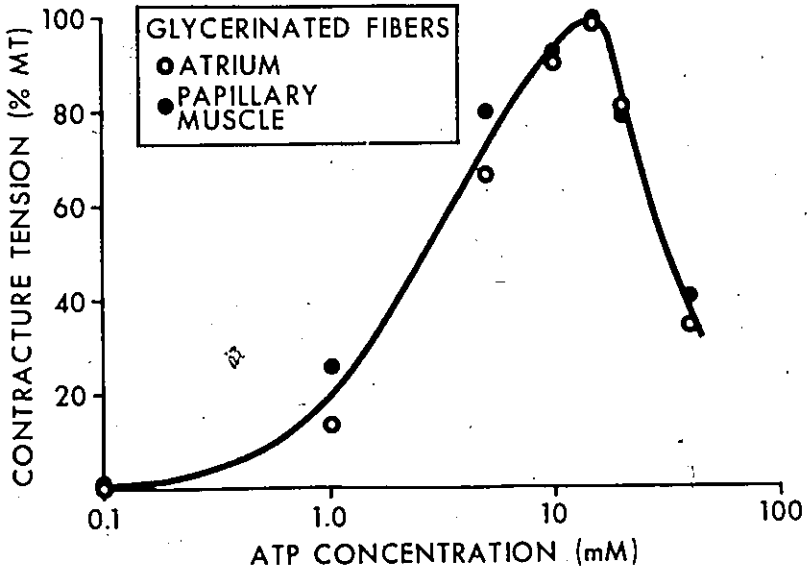


Figure 39. Calcium and ATP dose-response curves for glycerinated A and P.

a.) contracture tension as function of calcium concentration (6 atria, 6 papillary muscles); 10 mM ATP, 10 mM $MgCl_2$.

b.) contracture tension as function of ATP concentration (1 representative A and P); 6.5 x 10^{-6} M $CaCl_2$, 10 mM $MgCl_2$.

iii) Normal Contracture Tension Development

Data from the experiments on glycerinated muscles shows that the average weights and lengths are similar (Table 18); however, the absolute amounts of tension developed in the P are 3 x that in the A. Consequently, with larger tension in P, there was a greater initial and mean rate of tension development. It seemed only reasonable to compare A and P by using the rate of tension developed per maximum tension as was done in the EDTA studies. Calculation and comparison of the $\frac{\text{initial rate}}{\text{maximal tension}}$ provides evidence that this rate index is not different between A and P. This may also suggest that the contracture time course is not different when calculated as % of MT for A and P. The results in Figure 40 confirm this suggestion where no significant difference is shown.

iv) Recovery of Tension After Release to Shorter Muscle Lengths

Diffusion limitations may affect contracture time course and rate, particularly diffusion of ATP. Therefore, in one series of experiments, the contractures were allowed to develop maximally, that is, to the maximum active state where any ATP diffusion problems may be minimal. At this peak tension the muscles were allowed to shorten suddenly to -5 or -10% of their lengths and then redevelopment of tension was followed (Figure 41). After such a manipulation the curve shapes were not different comparing A to P.

TABLE 18

Summarized Data from Experiments with Glycerinated Rat Heart Fibers

		ATRIA	PAPILLARY MUSCLES	
Weight	(mg)	.40±.030	.33±.049	N.S.
Length	(mm)	3.26±.152	2.98±.095	N.S.
Cross-section	(mm^2)	.12±.012	.11±.016	N.S.
Tension at 10 min	(mg)	54±5.7	166±17.3	$p < .001$
Initial rate	(mg/min)	30±5.8	113±28.0	$p < .01$
Mean rate	(mg/min)	5.4 ±.57	16.5±1.73	$p < .001$
$\frac{\text{Initial rate}}{\text{Tension at 10 min}}$	(min^{-1})	.53±.076	.73±.131	N.S.

Mean ± S.E.M., n=12, N.S. = not significant

Initial rate is calculated through the first 60 seconds and mean rate through 10 minutes.

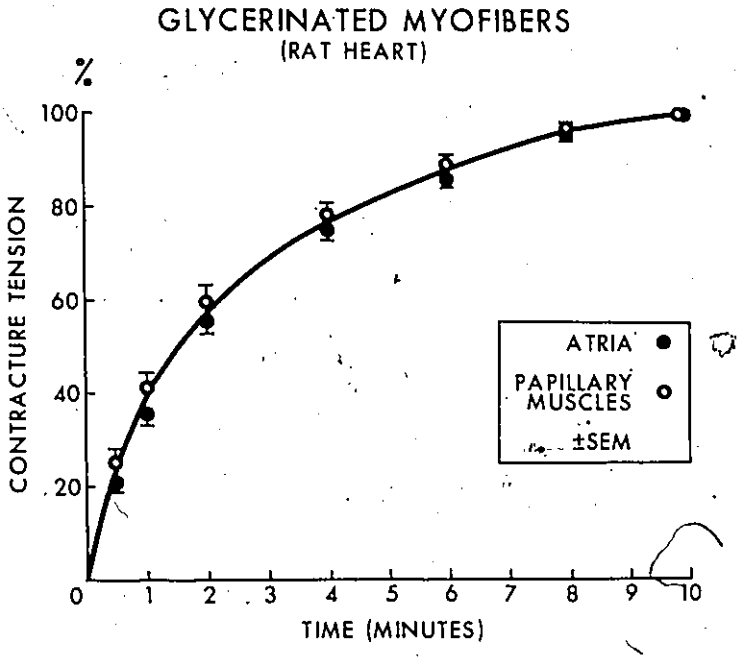


Figure 40. Time course of contracture development in glycerinated myofibers. Tension is calculated as a % of peak tension during 10 minute for A (o) and P (o) at each minute following the start of contracture. Mean values ± SEM are shown for 12 atria and 12 papillary muscles.

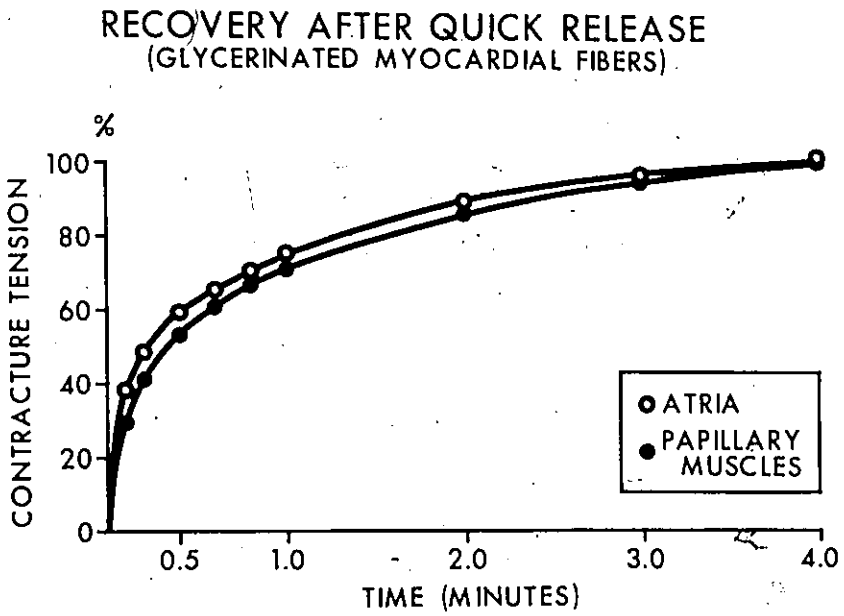


Figure 41. Redevelopment of contracture tension in glycerinated fibers after release to -5 or -10% of the length at which the contracture was developed. Mean values for 7 atrial and 7 papillary muscles from rat.

