MECHANICAL AND ELECTRICAL INTERRELATIONS

IN

NORMAL AND ISCHAEMIC HEART

BY

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This work has been approved by the University of London
for the degree of M.D.

ABSTRACT

Introduction. Excitation-contraction coupling in cardiac muscle has been extensively scrutinized. The converse link, however, has received little attention despite its potential physiological and clinical relevance This thesis bears on the converse link: i.e. the effects of mechanical events on the electrical events in ventricular muscle under a variety of experimental and simulated clinical situations.

Method. Superfused and perfused preparations of isolated hearts as well as intact hearts in situ were studied. Electrophysiological measurements, force and motion were recorded while mechanical or ischaemic interventions were imposed.

Results. In general, the following observations obtained:

1. Stretch of *resting* muscle produced depolarization which could reach threshold for an "extrasystole".

2. Stretch *during* the action potential produced no change whereas muscle shortening prolonged the action potential: a prolongation of repolarization processes.

3. A delayed muscle shortening produced an afterdepolarization which could cause an extrasystole (cf also point 1).

4. All the electrophysiological changes had their equivalent in the e.c.g of the intact heart.

5. Changes in left ventricular mechanics of intact hearts in situ also produced changes in ventricular repolarization gradient, and thus the T-wave of the e.c.g.

6. Dyskinesia of ischaemic areas of the ventricle were also associated with transient depolarization and extrasystoles were seen during the 1st hour of regional ischaemia, sometimes leading to ventricular fibrillation.

Possible Mechanisms. (1) Passive architectural changes could affect membrane permeability or intracellular spaces to influence ionic movement and cause membrane depolarization. (2) The calcium ion could mediate in the contraction-excitation feedback. Calcium can affect ionic movement and conductance across membranes in several ways and includes a system invoking c-AMP and phosphorylation reactions.

Clinical relevance. As yet the, curiously, "upright" T-wave of the ecg is unexplained. The length-tension distributions in the normal ventricle could influence the action potential differently in different regions, to contribute to this T-wave (see points 2 & 5 above). Similarly the inhomogeneous contraction patterns could produce afterdepolarizations to form or influence the U-wave.

The cause of the extrasystoles that precipitate very early fibrillation following myocardial infarction is also unexplained. Regional ischaemia produces contraction abnormalities which could cause extrasystoles to precipitate ventricular fibrillation. It is possible that the management of these arrhythmias may have to be reconsidered.

ACKNOWLEDGEMENTS

My special thanks to Professor A. Guz, friend, collegue, and teacher, whose passion for research is a positive inspiration. I also place great value on my collaboration with Professor R. Kaufmann, Drs. R Hennekes and K Woollard. I thank Mrs R Kingaby for her expert technical assistance in some experiments.

I acknowledge selections, figures, and modifications from:

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CHAPTER I

INTRODUCTION

BACKGROUND AND RELEVANCE OF PROJECT.

This investigation is concerned with the effects of changes in mechanical behaviour of cardiac muscle on its electrical activity. Until recently mechanical and electrical interaction in myocardium has been thought to be unidirectional: beginning with the "initiation" of heart beat, via the pacemaker potential or diastolic depolarization, going through a process of excitation-contraction coupling and, finally, resulting in contraction of heart muscle. However, the evidence to be presented in this dissertation supports the existence of a mechano-electric interaction or contration-excitation feedback which has several aspects. First, a reverse coupling in which the process of contraction feeds back to affect the action potential that initiates the beat. This feedback will be termed contraction-excitation feedback and can take place chronologically because the duration of action potential and mechanical activity are of the same order of magnitude. It will be demonstrated that the duration of the action potential is prolonged when the tension developed by the muscle is low and shortening is unhindered. Conversely, the action potential duration is abreviated when contraction is isometric and no external muscle shortening occurs. The relevance of the mechanical influence on the action potential duration is concerned with the observation that changes in action potential

duration affect the subsequent mechanical behaviour of the myocardium. The latter influence occurs by virtue of more or less calcium (Ca^{2+}) entering the cell during the plateau phase of the action potential. A feed back control system is thus set up on a beat to beat basis which can regulate myocardial contraction. Secondly, changes in duration of the action potential will inevitably reflect in the ventricular repolatization gradient. This will influence the T wave of the electro-cardiogram of the intact ventricle.

The final broad aspect of the mechano-electric interaction which is presented is that mechanical behaviour of myocardium which is temporaly out of phase with that normally expected at that time can cause depolarization of the myocardial membrane. This depolarization is capable of initiating extra beats. It will be shown that either sudden releases or sudden stretches of the myocardium under defined circumstances can cause extrasystoles. In the intact ventricle this phenomenon may have profound clinical relevance since it will be demonstrated that out of phase mechanical behaviour of segments of the ventricle can occur with myocardial ischaemia and the inhomogeneous pattern of contraction is often found together with apparent depolarizations; and with accompanying ectopic beats. The mechanical induction of extrasystoles is thus an alternative explanation for ectopic impulse formation to the re-entry mechanism and ectopic enhancement: both the latter hypothesis are based purely on altered membrane properties as a result of ischaemia. The newly discovered possible cause of arrhythmia may have far reaching implications in the rational prevention and treatment of early ischaemic, fatal arrhythmia. At this stage the

reader may wish to peruse the final chapter of this thesis for a more comprehensive summary of the existence of contraction-excitation feedback, and its clinical role and content.

STUDY SYSTEMS EMPLOYED: ADVANTAGES AND LIMITATIONS

Cat papillary muscles

Papillary muscles from the right ventricle of cats are one of the best preparations for use in rigorous study of mechano-electric properties of mammalian myocardium. Length and tension were accurately monitored and controlled while micro-electrodes were used for recording transmenbrane potentials. As a supplement to the microelectrodes action potentials obtained via a sucrose gap recording chamber were used and this can provide a more uniform indication of electrical activity in the muscle as a whole than micro-electrodes.

Intact isolated frog ventricle

Perfusion through the coronary circulation is mechanically dependent, and during contraction of the intact ventricle flow through the vessels can be profoundly affected. It will be shown that ischaemia affects mechanical activity of segments of ventricle

within seconds and the present study involves large mechanical changes imposed on the heart. Thus a preparation without a coronary circulation such as the intact frog ventricle is worth studying since it affords a means of differentiating pure mechanical effects on electrical events, from the electrical effects of changes in coronary perfusion. This preparation however precluded rigorous control and monitoring of mechanical and electrical events since stress and strain in intact segments of heart were difficult to assess and microelectrode recordings from the small cells of a vigorously beating heart intact frog heart were almost impossible to obtain. Ventricular volume, pressure and segment lengths were thus measured in this preparation together with the monophasic action potential from the segment. The latter electrical recordings,however, were subject to difficulties in interpretation. In consequence confirmatory microelectrode measurements were made in isolated frog ventricular strips.

The above two preparations were perfused by cell-free solutions. Some questions have been raised as to the validity of using the results obtained from these preparations to predict the behaviour of the intact heart in situ. (Reichel 1976). For example in the isolated papillary muscle of mammalian ventricle excessive amounts of potassium can be lost over a period of time - particularly if the frequency of stimulation was high. The force frequency relations were also different between the intact and isolated preparations: the isolated preparation had a steeper force-length relation than one which was blood-perfused (Kavaler 1972). Segments of epicardium of the intact ventricle in situ were thus also studied in the context of the mechano-electric interrelations outlined above.

Normal segments of the intact mammalian ventricle in situ

The movement of epicardial segments in the ventricle in situ, together with surface electrograms and action potentials, provide a convenient preparation which can supplement, at least qualitatively, the studies with the isolated preparations. The mechanical behaviour of the segment was not easily controlled or monitored because of its interaction with the surrounding myocardium. However this limitation was apparent and not real, for in fact the mechanical behaviour of segments, in parallel and in series with the segment studied was germaine to the understanding of the performance of the wall of the ventricle as a whole. The electrical activity at a cellular level was also difficult to obtain in these preparations and action potentials recorded by other methods, such as suction electrodes have inherent pitfalls. These will be described later . Nevertheless the action potentials used mainly in the study of the intact mammalian ventricle in situ had some distinct advantages in that the representative electrical activity of a number of cells was sampled.

The intact Pig heart was used which has a coronary circulation and ventricular anatomy remarkably similar to that of the human. Extrapolation of these results to humans may thus be done with greater confidence than when using dogs, where for example, the

collateral coronary circulation is abundant,

ISCHAEMIC SEGMENTS OF INTACT VENTRICLE IN SITU

Segments of the ventricle were made ischaemic by occluding the supplying coronary artery. Although this procedure may approximate the pathological process of the acute coronary occlusion in some clinical situations, in most cases the pathogenesis of the occlusion and subsequent infarct is different. The experimental model was however useful to initially study mechano-electric interrelationships during acute ischaemia. In fact, no previous simultaneous measurements of epicardial mechanical movement, electrograms as well as action potentials have been documented using any other models. The use of a better model, for example the study of wall motion, epicardial ECG and action potential in unrestrained conscious animals during gradual occlusion of coronary arteries, would be technically very difficult, and perhaps premature at this stage of the investigation.

CHAPTER II

INTRODUCTION TO CELLULAR PHYSIOLOGY (Huxley 1974; Noble 1975; Corabboeuf 1978; Carmeliet 1978; Vassalle 1979; Reuter 1979; Chapman 1979; Nayler & Williams 1978; Fabiato & Fabiato 1979)

The subject matter covered in this thesis is wide. It deals with aspects of electrophysiology, excitation-contraction coupling and mechanics of both isolated and intact myocardium in normal situations. In addition all three aspects were studied under abnormal (ischaemic) conditions. Clearly to go thoroughly into the background of these studies will result in a cumbersome tome. Therefore only those aspects that have direct application to the experiments in this dissertation will be dealt with and even these will have to be briefly covered.

THE RESTING POTENTIAL (Fig.II-1)

At rest the transmembrane potential difference is about 80 mV, inside negative. The ion that is mainly responsible for the potential difference is potassium which diffuses out of the cell along its concentration gradient. The negatively charged balancing anions remain in the cell and a potential difference, 'pulling' potassium back, is thus set up. Theoretically this

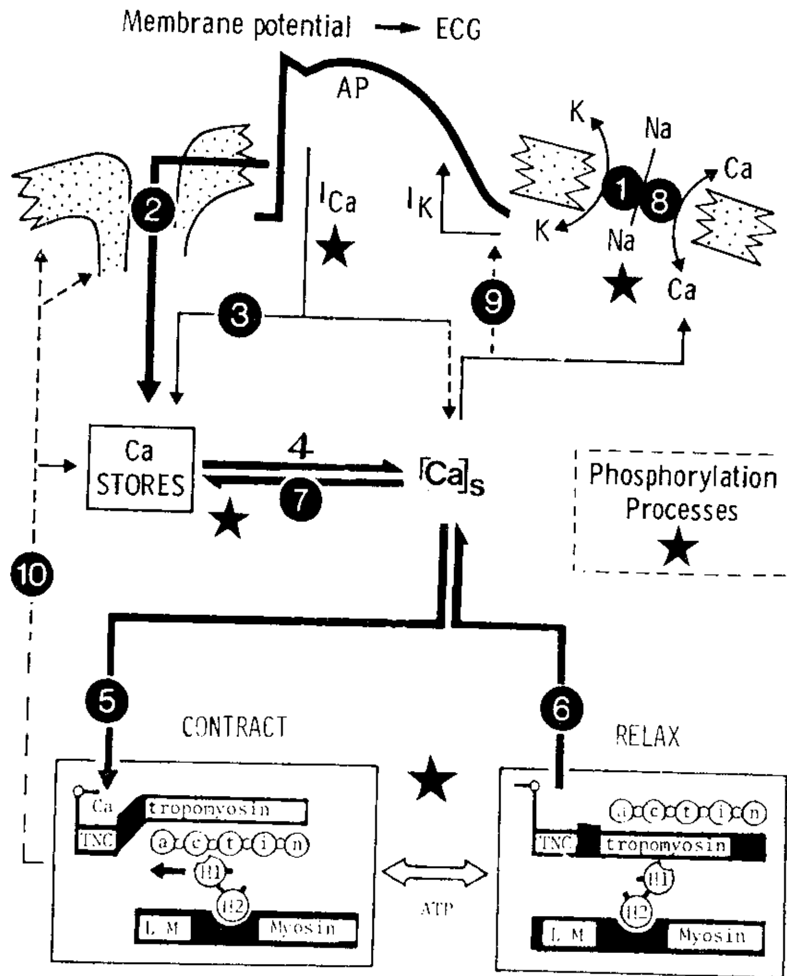


Figure II - 1

Figure II-I

Schematic diagram of some interactions between action potential (AP) and contraction in heart. The interactions include phosphorylation processes which are indicated by the asterisks, each denoting a different reaction. During rest sodium conductance (g_{Na}) is low. However g_K is high and is the major immediate contributor to the negative resting potential which is maintained in the long term by an ATPase dependent Na/K pump (1) which keeps the internal Na^+ concentration $[Na]_i$, low and $[K]_i$ high. Extracellular Ca^{2+} $[Ca]_o$ is relatively high, while sarcoplasmic $[Ca^{2+}]_s$ is very low. With the upstroke of the action potential g_{Na} rapidly increases and the fast inward sodium current, I_{Na} reverses the transmembrane potential. Despite the consequential increase in the driving forces for potassium, g_k decreases and the outward repolarizing K^+ current, I_k, is less than expected. The cardiac action potential is thus prolonged. A slow inward current, carried mainly by Ca^{2+}, I_{Ca}, also prolongs the plateau. The channels for I_{Ca} are influenced by cAMP dependent protein kinase (I_{Ca} probably generates action potentials in the sinus node and partially depolarized cardiac muscle (Cranefield 1975.) In mammalian muscle I_{Ca} does not normally contribute directly contraction to any significant degree unless the plateau is long. The depolarization increases sarcoplasmic calcium $[Ca^{2+}]_s$ from the stores (4), directly (2), & by Ca - induced Ca^{2+} release (3, Fabiato & Fabiato 1979) as well as via I_{Ca} (dotted line). When $[Ca^{2+}]_s$ is high enough contraction begins (5). Tension production needs ATP ase is influenced by phosphorylation of acto-myosin which involves cAMP. As or probably before the membrane repolarizes during relaxation the reticulum (SR) sequesters Ca^{2+} (6,7). This event comprises a rapid binding process and a storage process. An SR bound ATPase, which is Ca^{2+} activated, mediates the transport of Ca^{2+} by the sarcoplasmic reticulum. A membrane Ca^{2+} carrier is thought to be phosphorylated to bind and translocate it. Once inside, the Ca^{2+} is released on dephosphorylation of the carrier. The phosphorylated intermediate is regulated by protein kinase which in turn is regulated by cyclic AMP. The SR membrane is thus complex and incorporates the receptor protein for kinase catalyzed phosphorylation as well as the ATPase. Ca^{2+} can also leave the sarcoplasm (8) by a metabolically dependent calcium pump or by Na-Ca exchange. The latter system is important for contraction in the frog. Changes in $[Ca]_s$ may influence outward currents (9). The asterisks and dashed lines also imply processes that could link contractile activation with membrane potential, and also encompass length-dependent activation. (Jewell 1977).

equilibrium potential may be calculated (using the Nernst equation) and is equal to the potassium equilibrium potential E_K. In practise the resting potential is not equal to E_K. This is due to the fact that small amounts of Na^+, which has a higher external concentration than internal, can pass through the membrane thus neutralizing some of the internal negativity. The sodium pump, energetically mediated, then extrudes this Na^+. The resting cell is thus in a steady state, not an equilibrium state, in which K^+ determines the immediate resting potential and the Na^+ / K^+ pump maintains it in the long term.

CURRENTS ASSOCIATED WITH DEPOLARIZATION. (Fig II 2)

During activation sodium 'explosively' enters the cell as excitation renders the membrane permeable to it. This is because there is both an electrical and chemical gradient directing it inward. The process slows when the transmembrane potential nears E_{Na}, the equilibrium potential for sodium. There is considerable evidence from voltage clamp studies that Na^+ carries the depolarizing current in cardiac muscle and purkinje fibres. One example of this evidence is mentioned since it is relevant to some experiments in the thesis: under voltage clamp conditions when the membrane is partially depolarized (e.g. to about -50mV) a depolarising step produces no inward Na^+ current i.e. the Na^+ current is inactivated.