v) Creatine Phosphokinase System and Deoxycholic Acid

In another effort to remove from suspicion the concern that there may be limited diffusion of ATP into the bundles of fibrils, an "ATP regenerating system" was utilized. This procedure has been used to improve speed of contracture and/or maximal tension (Bowen and Martin, 1963; Wise et al., 1971). The basic equation describing the system is:

$$CP + ADP \xrightarrow{CPK} C + ATP$$

Where CP = creatine phosphate (5mM)

ADP = adenosine 5' diphosphate (5mM)

CPK = creatine phosphokinase (0.1 mg/ml)

C = creatine

ATP = adenosine 5' triphosphate (5mM)

Several variations in procedure were used:

1) muscle bundles were soaked in CPK for 5 minutes to 2 hours, then ADP, ATP and CP or only ATP were added,
2) muscle bundles were soaked in ADP and CP prior to CPK,
3) CP, CPK and ATP were added simultaneously to evoke contracture,
4) prior to contracture with ATP, bundles were soaked in deoxycholic acid (7.5 mM) and/or CPK.

The rationale for using deoxycholic acid is based on the evidence that this detergent evoked higher rate of tension development and absolute amount of tension in aortic strips and skeletal muscles after glycerination (Cherney et al., 1971).

No improvement in the rate of contracture development was indicated for A or P after either CPK or deoxycholic acid (Figure 42).

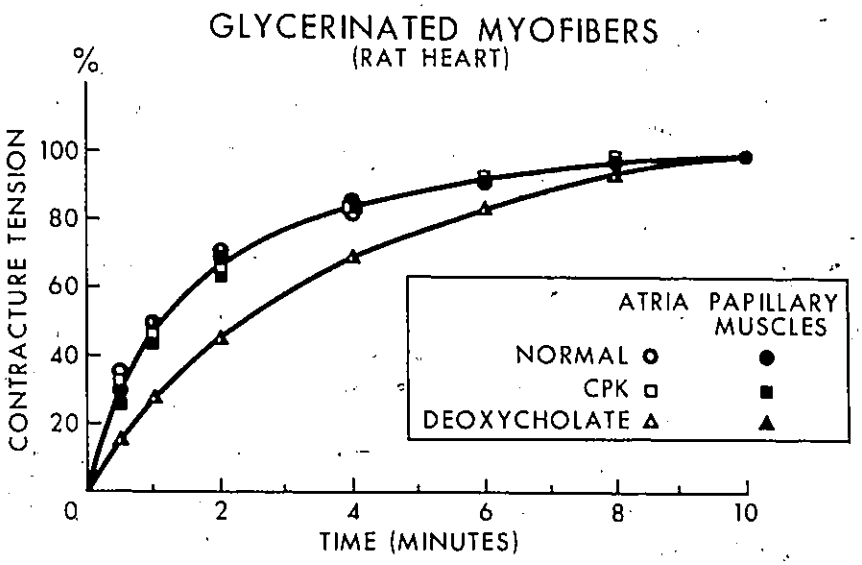


Figure 42. Comparison of contracture tension development in glycerinated muscles: normal, CPK-treated, and deoxycholic acid-treated. Tension is calculated as a % of peak tension during 10 minutes.

vi) Change in Tension After Muscle Shortening to a New Length

In several experiments, after the glycerinated muscles had achieved full contracture development, they were released to shorter lengths. After 5 minutes at this length, the redeveloped tension was recorded. In these experiments the atrial tension dropped more per % of length decrease than did the P. For instance, the redeveloped contracture tension in P was approximately 20% of the peak contracture tension (PCT) after the muscle was allowed to shorten by 30%. On the other hand in the A the redeveloped contracture tension was approximately 20% of the PCT when released only by 15% of the original length.

As mentioned previously, one method for roughly estimating the SE compliance is by releasing a muscle to zero tension from a maximal active state after tetanizing and measuring the resulting % length change. However, high stimulation rate does not tetanize cardiac muscle as it does skeletal muscle. It is attractive to consider that the glycerinated cardiac muscle fibers presented here are at maximal "active state" when at peak of contracture. Therefore on release to zero tension the % length change should estimate the S.E. Consequently, after such a release (300 msec time duration) to zero contracture tension, the % length change was 10.5% in P (n = 6) and 6.1% in A (n = 6); however, there was no statistically significant difference between these values. The higher % values would indicate more compliance and conversely less stiffness. This may indicate that the atrial values tend to show greater stiffness of the S.E.

E. MYOFIBRILLAR ATPase ACTIVITY

The activity of the contractile protein ATPase was measured in A and V to investigate the possibility that this biochemical process may be related to the velocity of shortening in these two muscles.

In the non-glycerinated fibers the ATPase activity uMPi/mg protein/minute was higher in the right V than in the A but higher in the A than in the left V (Table 19). However, in the glycerinated myocardium no statistically significant differences could be found on comparing various samples. Combining the activity from all atrial and all ventricular samples, respectively, and subsequent statistical analysis revealed no difference between A and V.

TABLE 19

Myofibrillar ATPase Activity of Rat Heart

	No. of Samples	uMPi/mg protein/minute
A. Glycerinated fibers:		
Cardiac Muscle		
Left atrium	7	.17 ± .015
Right atrium	7	.20 ± .015
Left and right atria	8	.21 ± .025
Left ventricle	10	.20 ± .013
Right ventricle	12	.19 ± .001
Skeletal muscle	6	.435 ± .035
B. Normal fibers		
Atrium	10	.22 ± .009
Right ventricle	20	.24 ± .013
Left ventricle	40	.15 ± .045

N.S. (Left atrium vs. Right atrium)
N.S. (Left atrium vs. Left and right atria)
N.S. (Right atrium vs. Left and right atria)
N.S. (Left and right atria vs. Left ventricle)
N.S. (Left ventricle vs. Right ventricle)
.198 ± .012
.194 ± .008
N.S. (.198 ± .012 vs. .194 ± .008)
p<.001 (Right ventricle vs. Left ventricle)
.18 ± .008
p<.05 (Atrium vs. .18 ± .008)

V. DISCUSSION

A. CONSIDERATIONS OF TWITCH CONTRACTION CHARACTERISTICS

1. Tension

Less tension is developed in the atrial myocardium in reference to the ventricle. A similar relationship has been observed on comparing cardiac muscle which developed only 4-8 g/mm^2 to skeletal muscle which developed 20-30 g/mm^2. Evidence was subsequently presented that the area of tissue occupied by contractile mass in cat papillary muscle is 58% and in rat soleus 90% (Sonnenblick et al., 1968). Therefore, when corrected for non-contractile mass the force developed is similar in the two muscles. A situation analogous to this may exist in the A as compared to the V. There is no evidence of a study concerned with % contractile mass in these two muscles, however, as pointed out previously the actin and myosin concentration was twice as high in the V as in the A muscle. If this experimental finding holds for other species, then the tensions in rat A and P may also be very nearly the same when normalized per quantity of contractile proteins in each muscle.