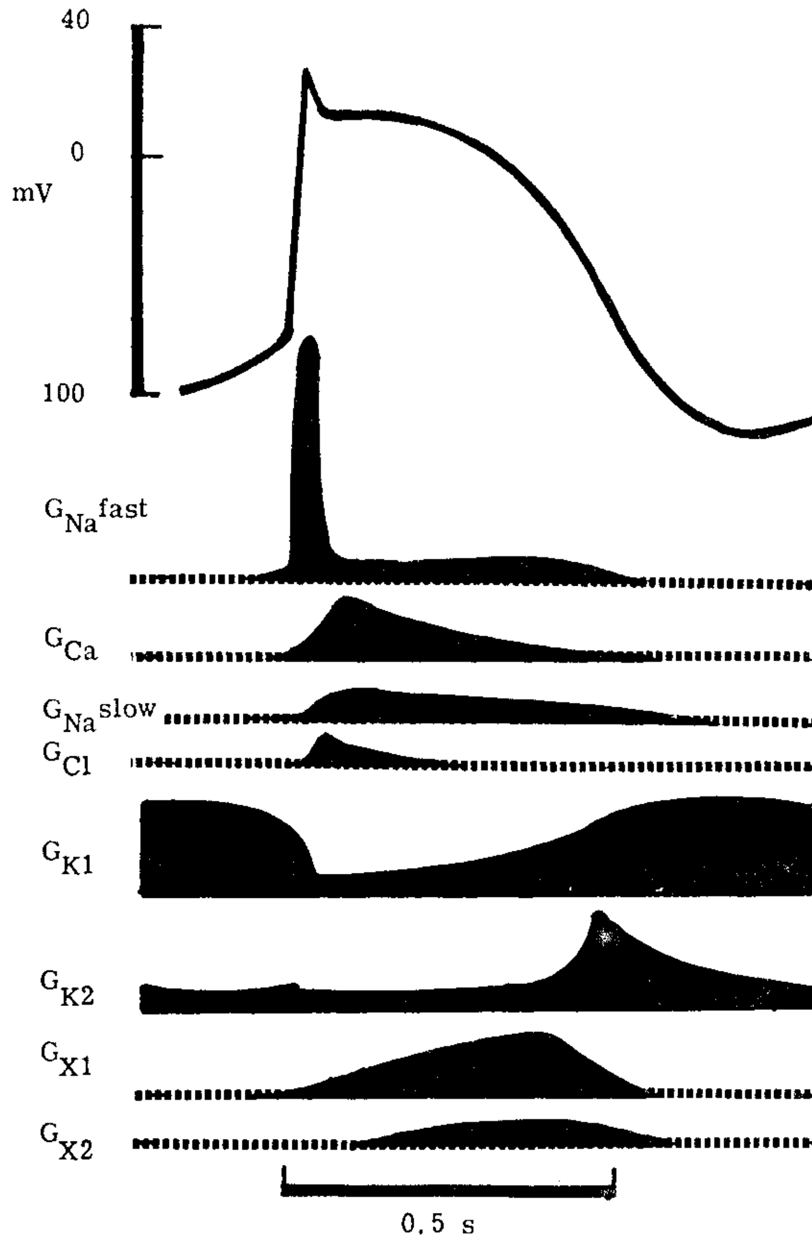


Fig II-2 Some ionic Conductances (G) during the action potential. The thicker the black bands representing the conductances, the greater the "permeability". For example GK_1 is high during rest and decreases with plateau potentials. GK_2 is high near the end of repolarisation and the maximum diastolic depolarisation, and decreases in Purkinje fibres to produce the characteristic pacemaker potential of this tissue. See text for further discussion.

On depolarization the outward driving forces for K^+ increase because the inside of cell is now relatively positive. However, the potassium conductance G_k , decreases (anomalous rectification) and one of the outward potassium currents $\dot{I}_{ki}$ is less than expected. The cardiac action potential thus prolongs as compared with nerve or skeletal muscle action potentials where an increase in G_k immediately repolarizes the membrane. At these 'plateau' potentials a second outward potassium current $\dot{I}_{x1}$ is activated.(Noble & Tsien 1972). A third outward current carried by chloride ions, is activated at transmembrane potentials near zero and which is thought to be responsible for the notch seen just after the spike of the action potential in purkinie fibres when they are stimulated at relatively slow rates.

In addition to the above outward currents, slow inward currents ha ve been shown to flow during the plateau of the action potential and which may be important for the genesis of action potentials in the sinus node and in partially depolarized cardiac tissues. This current passes through non-specific 'channels' in the membrane and is carried by both Na^+ and Ca^{2+} ions. The current appears entirely different to the fast inward $\dot{I}_{Na}$. being activated after inactivation of the fast inward $\dot{I}_{Na}$ at about -40mV, and peaking at zero potentials.

Thus, regenerative depolarization is due to the rapid inward Na^+ current carried by some 'activated' moeity in the membrane and which is available at resting membrane potentials. The carriers are then rapidly inactivated but only become available when the

membrane repolarizes: this is the cause of the absolute refractory period. Depolarizing the membrane to about - 50mV inactivates the carrier , but strong stimulation can activate a calcium mediated action potential. The latter may be the physiological case in sinus nodes.

RELATIONSHIP BETWEEN TRANSMEMBRANE CURRENTS AND REPOLARIZATION PHASES OF ACTION POTENTIAL

The interpretation of the role of the various ionic currents during repolarization is not as clear as the depolarizing currents. It appears fairly conclusively,that the slow inward currents carried,by Na^+ and Ca^{2+} go through a separate channel to the fast inward current, and that G_K decreases with depolarization. It is their relative contributions to the repolarization process that is being debated. One interpretation is that the activated slow inward currents decay with time and thus even if the outward potassium current remains constant there is a net increase of outward positive charges with time and the cell repolarizes to a level where there is not such a decrease in G_K i.e a smaller anomalous rectification, and the membrane repolarizes. The second interpretation is that the inward currents are constant whilst there is a time dependent increase in a potassium current - I_{X_1} . The two mechanisms however, are not mutually exclusive. The action potential is probably the result of a series of events in which currents bring the membrane potential to values which activate other currents in orderly fashion

PACEMAKER POTENTIALS AND DIASTOLIC DEPOLARIZATIONS.

One aspect of the studies to follow this chapter concerns the

formation of ectopic impulses. One acceptable mechanism for the formation of these impulses is that related to pacemaker activity. This form of activity will thus be beiefly described. There is no resting potential in pacemaker fibres of the sinus node. The maximum diastolic potential reached after an action potential is not maintained and a gradual diastolic depolarization results in the cell reaching threshold. The spike take-off is gradual in dominant pacemaker cells and abrupt in latent pacemaker cells. In Purkinie fibres the diastolic depolarization which is observed in the range of 90-60 mV is due to a modification of an outward K^+ current $\dot{I}K_2$. After repolarization this current shows a time dependent decrease which results in a slow depolarization. Later the decay in the potassium current becomes voltage dependent and at the same time the inward Na current is activated resulting in a complete depolarization and thus an action potential. The atrial and sinus pacemaker activities which occur at a less negative transmembrane potential of -60 to -40 mV are due to a different current although it is also mainly carried by K^+ . A decay of the current termed i_x results in the slow diastolic depolarization to threshold of this tissue. If the pacemaker is made quiescent, as when Purkinie fibres are bathed in about 2 x $[K]_o$ which increases background G_K to decrease the nett inward current, the maximum diastolic potential is more negative than the resting potential which the cell eventually attains. Ventricular myocardium by contrast has a stable resting potential unless for example $[K^+]_o$ and/or $[Ca^{2+}]_o$ are zero, or steady depolarizing pulses are applied.