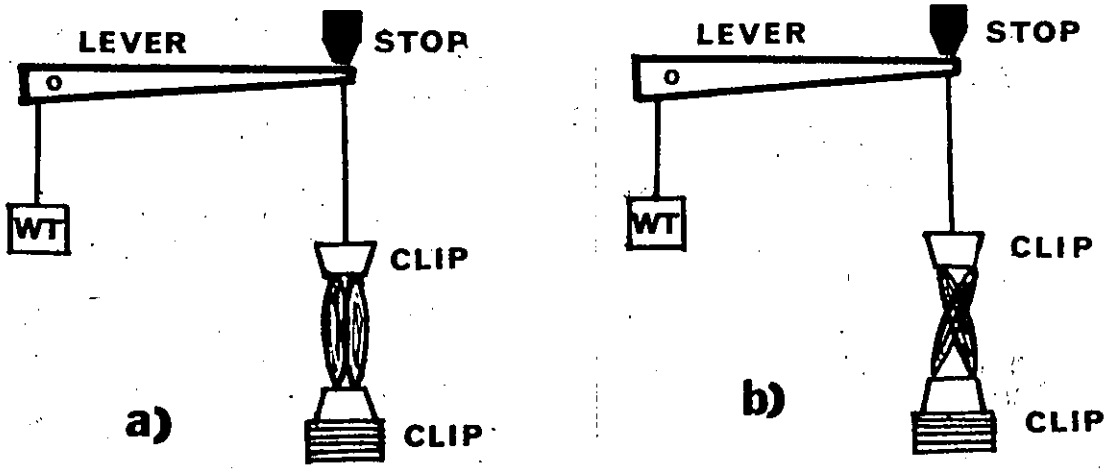
It has been mentioned (Fawcett and McNutt, 1969) that the boundary between cells in cat ventricular muscle was quite rectilinear, that is, running perpendicular or parallel to the fiber axis. In contrast, the intercalated disks of atrial cells appear more variable including an oblique arrangement. It is well known that pressures developed within the atrial heart chamber are less than in the

corresponding ventricle, and the less rectilinear outline of atrial cells may be related to the decreased mechanical tension. Consequently, in the isolated muscle preparation this structural uniqueness of the atria may also contribute to lower absolute amount of tension generated.

2. Contraction Velocity

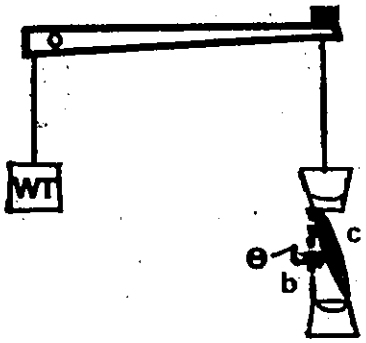
Comparison of the data from isometric twitch contractions conclusively show that the A have a higher dT/dt per unit of force development than the P; also, in isotonic contractions the dL/dt is greater per unit of shortening in A. Even in cases where the atrial strips and papillary muscles have similar weights (Figure 3) and/or maximum developed tensions (Table 13), the maximum rate is greater for atria. This finding is especially significant in view of the possible difference in fiber orientation in the two muscles.

In contrast to the more or less parallel arrangement of fibers in a papillary muscle (Diagram a), the individual fibers within an atrial bundle may be oriented in a non-parallel fashion relative to the long axis of the muscle bundle (Diagram b).



Then, the question becomes: In muscle bundles where individual fibers may be oriented in a non-parallel manner, is the velocity of shortening of the whole bundle an accurate measurement of the velocity of each fiber? In effect, some atrial fibers may be pulling the lever at a velocity which over-or-under estimates their true capability.

Approaching this problem using simple trigonometric functions and considering the orientation of one fiber:



The hypothetical fiber C is arranged in a non-parallel manner at an angle of deviation (θ) from the long axis of the muscle bundle. The formula becomes:

$$\cos \theta = \frac{b}{c} \quad \text{or} \quad C' = \frac{b}{\cos \theta}$$

b = the measured muscle contraction velocity

C' = the actual velocity of the fiber C

Arbitrarily assuming a situation where θ equals an angle of 30°, and the total muscle shortening velocity is 50 mm/sec, the corrected velocity (C) of the non-parallel fibers is:

$$\cos 30^{\circ} = .866 \quad C' = \frac{50 \text{ mm/sec}}{.866} = 58 \text{ mm/sec}$$

Therefore, the actual speed of shortening or force developed by diagonally arranged atrial fibers would be equal to the observed values divided by cosine θ, thus causing an increase in the absolute values. The application of the above formula would amplify the differences already seen between atrial and papillary muscle contraction velocities and therefore was not considered imperative in this work.

Close (1964) also suggested that when a cell or bundle of cells contain some fibrils not oriented along a single axis, the resultant influence is antagonistic rather than synergistic and consequently acts to decrease the measured muscle velocity.

3. Time to Maximum Tension

a. Young Animals

Histogenesis of ventricular cardiac muscle of rats and birds provides evidence that the formation of T-tubules occurs between the 14th and 20th day after birth (Schiebler and Wolff, 1966; Manasek, 1970). By the 24th day the S.R. has acquired its characteristic differentiation and arrangement. By the 31st day the T-system showed its typical structure but was not quantitatively fully developed. Ventricular muscle cells during the first two weeks of postnatal life are very similar microscopically to the atrial cells of the adult rat. If the T-system determines the time course and/or rate of contractions, then mechanical measurements recorded during this early post-natal

period should be reflective. However, the results presented here show that in rat and dog the atrial contraction time course is definitely of the same magnitude of difference away from the ventricular contraction time course as seen in the adult animals. This suggests that lack of the T-system in ventricular fibers does not create a faster contraction.

b. Myofibril Organization

Fawcett and McNutt (1969) emphasize in their E.M. studies on cat heart that the cardiac muscle fibers had a single coherent bundle of myofilaments extending throughout the fiber cross-section. This contrasts with the discrete myofibril units of uniform size as seen in skeletal muscle and also reported by others in cardiac muscle. It was suggested that the unique contractile mass geometry in cat heart might be causally related to its slower time course of contraction and prolonged contractile state, as compared to skeletal muscle. An extension of this idea to account for the faster contraction in atrium relative to the ventricle does not seem warranted since the absence of discrete "myofibrils" was observed in both atrial and ventricular sections of the cat heart.

c. S.R. and Relaxation

The role of the S.R. in relaxation of skeletal and cardiac muscle is well accepted. In guinea pig the fragmented S.R. of the "fast" vastus lateralis (TMT of 19 msec) had a higher steady-state

rate of calcium uptake than the "slow" soleus (TMT of 82 msec). This rate of calcium accumulation was correlated with the half-relaxation times for vastus (18 msec) and for soleus (114 msec)(Fiehn and Peter, 1971). Experiments of this kind to determine the calcium uptake rate of the S.R. as well as the mitochondria from A and P should be very informative.

4. dT/dt/MT

Results obtained after normalization of dT/dt per MT developed in a particular twitch further substantiates the faster rate in A relative to P. This index expresses the maximum rate of proportional rise of developed tension, has a dimension of time^{-1} and is independent of crossectional area and preload (Figure 26). The data derived by plotting the different values of this index at various temperatures as a function of TMT indicate strongly that the atrial contraction may be a special form of the ventricular contraction or vice versa. Thus certain atrial contraction characteristics may be predicted from the mechanical features of the ventricular contraction according to the general equation $\frac{dT/dt}{MT} = K\left(\frac{1}{TMT}\right)$ where the K value appears to be the same for atria and ventricles in the same species. In an actual experiment, atrial and ventricular twitches with identical TMTs were produced by two different temperatures, and the twitch contraction curves became reasonably superimposed (Figure 15).

In response to an increase in temperature the P and A are

shifted upward along a common slope described in the plot of $\frac{dT/dt}{MT}$ against $\frac{1}{TMT}$. It can be postulated that temperature may exert its influence by altering the speed at which calcium ions diffuse to the myofilaments as well as the rate of chemical reactions associated with the contractile proteins and calcium sequestration.

In contrast to the typical inotropic responses caused by temperature change, the rate of electrical stimulation has little influence on the atrial position; however, it causes the papillary muscle values to shift upward along the common slope K. In fact, at a selected temperature (for instance, 28°C) and with an increase in frequency (5 to 300/minute) the P are shifted to the region of the next higher temperature studied (33°C) (Figure 25). It has been suggested that an increase in contraction frequency may make greater quantities of calcium available to the contractile system (Nayler, 1967; Langer, 1971) and this is associated with a positive inotropic response. The proposed scheme is such that an increase in frequency of contraction causes more calcium influx across the cell membrane to the myofilaments where it binds to troponin sites and more completely inhibits the troponin-tropomyosin interference with actin-myosin coupling. The result is a greater rate and amount of tension development.

5. Resting Tension and the Length-Tension Curve

The resting tension in the atrial and papillary muscles was expressed as a % of the MT produced in the twitches as length was increased. It was noted that this RT was consistently a greater

fraction of the developed tension in the A than in the P (Figure 9). This greater RT may be expected in light of the higher collagen content reported. Jewell and Blinks (1968) alluded to a higher PE component in atrial muscle but no further information was presented. It must be cautioned that a statement indicating a greater quantitative amount of the PE should be made only after analysis of normalized tension/cross-sectional area as a function of % length change from Lo; Lo being defined as the unstretched muscle length at 0 tension. On this basis, a brief comparison of an A and a P of rat which have similar weights and lengths suggest that the RTs may not be different. This type of analysis to quantitatively compare atrial and papillary muscles may prove very fruitful in the future particularly utilizing very carefully chosen muscles from larger animals such as cat or dog where the atrial trabeculae may have a fairly parallel arrangement of fibers and normalization by cross sectional area is possible. This type of analysis assumes that the CE is freely extensible at rest and that the passive L-T curves reflect the characteristics of PE present.

B. ACTION POTENTIAL

1. Shape

There are obvious differences in the shape of the A.P. considering A and V. However, no complete explanation for this has been forwarded either based on an ultrastructural or biochemical basis. Information remains incomplete as to the specific ions and their respective rate of transport across the cell membrane during the

excitation and return to resting state. The A.P. difference appears to suggest structural or conductance differences in the membrane associated with excitation but as mentioned in the Literature Review further caution must be exercised in relating the A.P. to the resulting twitch contraction.

With alteration of the Na^+ and Ca^{2+} levels there was no evidence of significant TMT or total contraction time change in rat atria or papillary muscles even though the MT and dT/dt decreased to very low values. In all instances, the direction of the changes appeared to be the same in both types of myocardial tissue and no indication of possible transformation of the shape of atrial into ventricular twitch or vice versa was achieved. Fairly consistent relationships between A.P. duration and TMT was reported by various authors as reviewed in an early section of this dissertation and a brief consideration of the effect on the A.P. of external calcium or sodium fluctuation should be of value.

Lowering the external Na^+ concentration shortened the duration of the A.P. and increased the steepness of phase 2 of the AP in ventricular fibers of several species (Deleze, 1959; Brady and Woodbury, 1957). A four fold increase in Ca^{2+} resulted in near abolition of phase 2 so that the A.P. resembled the "atrial type" of potential, but 1% of normal calcium was without any great effect in dog papillary muscle. (Hoffman and Suckling, 1956). Low concentration of Ca^{2+} in the extracellular fluid prolonged phase 2

in dog atrial muscle; in addition, with both Ca^{2+} and Mg^{2+} reduced, the A.P. assumed a shape identical with that seen in the ventricle and phases 1, 2 and 3 were clearly demarcated.

It appears quite convincing that Ca^{2+} as well as Na^{+} contribute to the cardiac A.P. Slow inward currents of Ca^{2+} have been identified in mammalian ventricular muscle (Mascher and Peper, 1969; Beeler and Reuter, 1970) in addition to other muscles. Furthermore the slow inward current has been implicated in phase 2 plateau production in cardiac muscle. Interestingly, strontium apparently has the ability to replace Ca^{2+} in the plateau phase, (Niedergerke and Orkand, 1966). In the present study Sr^{2+} greatly prolonged the TMT in both rat atria (Figure 21) and papillary muscles and one ultimate effect may have been in prolonging phase 2 of the A.P. This is not suggested to the exclusion of the possibility that there may be a decrease in the ability of the S.R. to sequester Sr^{2+} in comparison with Ca^{2+} (Hajdu, 1970).

Atrial strips respond sooner to change in bath ionic composition than do ventricular strips. Both MT and dT/dt decrease or increase more rapidly in atria with large ionic changes. Brooks et al., (1955) also commented on a similar phenomenon which occurred in cat and dog heart where atrial fibers became rapidly inexcitable in a solution nearly calcium-free but papillary muscles remained normally excitable for hours.