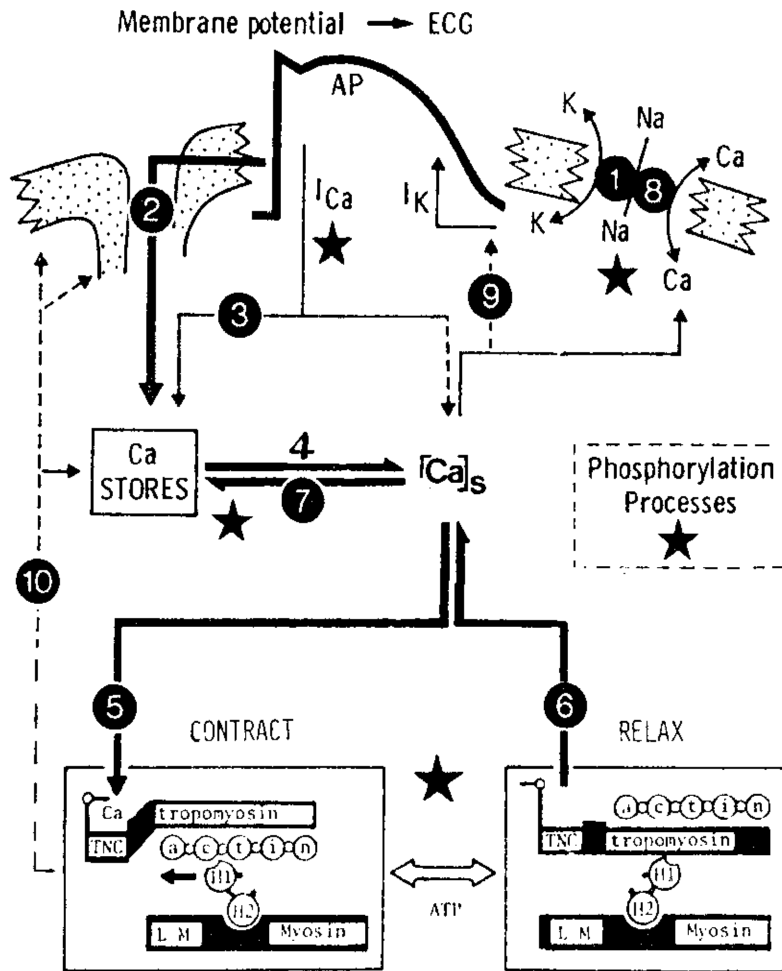


Figure II - 1A

It seems that the channels of the pacemaker current $\dot{i}_{K2}$ in Purkinje fibres not only close with time but show anomalous rectification, inactivation with the fall in potential It is fully inactivated at -90mV and fully activated at -60mV. A dominant pacemaker can inhibit the latent ones and this has been termed overdrive suppression which is observed as quiescences when the dominant pacemaker ceases.

In the experiments in this thesis diastolic depolarizations were also seen in ventricular muscle but these were mechanically induced. The nature of these depolarizations which can reach threshold, will be a subject for speculation. It may be pertinent to compare the different mechanisms for pacemaker activity in different tissues.

SLOW IN WARD CURRENT $\dot{i}_{si}$ (See also Fig. II - 1A)

Since the slow inward current may be intimately concerned in the feedback interactions studied it will be described in a little more detail here.

$\dot{i}_{si}$ determines the plateau phase of cardiac action potentials (Mcallister et al, 1975; Beeler & Reuter, 1977) and is the primary inward current of the spontaneous sinus node action potential. In addition it can also be the major inward current when the membrane of atrial (Brown et al, 1975) and ventricular muscle (Beeler & Reuter, 1977; Grant & Katzung, 1976) is depolarized. That is, when the fast inward Na^+ current is inactivated. $\dot{i}_{si}$ is largely carried by Ca^{2+} the channels being more than 100 x more selective for this ion than

for Na^+ or K^+ (Reuter & Scholz 1977) and for this reason the channels passing this current may be referred to as Ca^{2+} channels. Despite the fact that the reversal potential of i_{si} is more negative than the calcium equilibrium potential using the Nernst equation, a clear approximation can be obtained using the constant field equation. Nevertheless i_{si} does not proportionally follow changes in $[Ca^{2+}]_o$ and at low concentration i_{si} is carried mainly by Na^+ (McAllister et al, 1975). Further, intracellular injection of Ca^{2+} can in fact increase I_{si} in Purkinje fibres when the membrane potential is about -50mV (-35 to -65). (Eisenberg, 1977). I_{si} may be increased by adrenergic drugs (Brown et al, 1975: Tsien, 1977) whereas acetylcholine can reduce it (Ten Eick et al, 1976; Giles & Noble, 1976). However, epinephrine has no effect on the kinetics of I_{si} but may cause an increase in the number of conducting channels (Reuter & Scholz, 1977). An increase in cAMP dependent phosphorylation may increase the number of conducting channels and provide a mechanism for the action of catecholamines (Kolhardt & Kubler, 1975: Reuter & Scholz, 1977: Niedergerke & Page, 1977: Tsien, 1977).

An intracellular injection of cGMP reduces the Ca_i^{2+} (Ikemoto & Goto, 1978) and decreases diastolic depolarization (Tuganowski et al, 1977). Generally both acetylcholine and catecholamines reduce the action potential duration (APD) (Lipsius & Vassalle, 1977: Reuter, 1967) but catecholamines shift the plateau in the depolarizing direction via the increase in I_{si}.

Finally, the role of internal $[Ca^{2+}]$ may prove to be even more important than hitherto realized. If "contraction excitation feedback", which is the main subject of this thesis, is Ca^{2+} - mediated, one may have to invoke this ion either as some sort of internal "transmitter" substance which can change current-leak channels or as an activator of other transmembrane currents.

EXCITATION-CONTRACTION COUPLING AND THE ROLE OF Ca^{++} (FigII-IA)

Considerable attention will be paid to the role of calcium in membrane and contractile activity because it may be germaine to the explanation underlying the observations which follow in some of the chapters

Calcium extracellularly is of the order of 2.5 m μ whereas intracellularly its concentration is about 0.0001 m μ The amount of Ca^{2+} getting into the cell during the plateau is small (0.5-2.7 μM/Kg) and, as 60 μM is needed in mammalian cardiac muscle, the internal free calcium does not normally and directly cause contraction. Under some circumstances this calcium can possibly contribute to tension development. For example Ca^{2+} entry is high in frog myocardium when $[Na^+]$ o is low. It has been demonstrated that when commencing stimulation of a resting cat papillary muscle, the first action potential is the longest of the train and enough calcium appears to enter directly to activate the muscle (Allen et al, 1976). This contraction is small and late. Subsequent action potentials are shorter with first a two-component contraction then a single monophasic and early contraction of normal amplitude. The degree of calcium entry with each normal action potential depends on the extracellular $[Ca^{2+}]$, the ratio of $[Ca^{2+}]:[Na^+]$ and frequency of activation. (e.g. Winegrad & Shanes 1962)

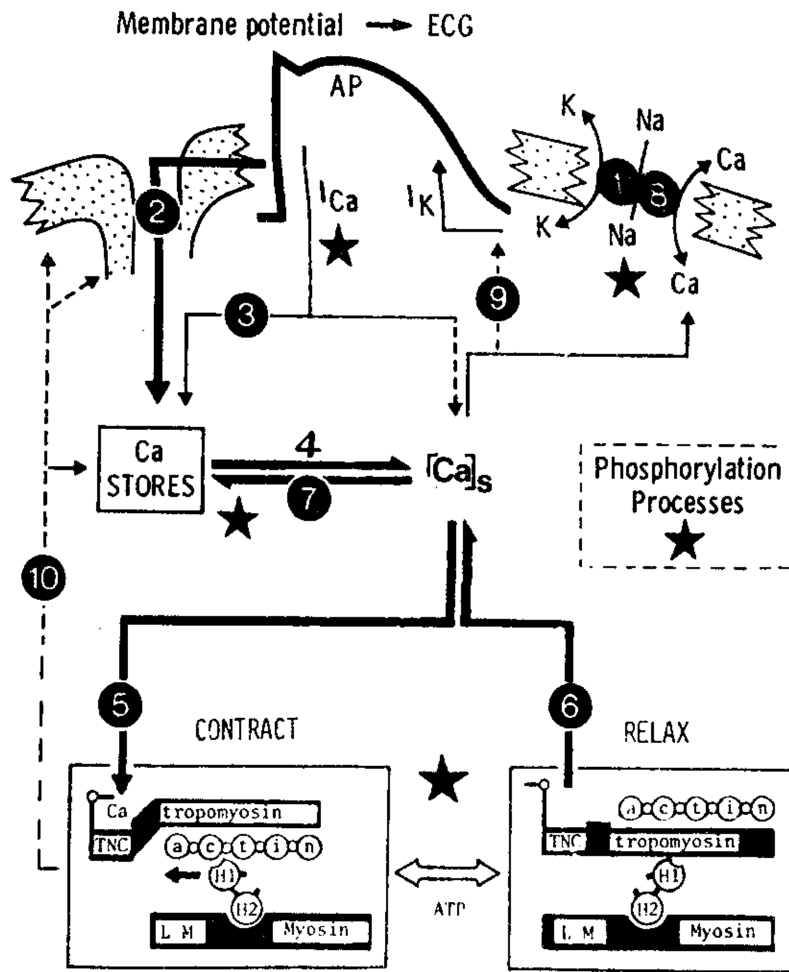


Figure II - 1 B

The electrical activity penetrates into the cell via the T system. The Na^{+} and Ca^{2+} flux may be involved in the depolarization of the S.R. which releases Ca^{2+} (Constantin & Taylor, 1973: Fabiato et al, 1971:Nakajima & Endo, 1973). If the membranes of cardiac muscle are disrupted (Fabiato & Fabiato, 1973) a very small amount of Ca^{2+} may be able to release larger amounts from the S.R. which can then go on to activate the contractile machinery the "calcium-induced calcium release" or regenerative calcium release (Ford & Podolsky, 1970). Thus the action potential triggers the activation of the muscle as well as influencing the force of contraction by its character and duration which influences the internal calcium concentrations.