A great deal of evidence has been accumulating relating the A.P. to energy metabolism but until recently the studies have used

only papillary muscles or various trabeculae. Hyde et al., (1972) used cultured rat heart cells and studied the affects of energy deficiency on the transmembrane potentials and contractions. Inhibiting the energy production or increasing the rate of utilization greatly reduced the duration and amplitude of the action potentials. Their conclusion was that the drop in energy-rich compounds in or near the plasma membrane modulated the Ca^{2+} influx associated with the plateau of an A.P. The results from this study suggest that a cell with a higher ATP content or higher energy producing capacity may indeed have a differently shaped A.P. Directly related to this report, McDonald and Macleod (1972) published a study indicating that A.P. duration declined at a faster rate in anoxic guinea pig ventricular muscle than in atrial muscle. During the anoxic incubation, atrial muscle maintained a higher glycolytic rate, ATP content, glycogen content and less potassium loss than ventricular muscle. The situation in anoxic A and V seems partially agreeable with the hypothesis of Hyde. That is, since the A maintain higher ATP stores during anoxia the A.P. will be maintained near normal for a longer period of time; however, as the energy storage decreases the Ca^{2+} would rapidly accumulate at the plasma membrane or its vicinity during the A.P. and cause shift of E_{CA}^{2+} toward E_m to occur earlier and therefore shut off the I_{Ca}^{2+}. The result should be a shorter A.P. which is not in agreement with McDonald and Macleod who observe a longer A.P. with anoxia. Since normally the ventricle contains more ATP than the atrium, the hypothesis of Hyde may still be applicable in non-anoxic conditions. The higher

ATP store in the ventricle would hypothetically cause a longer A.P. duration because the I_{Ca}^{2+} is maintained longer. This hypothesis probably is greatly oversimplified but seems attractive as reference for future experiments.

2. Rate of Depolarization

With a similar sarcoplasmic composition a fiber with a larger cross-sectional area would be expected to conduct more rapidly than a smaller fiber. Extending this basic concept to fibers from the atrium and ventricle, the faster rate would occur across the ventricular fiber since it is larger in diameter. However, this is not consistent with the longer TMT in V and probably is not a valid conclusion since ventricular cells have a large array of T tubules which are lacking in atrial cells. Variation in tubular mass has been shown to alter the electrical capacitance as in skeletal muscles (Adrian and Peachey, 1965):

	Capacitance	Resistance
slow fibers - no triads or T system	C	R
fast fibers - triads + T system	3C	0.1R

Hypothetically, if the fibers from the V and A were the same size, except one fiber with and the other without T-system, then there would be an increased capacitance in the cell with tubules and a slower A.P. conduction. In effect there would be more membrane area to depolarize before an A.P. could be propagated across the fiber. In actual fact, the atrial cell is approximately 1/2 the size of a ventricular cell but it has no T system. Therefore the A probably

has lower capacitance and this may be instrumental in faster depolarization and calcium release.

C. KCl CONTRACTURES

Many experiments have dealt with contracture development in muscle initiated by compounds such as caffeine, acetylcholine and KCl. Although no experiments with KCl contractures were presented in this dissertation, the method does offer a worthwhile step between the electrically stimulated twitch contractions of normal fibers and the Ca^{2+} - induced contractures after EDTA treatment. In other words, KCl contractures are initiated by the effect of high potassium instead of a direct electrical stimulation.

The procedure involves a sudden increase in potassium concentration, and this causes, in order, membrane depolarization, calcium flow to the myofilaments and contracture. The contracture time course has been analyzed in only a few instances and there is great paucity of experiments on mammals. However, Fozzard and Gibbons (1971) have studied the potassium contractures of sheep atrial and ventricular trabeculae. After I visually inspected an atrial contracture and one from the ventricular muscle, it was apparent that the A reached peak contracture in approximately 10 seconds and the V in 30 seconds. Therefore, the ratio of $\frac{V}{A}$ is 3 and this is similar to the TMT ratios of normal twitches of several species (Figure 23).

Even though no experiments were presented on KCl contractures of rat heart muscle, one might expect similar results regarding the

contracture time course differences between A and V. Indeed, the EDTA-treated Ca^{2+} induced contractures showed a $\frac{V}{A}$ time to peak contracture of approximately 2.5. Both of these experimental procedures (KCl and EDTA-Ca^{++} treatment) to evoke contracture have eliminated the requirement of an electrical stimulus.

It is most probable that the contractures are the result of calcium release from internal pools and/or displacement from membrane sites associated with the sarcolemma. Consequently, the fact that the time course differences of normal A and P twitch contractions and of these contractures are similar, strongly suggests that transport and binding rates of calcium and/or sites further along within the fiber are capable of determining this difference.

D. CONTRACTURE RATE AFTER EDTA

After EDTA treatment it may be postulated that the diffusion of calcium, magnesium or ATP may be restricted and this may cause a slower rate of contracture force development. However, since the subcellular structures involved in metabolic processes are still intact and assumed functionally active, it seems likely that ATP may be produced within the cells and consequently ATP supply by diffusion is of limited importance. Nevertheless, both calcium and magnesium were drastically depleted from the muscle bundles by EDTA. Therefore, the re-entry of required calcium and magnesium may retard activation of the contractile proteins.

In physically skinned skeletal muscle fibers, Hellam and

Podolsky (1969) used the $CaEGTA^{2-}$ buffer with pCa 5.0 in the bathing solution to facilitate activation of the fibers to maximum force development. When they used the same pCa 5.0 with no EGTA buffer, the fibers developed very little force. In this latter case, it was surmised that the calcium ions were taken up by the sarcoplasmic reticulum as quickly as they diffused into the fiber. If this is so, then with use of the EGTA-calcium buffer system, the $CaEGTA^{2-}$ probably diffused through the fiber without interacting with the peripheral myofibrils or sarcoplasmic reticulum.

The present studies of atrial and ventricular contractures post-EDTA treatment did not incorporate an EGTA buffer system for calcium. Since a maximal rate and time course of force development was sought, a bath calcium concentration (pCa 2.60) was used to give a high concentration gradient into the fibers. This pCa should be sufficient to saturate the sarcoplasmic reticulum, mitochondria, and any other calcium binding sites as well as the myofilaments. The diffusion of calcium, not complexed with EGTA, should be more rapid. In addition, with free calcium there is no necessity for the time dependent process of $CaEGTA^{2-}$ dissociation to release calcium at the myofilaments.

E. EFFECT OF GLYCEROL TREATMENT

1. Short-Term in Glycerol

The results presented here for rat atria and papillary muscles

indicate that the A react differently to brief glycerol treatment than the V. The loss of cardiac contractility in A was transient but V twitch tension was never lost. Similar response has been seen in rabbit (Schmidt et al., 1971) and guinea pig atria (Duckles and Jensen 1971). The latter authors found no electrical response during the time when developed tension was lost. This contrasts with skeletal muscle data where normal A.P.s are seen even when the tension is lost. Removal of the glycerol appears to be the time when ultrastructural damage occurs, that is a swelling of the S.R. and mitochondrial disintegration possibly because of a large concentration gradient between intracellular regions and the bath solution. It is interesting that Niemeyer and Forssman (1971) observed no T tubule disruption in the rat ventricle after glycerol removal and only minor ultrastructural change. This may suggest that in A the lack of T tubules lessens the ability of the fibers to rapidly equilibrate when placed back into normal bath solution from glycerol. Thus, ultrastructural damage as well as ionic imbalance may contribute to loss of both A.P. and contraction.

2. Contracture Rate After Long-Term Glycerination

In glycerinated psoas the rate of contracture development was increased progressively as the ATP concentration was raised from .14 mM to 2.3 mM. (Ranney, 1954). This general trend was also observed by Edman (1957). In the present experiments on rat

myocardium the maximal tension was produced by 10 mM ATP. This concentration was optimal for maximal rate and amount of tension in both A and P. The diffusion of ATP and the breakdown by ATPase evidently reach an equilibrium at this concentration.

Depth and rate of ATP diffusion into a fiber bundle is important when dealing with contracture development in muscle because of the lack of substrates and metabolism within the fibers. In the case where the depth of diffusion equals the radius of the fiber and based on diffusion coefficients and rate of ATP splitting for skeletal muscle fibers,

$$r = \left(\frac{N.C.D.}{A}\right)^{1/2} \quad \text{(Meyerhof-Schulz, 1927)}$$

Where r = radius of fiber, also depth of diffusion

C = external ATP concentration

N = 4 = integer used when diffusion distance approaches r (Edman, 1957)

D = diffusion coefficient

2×10^{-8} cm^2/sec (Hasselbach, 1952)

$1\text{-}2 \times 10^{-6}$ cm^2/sec (Bowen and Martin, 1963)

2×10^{-7} cm^2/sec (Mannherz, 1968)

A = rate of ATP splitting

$505 \times 10^{-6} g/cm^3/sec$ (Hasselbach, 1952)

$100\text{-}125 \times 10^{-6} g/cm^3/sec$ (Hayashi et al., 1968)

in $moles/cm^3$ tissue or moles/cm fiber length

Therefore, if: $C = 10mM = 6.23 \times 10^{-3}\ g/cm^3$

$N = 4$

$D = 2 \times 10^{-6}\ cm^2/sec$

$A = 100 \times 10^{-6}\ g/cm^3/sec$

Then, $r = 0.0223$ cm or 223μ.

This value is within the range of radii used in the studies on glycerinated A and P muscle presented here. However, the calculated radii may be 1/10 of this value 223μ if other constants for A and D (shown above) are chosen. Diffusion coefficients have not been presented for cardiac muscle but may be different than for skeletal muscle fibers. Consequently the diffusion radius may be meaningful only in reference to skeletal muscle and even with this restriction the variations in values for D are very great.

One assumption has been that there is just one fiber, whereas in reality there are 10 - 50 fibers per bundle which are separated by an intercellular matrix. Consequently diffusion rates through the bundle may be different from that through a single fiber. In fact, in a skeletal muscle bundle of 7 single fibers, the diffusion gradient was only twice as much as for a single fiber (Mannherz, 1968). This suggests that a bundle does not behave as a tissue cylinder with uniform ATP diffusion coefficient. Ruegg (1971) suggested that bundles no larger than 200-300μ in diameter be used in skeletal muscle.

The creatine phosphokinase system has been shown to increase the ATP concentration in the core of glycerol-treated skeletal muscle fiber bundles which range from 125 to 700μ diameter (Bowen and Martin,

1963). The contractures of A and P from rat are not affected by the use of this systemor deoxycholic acid. This suggests that the glycerol-treated fibers are sufficiently small so that the maximum rate of actin-myosin interaction and subsequent tension output are not slowed by an ATP deficient core.

As mentioned previously, there are fast and slow skeletal muscles which are attractive as comparisons to the heart "fast" atrial and "slow" ventricular muscle. In one study (Sexton and Gersten, 1967), evidence was presented to show that the rate of onset of tension development in glycerinated gastrocnemius (fast muscle) exceeded that in the soleus (slow muscle). This difference was attributed to the number or nature of actin-myosin cross-links formed since this difference was seen not only at the glycerinated fiber level but also in in vivo contraction. However, there was no mention or comparison of total time courses.

In another study, peak contracture tension and rate of tension development have been determined in hyperthyroid versus normal rabbit right ventricular papillary muscles after glycerination (Su et al., 1970). It was concluded that the contractile proteins from the hyperthyroid animals developed greater tension and had a higher rate than the normal.

In contrast to the difference in contracture rate seen with fast and slow skeletal muscles, the rate of contracture development per maximal tension and overall time course of "fast" atrial fibers

are not significantly different from the "slow" papillary muscle fibers. In addition, the ATPase activity of the myofibrils from these glycerinated muscles are not significantly different. This seems to indicate that the difference in time course of contractions seen in EDTA treated fibers or in normal twitch fibers is not mediated by the contractile protein hydrolytic activity.

F. MYOFIBRILLAR ATPase

In a myofibril preparation the contractile proteins should more closely represent the natural contractile system, as compared to the more purified actomyosin or myosin preparations. The major disadvantage is that total ATP hydrolysis is only partially associated with the myofibril contractile response. ATPase activities of cell structures such as mitochondria are also included. However, sodium azide has been shown to inhibit mitochondrial ATPase. In fact, Fanburg (1965) showed that after abolishing the contaminating ATPases with azide, the remaining activity closely resembled that of actomyosin ATPase. It was suggested that actomyosin is probably the active moiety of myofibrillar ATPase (Chandler et al., 1967).

Values reported in this paper for rat ventricular fibrils at ionic strength .17 are similar to those reported by Aoki et al. (1971), where values ranged between 0.18 and 0.12 uMPi/mg protein/min. at ionic strengths, respectively, 0.1 to 0.2. Glycerol-extracted, azide-treated myofibrils indicate a slightly lower activity but certainly

still near the range of Aoki's work mentioned above. Other workers, including Henry et al. (1972) found that glycerination of muscle fibers did not eliminate inherent physiological differences in ATPase activity. Specifically, they reported that ATPase activity of glycerinated fibrils from hypertrophied rabbit heart was lower than from glycerinated normal rabbit heart.

The myofibrillar ATPase activities reported here and by most other authors are steady-state values measured through time in minutes. In fact from approximately 1 minute to 8 minutes the activity rate increases linearly in A and P. Recently, Takauji and Honig (1972) have emphasized that bundles of myofilaments isolated by homogenization procedures are invariably preshortened and offer little advantage over myosin B. These workers perfuse the heart with EGTA at the outset to deplete calcium more thoroughly. Their experiments present evidence that the individual sarcomeres are not preshortened and that the ATPase activity during the first several hundred milliseconds of contraction is more representative of the true <u>in vivo</u> contractile protein activity.

This kind of experimental procedure should be most beneficial in extending the myofibrillar study which I have started in comparing A and P. Since normalization by cross-sectional area or protein is problematic with A, the use of a few distinct sarcomeres with a monitor of length change and simultaneous ATPase activity would contribute a great deal. Furthermore, the definition of the initial kinetics of

this hydrolytic activity in A and P may reveal interesting information.

In formulating the relationship between ATPase activity and shortening velocity, Barany used purified myosins. In recent conversations with me, Dr. Barany has suggested a schema which should yield actin and myosin from atria in a pure state. The information gained at this level of chemical activity should be very useful in comparing to the myofibrillar ATPase activity of A and V presented in this dissertation.