At the normal resting internal $[Ca^{2+}]$ which is at about 10^{-7} M (Fig. II - IB) actin and myosin molecules do not interact because the actin molecules are inhibited by tropomyosin, since troponyosin lies near the grooves of the actin helix (Huxley 1973). The tropomyosin is controlled by troponin complexes periodically arranged along the thin filament. Under these circumstances the ATP-ase activity of the myosin is low. When the myoplasm $[Ca^{2+}]$ increases to about 10^{-5}M, 2 molecules of Ca^{2+} bind, with a high affinity of some 1×10^{6} M^{-1}, to the TN-C component of the troponin complex. This binding causes the TN-1 component of troponin to stop inhibiting actin. One such binding of Ca^{2+} to a troponin complex results in seven units of actin becoming capable of interacting with myosin.

The stiff cross-bridges of the myosin thick filament consists of the heavy mero myosin(HMM)sub fragments S_{I} and S_{2}. The light

meromyosin (LMM) part of the myosin molecule is embedded in the thick filament. There is an apparent hinge between LMM and HMM and another between the heads, HMM S_I and HMM S_2. The myosin ATP-ase is in the globular S, portion.

A bond between this portion and actin is formed in the form of a complex with Mg^{2+} and ATP. Splitting of the ATP supplies the energy for the conformational change which then results in a pulling action of the S_I fragment on the S_2 fragment (Huxley, 1973, Huxley and Simmonds 1971). This movement breaks the bond. ATP is resynthesized to reposition the head (S-I subfragment). The traction has now opposed new sites since the fila ments slide over one another in opposite directions, and the process repeats. There is some elasticity in the bridge (Huxley & Simmonds 1971) which may reside in the S_2 subunit.

The above series of events is a simplified version of the current state of affairs and the scheme in Fig II-1is an even simpler summary. For example in heart muscle the problems in the regulation of the myosin-action-ATP system by Ca^{2+} and the regulatory proteins are unresolved and complex (Murray & Weber, 1974; Tonomura 1973). However one mechanical aspect of this regulation needs some expanding as, first, much of the work in this thesis is concerned with mechanically dependent changes in membrane potential and secondly, recent evidence is accumulating that internal Ca^{2+} and cardiac muscle activation is mechanically dependent.

In essence, the tension developed by the intact cardiac muscle, as opposed to the skinned fibre, at short lengths appears to be less than can be explained by the sliding filament theory (see Jewell 1977 for a review). It has been suggested that this observation is due to less activation of the contractile machinery. The smaller activation is most likely related to Ca^{2+} either by decreasing its affinity for tropnin (Huxley, 1963) or by reducing its sacroplasmic concentration, either by an increased uptake or reduced release. No evidence is available for the troponin mechanism in cardiac muscle but there is evidence for length-dependent changes in Ca^{2+} release. This possibility, that changes in mechanical conditions are associated with changes in internal $[Ca^{2+}]$, is central to the theme in this dissertation. The regulation of this $[Ca^{2+}]_i$ will therefore also be discussed in further detail below.

MAINTENANCE OF LOW $[Ca]_i$ (Fig. II - IC)

A raised intracellular $[Ca^{2+}]$ associated with tension development and it is essential to keep $[Ca]_i$ low during diastole so that the ventricles can expand and allow ventricular filling to take place. Several mechanisms contribute to maintaining a low Ca_i.

A trans-sacolemmal Na-Ca exchange exists in which 2-4 Na^+ exchange for one Ca^{2+}. The Na-Ca exchange system requires little or no energy. With such a system one can get an increase in Ca^{2+} efflux under some circumstances - but only when $[Na]_o$ exists in sufficient amount for an exchange. If there is no $[Na]_o$, resting tension increases and this can be equated to an increase in $[Ca]_i$. Na-Ca exchange however is a

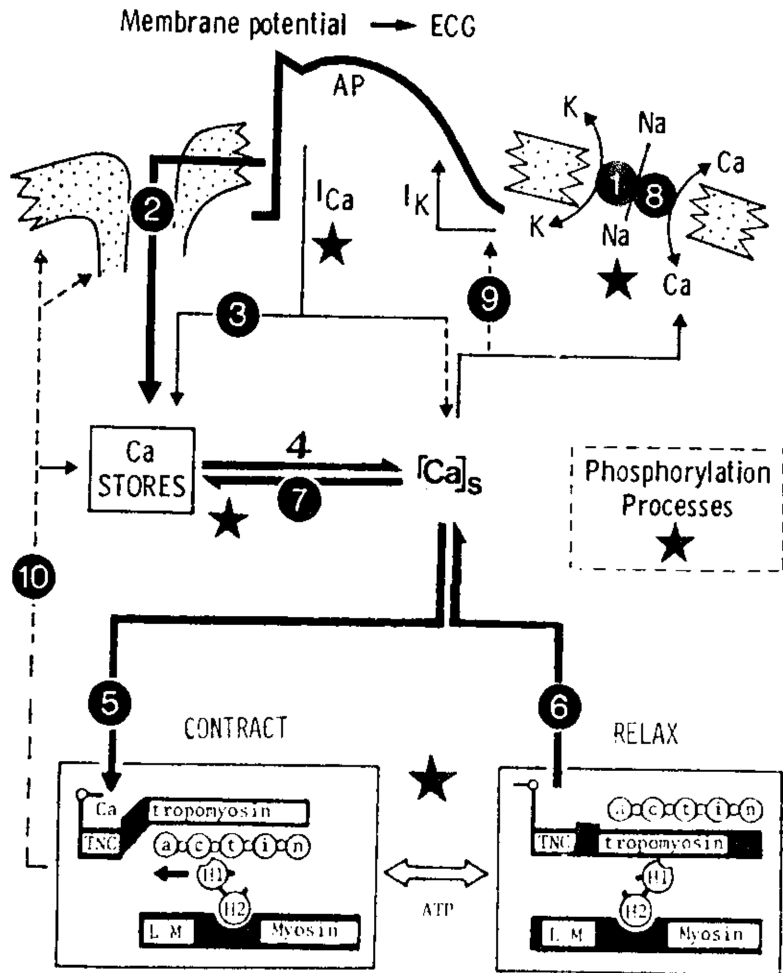


Figure II - 1 C

backup system for the other mechanisms for regulating $[Ca]_i$. In amphibian heart Na^+ - Ca^{2+} exchange is the most important mechanism for maintaining a low $[Ca]_i$. But this is not so in mammalian heart. In amphibian heart it was concluded that (a). Na-Ca exchange is fast enough to raise $[Ca]_i$ to cause contraction (b) the carrier must be charged or more than 2 Na^+ ions must exchange for 1 Ca^{2+}.(c) the exchange may not be electrically neutral ie. changes in transmembrane potential difference affect the exchange. Thus with depolarization Ca^{2+} moves in and with repolarization Ca^{2+} moves out. This Na-Ca exchange can explain many phenomena in amphibian heart but there are some apparently anomalous observations. For example in $[Na]_o$ = zero, a hundred times increase in $[Ca]_o$ produced no increase in tension - viz Na-Ca exchange was inactive (Chapman & Miller, 1972), but depolarization with K^+ still can (Vassort 1973; Benninger et al, 1976). The latter contracture can be blocked by local anaesthetics, as in a caffein contracture, thus suggesting another source of Ca^{2+} , probably the sarcoplasmic reticulum (Chapman, 1979). The Na-Ca exchange in mammalian heart is electrogenic.

Na/Ca exchange is not metabolic (Peuter & Seitz, 1968,Jundt, 1975). Any link with metabolism is due to a Ca^{2+} pump, which is another system maintaining a low $[Ca]_i$. This system is one in which Ca^{2+} binds to high affinity sites on the phosphorylated pump protein on the SR, is translocated across the membrane, and taken up by a high capacity low affinity protein - probably on the inner surface of the SR (Maclennan & Holland, 1975). Therefore Ca^{2+} sequestering may depend on the surface area rather than the volume of SR.

This may be important for contraction-excitation feedback.

Research is trying to establish a link between the sarcolemammal Ca^{2+} pump and phosphorylation of sarcolemmal vescicles by protein kinases. hese protein kinases may be cyclic AMP dependent or Ca^{2+} dependent (see Review by Sulakhe & St Louis, 1976). hus several enzymes are associated with SR; an adenyl cyclase, a protein kinase (c-AMP dependent) phosphorylase kinase, phosphorylase (Entman et al, 1976).

A third method of maintaining a low $[Ca]_i$ is via the SR. In amphibian heart methylxanthines eg. caffeine inhibit phosphodiesterase activity by acting on cAMP (Beavo et al, 1970). ension development increases consequent on the increased efflux of Ca^{2+} from the SR. Local anaesthetics can inhibit this development of caffeine contracture as can antazoline and chlorpromazine which block Ca^{2+} activated ATPase of the SR. These observations suggest that some Ca^{2+} is liberated by the initial depolarization of amphibian heart,as well as the later transarcolemmal calcium influx. In frog hypodynamic state cAMP levels fall, as C-GMP rises. (Flitney et al, 1977).

In mammalian heart, substances that liberate Ca^{2+} from SR are similar to amphibian hearts. Methylxanthines also inhibit phosphodiesterase activity here and result in Ca^{2+} release from SR which increases tension (Bechtold & Scholz, 1971). But caffeine at low temperature inhibits the heart beat, although its never complete, resembling a rested state contracture. i.e. Ca^{2+} in the SR is progressively depleted until there is none left and residual contraction must be from Ca^{2+} coming from another source - possibly

an inward Ca^{2+} current from the prolonged AP (my interpretation partly supported by the findings of Beresewicz & Reuter (1977).

Tension changes with changes in rate are not accompanied with changes in activity of cAMP dependent protein kinase. Therefore, a mechanism involving Ca^{2+} is more likely (Keeley & Corban, 1977; Ezrailson et al, 1977). Tension changes with isopreterenol are accompanied by changes in cAMP levels but need $[Na]_o$ for inotropy (Linden & Brooker 1978). This implies that cAMP operates via Na-Ca exchange and can be another pathway for Ca^{2+} entry during rested state contractions.

The fourth, indirect, method of regulation of Ca_i has already been discussed separately. The inward calcium current can vary the amount of Ca^{2+} gained intracellularly on a beat to beat basis.

The subcellular site at which Ca^{2+} translocation occurs across the sarcollemma is uncertain. Recently the glycocalyx an internal layer which forms an integral part of the sarcolemma, invaginating into the T-tubules has been implicated (Langer 1978 for a review). The layer contains many fixed negative charges, Sialic acid contributing to these. Removal of Sialic acid increases the membranes permeability to Ca^{2+}. It appears that Ca^{2+} bound to this layer can be of importance in myocardial contraction.

CYCLIC NUCLEOTIDES AND CONTRACTION.

There is abundant evidence that the cyclic nucleotide - protein kinase system plays several roles in cardiac contraction: on the trans-sarcollemal ionic currents, Na-Ca exchange, the contractile protein, and the Ca^{2+} uptake by the SR (see Tsien 1979, Chapman, 1979 for reviews).

There is also evidence that concentrations or activities of cyclic nucloetides can vary in heart on a beat to beat basis (Brooker et al, 1973; Wollenberger et al, 1976).

Intracellular nucleotide, by diffusion, produces effects similar to extracellular catecholamines (Tsien & Weingart, 1976). However no consistent tension - cAMP relationship appears in the literature. Keeley & Corbin (1977) have found a correlation between activation of the cAMP dependent protein kinase and increase tension in rat heart but no rise in cAMP. These workers also found that increasing $[Ca]_o$, and stretching the muscle, were not accompanied with changes in protein kinase or phosphorylase activation indicating that the cAMP-protein kinase system modulates contractile activation but is not germaine to it. In the context of contraction to excitation coupling, in which stretching produces no action potential changes whereas release does, it may be of interest to see whether release would be accompanied by a change in protein kinase activation.

Winegrad & Maclellan (1978) suggested from their evidence from rat myocardium treated with detergent, that non phosphorylated contractile proteins had a high Ca^{2+} sensitivity, e.g. when cAMP was included in the bathing medium - even in the presence of cAMP which phosphorylates the proteins and reduces Ca^{2+} sensitivity. cGMP either acted on the contractile proteins or a cGMP dependent protein kinase. Apparently the effect of detergent in this system was to inactivate nucleotide cyclases in the membrane. These cyclases were closely associated with the phospholipids in the membrane since the addition of these could, for example, lower the calcium sensitivity of the preparation.

Calcium controls the contractile proteins via calcium binding sites: either troponin, as described above, or light chain myosin. Light chain myosin can be phosphorylated by Ca^{2+} dependent protein kinase. When contractility was raised e.g. by adrenaline or Ca^{2+}_{o} the phosphorylation was reduced (Flearson et al, 1976).

In intact hearts a positive correlation between tension and phosphorylation of troponin I exists but the Ca^{2+} sensitivity of troponin I phosphorylation is not clear cut. Concerning this particular phosphorylation of the contractile protein, it appeared that an increase in tension or Ca^{2+} sensitivity was either accompanied by a reduction in phosphorylation (Flearson et al, 1976 for light chain myosin: McClellan & Winegrad, 1978, England, 1976, for ATPase of actomyosin) or no change (Flearson et al, 1976 for troponin). Adrenaline reduced the Ca^{2+} sensitivity of the contractile apparatus (Allen & Blinks,1978). That is, although the evidence is not entirely consistent it appears generally in favour of the proposal that mechanisms which cause phosphorylation of the actomyosin system may reduce Ca^{2+} sensitivity and hence contractile activation.

Thus in summary, cAMP can modify contractile response by:
a) increasing Ca influx with the AP; b) increasing amount of Ca^{2+} bound by the SR; c) affecting Ca^{2+} sensitivity of contractile proteins. It may be worth noting at this stage that the Ca^{2+} activated ATPase of the SR is not the same as the Ca^{2+} activated ATPase of the membrane Ca^{2+} pump

RELAXATION.

Relaxation, as is contraction, is related to the timing of the action potential. Relaxation is 'off' during depolarization and turns 'on' during repolarization. As the membrane repolarizes the sarcoplasmic reticulum rapidly accumulates Ca^{2+}
This process is energy dependent and ATP is hydrolyzed via an ATPase which is Ca^{2+} activated in the sarcoplasmic membranes. (Martones & Ferretos, 1964; Hasselbach & Mackinose, 1963). The sarcoplasmic reticulum has a great affinity for calcium and uses two processes in its uptake. A rapid binding process and a storage process which requires oxalate or phosphate (Katz & Repke, 1968; Ulrich et al 1977). The rapid binding system is adequate to explain the relaxation of cardiac muscle on a beat to beat basis as Ca^{2+} is removed from the sarcoplasm at the rate of about 25 nmol G^{-I} in 100ms (between 50-100 μ mol/kg^{-I} Ca^{2+} is needed for contraction), Oxalate and phosphate follow Ca^{2+} into the vesicles and precipitate when the concentrations exceed the solubility product. The stoichoimetry of the Ca^{2+} transport appears to be 2 mols of Ca^{2+} per carrier thought to be phosphorylated-which then binds Ca^{2+} and translocates Ca^{2+} across the membrane. Once inside the Ca^{2+} is released on dephosphorylation of the carrier. The phosphylated intermediate is regulated by a kinase which in turn is regulated by cyclic AMP. Thus catecholamines accelerate relaxation by activation of adenylcyclase through stimulation of beta receptors, and this raises intracellular cAMP to cause an increased rate of Ca^{2+} uptake. The SR membrane is thus complex and incorporates the receptor protein for kinase

catalyzed phosphorylation as well as the ATPase. After the Ca^{2+} has accumulated in the sarcoplasmic reticulum it is transported to the storage sites concerned in subsequent release of Ca^{2+} during activation.