VI. SUMMARY AND CONCLUSION

This study has characterized similarities and differences between the atrial and ventricular myocardium. Some aspects of cardiac muscle electrophysiology, ultrastructure and biochemistry have been major areas of concern as related to the contraction mechanics.

A. NORMAL TWITCH CONTRACTIONS

1. It has become clear that the atrial muscle contracts faster and has a shorter time to maximum tension than the ventricular muscle. This difference is present in very young rat where the T-tubules have not yet developed.

2. With an increasing stimulation rate (from 5 to 300/minute) the tension decreases similarly in A and V; however TMT does not decrease significantly in A as it does in V.

3. Between 18 and 38°C the dT/dt is higher and the TMT less in A than P compared at the same temperature. The temperature coefficients of MT, dT/dt, $\frac{dT/dt}{MT}$ and of TMT were not statistically different when comparing A and P throughout most of the temperature ranges studied.

4. The TMT is always significantly shorter in the A than in the V in rat, cat and guinea pig. This is also observed in cardiac muscle preparations which have similar maximum developed tension and persists after treatment with reserpine and/or propranolol. TMT is approximately 1/3 of the total contraction time in rat, guinea pig and cat at various stimulation rates and bath temperatures for both A and V. The TMdT/dt and TMVce appear chronologically later in the twitch of an A than a V

in both isometric and isotonic contractions.

5. Alterations in the ionic composition of the bathing solution reveal the following:

a) the A respond faster, as far as mechanical output change, after being placed in a solution which has low calcium or sodium.

b) increase or decrease in calcium did not alter the TMT significantly; however increase in muscle length after low calcium did lengthen the TMT.

c) replacement of calcium with strontium lengthened the TMT of A and P equally but increased the total contraction duration twice as much in the A as in the P.

6. An index, $\frac{dT/dt}{MT}$, was used to compare the A and P contraction mechanics and was always higher in A under the same conditions. This index was sensitive to inotropic agents such as temperature and isoproterenol. If $\frac{dT/dt}{MT}$ was plotted against the inverse of TMT, it increased in a linear fashion with decreasing TMT. The slope of this line was the same in A and P.

7. The force-velocity curves from isometric contractions appeared hyperbolic in the papillary muscle but more linear in the A. The A and V force-velocity curves were equally responsive to inotropic agents.

8. Measured isotonically the maximum dL/dt is significantly higher in the A than in the V compared at several preloads.

9. At Lmax the resting tension constituted 25-35% of MT in the P but 80-90% in the A. There was a greater resting tension relative to MT throughout the extent of the passive length-tension curves.

10. An indication of the SE extension was gained by allowing the muscles to shorten rapidly to zero tension from maximum developed tension. There was no difference in the change in length expressed as a % of muscle length comparing A to P.

B. EDTA-TREATED MYOFIBERS

After the EDTA treatment which caused high sarcolemmal permeability, the muscles were stimulated with calcium to develop tension.

1. The initial rate of tension developed normalized per maximum tension yield values which were significantly higher in A than P.

2. The average time to peak contracture tension in A was 2 - 3 times shorter than in P.

C. GLYCERINATED MYOFIBERS

1. Short-term (hours)

There is no major difference in TMT after glycerol treatment in A or P, but the A temporarily loses its ability to generate twitch tension after return to normal bathing solution following a period of 1 hour in glycerol.

2. Long-term (weeks)

After glycerination for at least 2 weeks which disrupted cellular membranes but left the contractile proteins intact, the fibers were stimulated with Ca^{2+}, Mg^{2+} and ATP.

1). Optimal concentrations of calcium and ATP for generating highest tensions were 6.5×10^{-6} M and 10mM, respectively.

2) Initial rate of developed tension normalized per maximal developed tension and the overall time course of A and P tension development was not significantly different in these 2 muscles.

3) Diffusion did not appear as a causative limiting factor in contracture development for the following reasons:

   a) the time course of tension redevelopment after shortening -5 or -10% was not significantly altered in A or P.

   b) Creatine phosphokinase or deoxycholic a. treatment did not improve the rate of contracture development.

4) The muscles were allowed to shorten rapidly from maximum contracture tension to zero tension. There was no statistical difference in the change in length expressed as a % of muscle length comparing A to P.

D. MYOFIBRILLAR ATPase ACTIVITY

The hydrolysis of ATP by contractile proteins indicated that the ATPase activity is not significantly different between A and V in glycerinated fibers. Normal fibrils, non-glycerinated, showed the highest rate in the right V, lowest in the left V and intermediate in A.

The differences in the time course of tension development between A and V were still observed in the contractures developed after EDTA treatment or after initiating the contractures with KCl instead of electrical stimulation. The experimental procedure which eliminates the tension time course differences between A and V is glycerination which causes sarcolemmal as well as subcellular membrane disruption. The biochemical activity of the myofibrils does not appear to reflect the differences between A and V shortening velocity or tension development of the whole muscle fibers. It is suggested that calcium diffusion time, binding or uptake may determine the time course differences between A and V.

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APPENDIX

TABLE A

Isometric Contractions of Papillary Muscles of Rat. Mean values ± SEM are given for 8-12 papillary muscles at various stimulation rates and temperatures.

Temperature	Stimulation rate	Resting length	Maximum tension (MT)	Time to maximum tension (TMT)	Maximum rate (dT/dt)	Time to maximum rate (TMR)	$\frac{TMR}{TMT} \times 100$
°C	/min	mm	g	msec	g/sec	msec	%
17-18	5	3.82±.319	2.48±.378	359±16.6	12.0±1.81	82±15.4	23
	50	3.82±.319	2.00±.301	269±12.7	11.5±1.72	77±10.3	29
	100	3.82±.319	2.29±.383	254±10.7	17.6±3.31	75±11.4	30
	200	3.80±.368	1.31±.275	202± 7.7	9.8±1.94	65±11.6	32
	300	3.87±.430	1.21±.356	219±15.8	9.0±2.72	96±12.5	44
22-23	5	3.79±.282	3.20±.352	248± 8.0	22.4±2.29	80± 7.8	32
	50	3.79±.282	2.45±.254	195± 7.3	21.8±2.51	69± 6.0	35
	100	3.79±.282	2.25±.187	172± 4.0	21.6±2.02	71± 4.7	42
	200	3.79±.282	1.71±.209	139± 9.0	22.2±2.33	74± 4.0	53
	300	3.79±.282	1.22±.126	134± 6.9	15.0±1.64	66± 3.3	49
27-28	5	3.62±.303	3.33±.347	160± 3.8	37.7±4.16	68± 4.3	43
	50	3.62±.303	2.52±.296	132± 4.0	31.5±3.75	62± 1.9	47
	100	3.62±.303	2.35±.245	117± 4.3	33.2±3.87	59± 2.5	51
	200	3.62±.303	2.00±.213	94± 2.5	36.3±3.92	53± 1.2	56
	300	3.50±.309	1.49±.170	91± 3.5	30.0±3.20	45± 2.8	50
32-33	5	3.63±.299	2.60±.366	81± 4.2	47.8±6.12	41± 3.0	50
	50	3.63±.299	1.71±.259	77± 3.5	33.1±4.20	38± 3.1	49
	100	3.63±.299	1.56±.194	70± 3.2	33.2±3.31	33± 3.6	47
	200	3.63±.299	1.81±.157	64± 2.5	42.9±3.82	26± 2.8	41
	300	3.63±.299	1.54±.130	59± 2.1	41.3±3.19	25± 1.8	42
37-38	5	3.64±.296	2.25±.248	62± 2.0	57.0±5.80	31± 3.3	50
	50	3.64±.296	1.46±.258	56± 1.5	40.0±6.02	26± 2.5	47
	100	3.64±.296	1.35±.208	56± 2.2	36.3±5.53	23± 1.7	41
	200	3.64±.296	1.33±.143	48± 2.7	43.5±6.85	22± 1.5	46
	300	3.64±.296	1.38±.151	46± 2.2	46.8±6.09	22± 1.7	48