In amphibian heart relaxation depends little on metabolic energy. What dependence there is,is probably related to Ca^{2+} uptake into the sparse SR via Ca^{2+} dependent ATPase. Relaxation in amphibian heart is mostly via Na-Ca exchange which is 'non-electrically neutral' (i.e membrane potential affects it). (Goto et al, 1972). By contrast relaxation in mammalian heart is not as sensitive to membrane potential as in amphibian heart. Prolonged depolarization produces phasic contraction (Beeler & Reuter, 1970; Morad & Goldman, 1973) with a fairly rapid relaxation phase. The SR is intimately involved since inhibition of Ca^{2+} uptake e.g. caffeine, prolongs relaxation (Blinks et al, 1972). Whilst substances stimulating uptake, e.g.cAMP, accelerates it (Tsien & Weingart, 1976).

ATP AND CONTRACTION

The ATPase enzyme forms an enzyme-substrate-calcium complex at the outer surface of the membrane which is then converted to a phosphorylated intermediate coincident with the translocation of Ca^{2+} from outside to inside of the membrane. As this happens the affinity of this complex for Ca^{2+} dramatically reduces while Mg^{2+} aids its decomposition. There is some evidence, however, for the alternative hypothesis to the above rotary carrier one, and that is that Ca^{2+} goes through a pore into the SR.

There are some similarities between ATPases concerned in ion transport and muscle contraction which may be interesting in the context of contraction-excitation feedback.

Na-K exchange across the sarcolemma, like Ca^{2+} transport across the SR membrane, and contraction of myofibrillis, is linked to the hydrolysis of ATP. The ion dependent ATPase is stimulated by Na_i in the former and $Ca^{2+}{}_i$ in the latter. There are also some similarities between the phosphorylated intermediates in the three enzyme systems and striking similarities in their thermodynamic properties. The actual energy transducing reactions are also similar (Tonomara, 1972).

<u>SUMMARY</u>

Contractile protein activation is brought about by the release of Ca^{2+} ions. This activity can be modified by phosphorylating enzymes which depend on cyclic nucleotides and Ca^{2+}. Removal of Ca^{2+} in mammalian heart by the SR promotes relaxation. A Ca^{2+} pump, depending on metabolism, as well as an Na/Ca exchange serve to reduce $Ca^{2+}{}_i$. In amphibian heart, the time lag between membrane potential change and contractile activity is consistent with activator Ca^{2+} coming from the sarcolemma. The mechanism is probably an $Na^+ - Ca^{2+}$ exchange which depends on Na_o , Na_i and membrane potential. Only if Na-Ca exchange is reduced does the SR play some role. In mammalian heart the SR releases Ca^{2+} at a rate which swamps the Na-Ca exchange. Why then do some agents

cause similar changes in these two types of tissues when the mechanism of activation of contraction and relaxation are so different ? One possible mode of action of these agents could be the cyclic nucleotide - protein kinase system, common to both.

As a taste of things to come concerning the mechanism of contraction-excitation feedback there are basically 3 pathways in the heart, the alteration each of which can give rise to membrane and contractile effects.

a) Change in Ca_i e.g. a reduction. This produces both a reduction in developed tension and a membrane depolarization. The latter may be brought about by an reduced $\dot{i}_K$, and increased $\dot{i}_{ca}$ because of the increased electrochemical gradient.

b) Change in hydrolysis of ATP via ATPase. ATPase activity contributes to the membrane potential via the Na/K and possibly Na/Ca pumps. Speeding the Na/K pump produces a nett extrusion of positive ions and hence a repolarizing current. Contractile events apart from Ca^{2+} involve the actomyosin ATPases whilst relaxation necessitates the removal of Ca^{2+}, aided by Ca^{2+} pumps or exchange mechanisms at the SR membranes.

c) Changes in cyclic nucleotides and cAMP protein kinases. cAMP has been implicated in the Ca^{2+} channels and thus $\dot{i}_{si}$ during the plateau of the action potential. It also has a regulatory function in tension development via actin and myosin interaction as well as Ca^{2+} uptake in the SR.

d) A reduction in binding of Ca^{2+} to TN-C will reduce force but this will tend to increase free $[Ca^{2+}]$ in the sarcoplasm. The increase could produce a membrane depolarization via the electrogenic Na/Ca exchange.

CHAPTER III

INTRODUCTION TO EFFECTS OF ISCHAEMIA ON CARDIAC MUSCLE

CHANGES IN ELECTROPHYSIOLOGY.

Having described the normal electrophysiology for ventricular myocardium as obtained from isolated muscle studies, the different effects of ischaemia on the electrical recordings from the intact ventricle in situ will be included in the following description, as the ischaemic studies in my laboratory were done on this type of preparation.

MEMBRANE POTENTIALS

The mechanism of the changes in electrophysiology with ischaemia are unclear. (See Elharrar & Zipes 1977 for a review). The effect of oxygen deprivation on myocardium depends on whether it is alone or in combination with a reduced blood flow. A decrease in oxygen stops ATP production via the β oxidative pathway of fatty acids and via the Krebs cycle. Glycolysis and glycogenolysis is enhanced. ATP thus comes from creatine phosphate and anaerobic glycolysis. This latter produces pyruvate which must be converted

under the above conditions to lactic acid which accumulates both intra and extracellularly resulting in acidosis. The energetic changes presumably affect ionic flux, for example through the ATPase Na^+ / K^+ pump, and the cell gains Na^+ and loses K^+. The precise relationships however remain concealed. Ischaemia thus produces it electrophysiological change via a combination of factors including; hypoxia, extracellular and intracellular acidosis, increased extracellular K^+ and other products of anaerobic metabolism which leak out of the cell.

A shortening in the duration of the plateau phase of the intracellular action potential with ischaemia or oxygen deprivation has been well described (Samson & Sher 1960; Kardesch et al 1958).
The resting potential also decreases ie. becomes less negative (Kardesch et al 1958) Together with these changes the upstroke velocity and amplitude of the action potential is reduced. The conduction velocity also decreased and there are changes in refractory period. The latter may actually prolong to greater values than control despite the reduced action potential duration. This post repolarization refractoiness leads to; alteration of low and high amplitude action potentials, 2:1 responses, and eventual total unresponsiveness. Extracellular studies have shown a reduction in refractory period but this can be explained by the fact that there is often a "dispersion of refractoriness" and, with the extracellular technique, the cells with the shortest refractory period would respond to a stimulus.

Hypoxia has different effects on myocardium to ischaemia. Although the action potential duration and amplitude may be reduced it takes hours to reduce the resting membrane potential. It has been

suggested that this observation is due to suboptimal but continuing ATP production, which together with the gain in intracellular Na^+ , keeps the Na^+- K^+ pump functional (McDonald & MacLeod, 1973) and this maintains the resting potential. The reduction in action potential duration has been shown to be related to a reduced inward Ca^{2+} current but it can also be due to an increase in outward K^+ current(Corabeouf et al 1976). However, hypoxia, with its concommitant changes cannot simulate the changes produced by ischaemia including the production of arrhythmias. Ischaemia, which is the situation studied in this thesis, caused hypoxia together with a reduction in nutrients, and an accumulation of metabolic byproducts. Potassium has received a great deal of attention in this context since it is rapidly lost from ischaemic myocardium and appears to be related to arrhythmia production (Harris 1966; Logic 1972). The precise relationship is not clear, as superfusing myocardium with venous blood containing about 9 mEq/I K^+ did not produce the same electro physiological changes as superfusion with blood from the ischaemic myocardium containing the same amount of K^+. (Downer et al 1977)

The role of acidosis in ischaemia is also difficult to define. An extracellular acidosis does not necessary indicate the intracellular pH, which may be lower. In addition extracellular acidosis can produce variable effects on action potential duration (Corabeouf et al 1976) and can even prolong it. In fact, when pH, K^+ and CO_2 in venous blood are appropriately combined and used to superfuse myocardium, it fails to mimic the electrophysiological effects of ischaemia. Clearly additional factors are responsible.

An action potential primarily due to the operation of the slow Ca^{2+} and Na^{2+} channels can be seen under certain condition and is worth briefly mentioning again (cf Chapter II). This "slow response" may be observed for example after raising K^{+} to 22mEq/L and the simultaneous addition of isoproterenol; or superfusion with Na^{+} - free, Ca^{2+} rich solutions. One can visualise the former type of situation arising during ischaemia. This action potential (Cranefield 1975; Wit & Friedman, 1975) has a low resting potential, small overshoot,and upstroke velocity which is less than 20 Vs^{-1} In addition the threshold for excitation and post repolarization refractioness is high. Automaticity can occur if the slow response occurs in the Ca^{2+} rich medium but if the K^{+} increases automaticity is abolished (Wiggins & Cranefield, 1976). As has been suggested above ischaemia may in fact shut the slow channels off: thus for the slow response to occur, a particular local environment has to obtain.