TABLE B

Isometric Contractions of Atrial Muscles of Rat. Mean values ± SEM are given for 8-12 atrial muscles at various stimulation rates and temperatures.

Temperature	Stimulation rate	Resting length	Maximum tension (MT)	Time to maximum tension (TMT)	Maximum rate (dT/dt)	Time to maximum rate (TMR)	$\frac{TMR}{TMT} X100$
°C	/min	mm	g	msec	g/sec	msec	%
17-18	5	5.21±.222	2.00±.179	147±8.0	25.3±3.38	48±3.6	32
	50	5.21±.222	2.08±.195	146±7.8	26.5±2.77	42±2.1	29
	100	5.21±.222	2.11±.192	136±4.6	30.1±3.04	49±2.4	37
	200	5.21±.222	1.84±.119	142±5.9	27.2±2.54	51±5.2	36
	300	5.21±.222	1.24±.197	147±11.9	15.5±2.68	55±2.2	38
22-23	5	5.00±.221	2.26±.150	88±4.9	46.6±3.61	31±2.1	36
	50	5.00±.221	1.90±.200	86±2.9	40.2±4.55	34±2.0	40
	100	5.00±.221	1.89±.191	82±2.8	42.0±5.27	34±1.8	42
	200	5.00±.221	1.64±.189	78±1.6	35.5±3.99	36±1.5	46
	300	5.02±.261	1.48±.158	78±1.8	33.9±3.56	40±3.1	51
27-28	5	4.80±.260	2.07±.143	54±2.6	66.4±4.88	25±1.4	46
	50	4.80±.260	1.24±.209	56±1.6	38.2±6.98	27±1.2	49
	100	4.80±.260	1.27±.200	54±1.0	41.4±6.95	30±0.9	55
	200	4.80±.260	1.07±.166	51±2.3	35.6±5.64	27±0.8	52
	300	4.82±.300	0.96±.172	51±2.3	32.0±6.10	29±2.0	57
32-33	5	4.82±.300	1.68±.135	36±2.1	86.9±8.61	19±0.7	52
	50	4.82±.300	0.90±.173	34±1.8	43.4±9.55	22±0.8	64
	100	4.82±.300	0.90±.169	36±1.3	42.9±8.31	21±0.7	58
	200	4.82±.300	0.81±.138	34±2.3	39.6±6.41	21±1.0	61
	300	4.76±.348	0.70±.113	36±1.7	33.3±5.16	20±1.1	56
37-38	5	4.80±.260	1.60±.112	26±1.5	100.0±7.21	17±1.3	66
	50	4.80±.260	0.90±.120	29±1.0	54.9±6.94	18±0.9	60
	100	4.80±.260	1.11±.233	27±1.4	61.3±9.62	17±0.9	63
	200	4.80±.260	1.09±.137	27±1.2	68.0±8.40	18±0.9	64
	300	4.80±.260	1.05±.108	27±0.8	66.2±6.77	19±2.4	73

TABLE C

The Effects of Change in Temperature and Stimulation Rate on the TMT $\left(\frac{\text{ventricle}}{\text{atrium}}\right)$ and $\frac{dT/dt}{MT}$ $\left(\frac{\text{Atrium}}{\text{Ventricle}}\right)$ in Rat, Guinea Pig and Cat.

Temperature (°C)	Animal	Stimulation rate (/minute)	TMT $(\frac{\text{ventricle}}{\text{atrium}})$	$\frac{dT/dt}{MT}$ $(\frac{\text{atrium}}{\text{ventricle}})$
18	Rat	5	2.44	2.57
		100	1.87	1.91
		300	1.49	1.71
	Guinea pig	5	2.82	3.22
	Cat	5	3.86	4.06
23	Rat	5	2.82	2.80
		100	2.10	2.20
		300	1.72	1.86
	Guinea pig	5	2.84	3.07
	Cat	5	4.98	4.59
28	Rat	5	2.96	2.86
		100	2.17	2.78
		300	1.78	1.63
	Guinea pig	5	2.64	3.44
	Cat	5	4.20	4.32

(Table C continued)

Temperature (°C)	Animal	Stimulation rate (/minute)	TMT ($\frac{\text{ventricle}}{\text{atrium}}$)	$\frac{dT/dt}{MT}$ ($\frac{\text{atrium}}{\text{ventricle}}$)
33	Rat	5	2.25	2.69
		100	1.94	2.14
		300	1.64	1.73
	Guinea pig	5	3.54	3.72
	Cat	5	3.55	3.33
38	Rat	5	2.38	2.45
		100	2.07	2.13
		300	1.70	1.82
		5	2.51	2.44
		5	3.45	3.55

TABLE D

The Maximum Rate of Developed Tension Divided by the Maximum Tension, and the Time to Maximum Tension in Isometric Contractions of Guinea Pig and Cat.

	TEMPERATURE	ATRIUM		VENTRICLE	
	(^{o}C)	$\frac{dT/dt}{MT}$ (sec^{-1})	TMT (msec)	$\frac{dT/dt}{MT}$ (sec^{-1})	TMT (msec)
Guinea pig	17-18	8.6±.28	200±8.9	2.7±.23	563±30.6
	22-23	11.7±1.10	145±3.9	3.8±.38	413±34.7
	27-28	19.4±1.16	99±3.2	5.6±.58	262±18.3
	32-33	31.0±1.48	46±4.8	8.3±.96	163±4.4
	37-38	41.6±2.12	39±1.0	17.1±1.99	98±4.9
Cat	17-18	10.0± .61	195±7.4	2.5±.52	752±130.2
	22-23	14.6± .67	132±8.6	3.1±.36	658±58.9
	27-28	21.1± .46	89±4.0	4.9±.24	374±32.8
	32-33	30.8±1.06	53±1.2	9.2±.69	188±6.6
	37-38	39.0±1.80	42±1.2	11.0±.67	145±5.0

Mean ± SEM of 3-8 atria or papillary muscles.