CHANGES IN CONDUTION AND EXCITABILITY

The consequences of the changes in action potential, just described, on other electrophysiological measurements are predictable and have been by and large experimentally verified. However there are some curious biphasic electrophysiological changes that can follow immediately on experimental occlusion. Studies in conduction in ventricular myocardium during ischaemia show an initial transient increase in conduction velocity (Gambetta & Childers, 1969; Holland & Brooks, 1976) before being followed by a reduction. The time course in excitability threshold is also biphasic and shows a decrease lasting a few minutes, followed by a progressive increase in the threshold (Brooks et al 1960; Tsuchida 1965 & Elharrar et al 1977).

The refractory periods are shortened (Lazarra et al 1975). Some studies, in contrast, have shown an increase in the refractory period (Elharrar et al 1977). In general, using bipolar electrograms, there is evidence of desynchronization of activity (Waldo & Kaizer 1973: Williams et al 1974). In fact some areas respond with a delay which is long enough to then reactivate adjacent recovered areas (Sherlag et al 1974). These then result in re-entrant beats.

Transmembrane potentials recorded from Purkinje fibres excised at various times after the onset of ischaemia show that electrophysiological changes do not occur until after approximately 20 min. (Friedman et al 1973; Lazarra et al 1974). It is interesting that severe rhythm disturbance can be seen during this period (Ten Eick et al 1976). At about 30 minutes (Lazarra et al 1974), the action potential duration is reduced and the diastolic depolarization disappears. Some workers (Friedman et al 1973) have found that 24 hours after coronary occlusion the Purkinje fibres just beneath the endocardium can survive the ischaemia. In the ischaemic zone the resting potential, action potential amplitude and upstroke velocity are all reduced. However the diastolic depolarization and action potential itself are prolonged. Ten days after occlusion subendocardial Purkinje fibre activity in the infarct area is remarkably similar to that expected in normal areas (Lazarra et al 1974).

RELATIONSHIP BETWEEN EPICARDIAL ELECTROCARDIOGRAM AND ACTION POTENTIAL DURING ISCHAEMIA

In the context of this introduction only the epicardial electrogram will be discussed and no attempt will be made to describe in detail potential changes in a volume conductor. The conventional AC coupled ECG amplifiers show characteristic S-T segment changes in recordings made from areas near or over ischaemic myocardium. Detailed study with D C coupled amplifiers have shown how these changes are related to transmembrane action potentials. Unipolar epicardial recordings over the ischaemic area demonstrate elevation of the S-T segment and an increase in the R-wave (Rakita et al 1954). S-T segment changes are thought to be due to currents of injury flowing across boundaries between ischaemic and normal areas. There is a potential difference across the boundary, and there are relatively low resistance pathways between the zones. As described above, during diastole ischaemic areas contain cells which are depolarized with respect to normal zones. Current, positive by convention, flows from normal to ischaemic areas. The unipolar epicardial electrogram records a potential which is negative to ground during this diastolic period and thus the recording shows a T-Q depression. (Samson & Sher 1960). During activity the ischaemic action potential duration is abbreviated. Thus in contrast to the resting period, the intracellular potentials of ischaemic cells are now more negative than the normal cells (Fig. III - 1). The flow of current between the areas is opposite to that during rest and the unipolar epicardial ECG electrode shows a potential positive to ground and thus an S-T elevation. The AC coupled, conventional ECG would simply show a raised S-T segment elevation which is a combination of the true S-T segment elevation and T-Q depression.

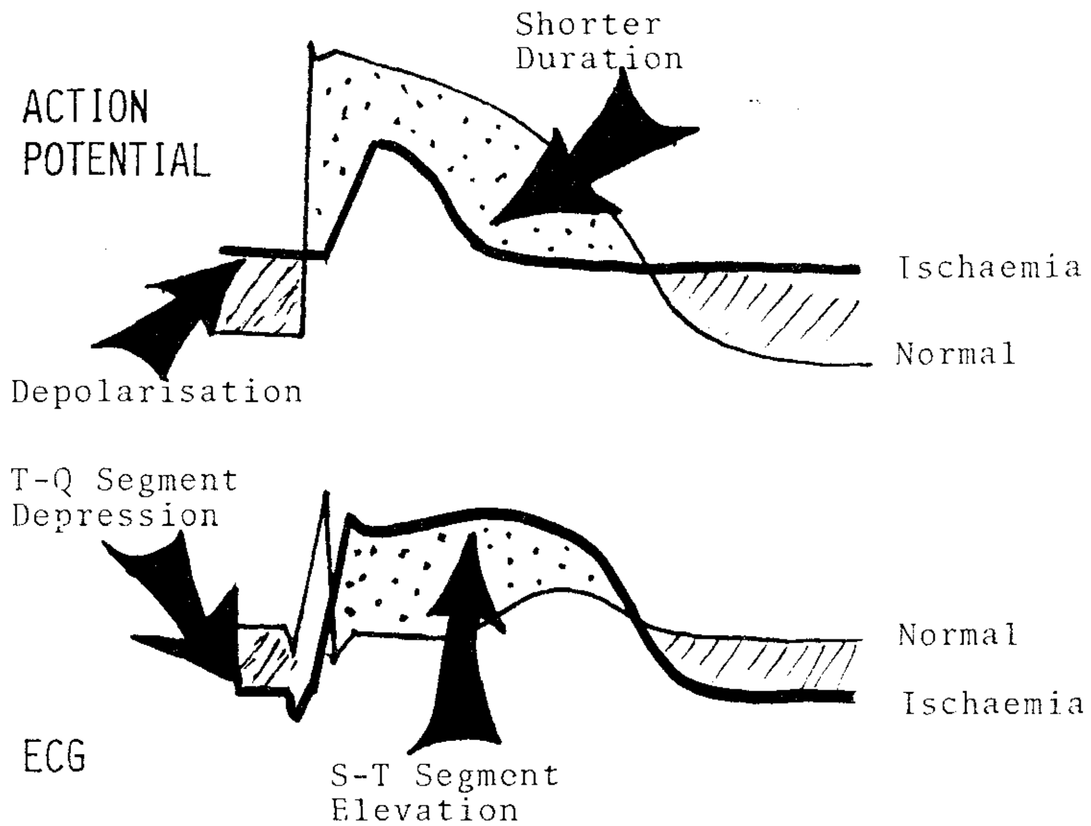


Fig III-1 Diagram of action potentials and ECG under normal and ischaemic conditions

With ischaemia depolarisation of the membrane at rest produces a current flow that causes a T-Q depression in the ECG. A shortening of the action potential duration gives rise to an opposite direction of current flow manifesting in the true S-T segment elevation. The overall change in the ECG is described as an S-T segment elevation

More recent studies indicated that T-Q depression provides the predominant contribution to the apparent S-T elevation during ischaemia. It has been suggested that S-T segment elevation occurs within seconds of the coronary occlusion and could result from inhibition of the active transport of K^+ with accummulation of K^+ in the transverse tubules. This suggestion is not in keeping with the observations of Downer et al (1977) who could not mimic this situation by perfusion with venous blood containing K^+ in the appropriate concentrations (see above).

THE GENERATION OF VENTRICULAR ARRHYTHMIAS IN MYOCARDIAL ISCHAEMIA.

During experimental and transient myocardial ischaemia three phases of arrhythmias may be found: Early and late phases, and a phase immediately on reperfussion. The mechanisms behind the initiation of the arrhythmia in each of the phases may invoke all or one of the following explanations: alterations in impulse conduction and propagation (re-entry), alterations in automaticity and alterations in excitability. These phases and mechanisms have been briefly reviewed by Arnsdorf (1977), Bigger et al(1977) Lazzara et al(1978):Cranefield & Wit(1979). The latter authors provide several references to further reviews.

REENTRY as the name implies is when an impulse re-enters an ischaemic area to produce a propagated action potential. A pre-requisite is the existence of at least one area of myocardium displaying a complete conduction block in the normally conducting antegrade direction (Fig III-2) but a partial conduction defect with a delayed conduction

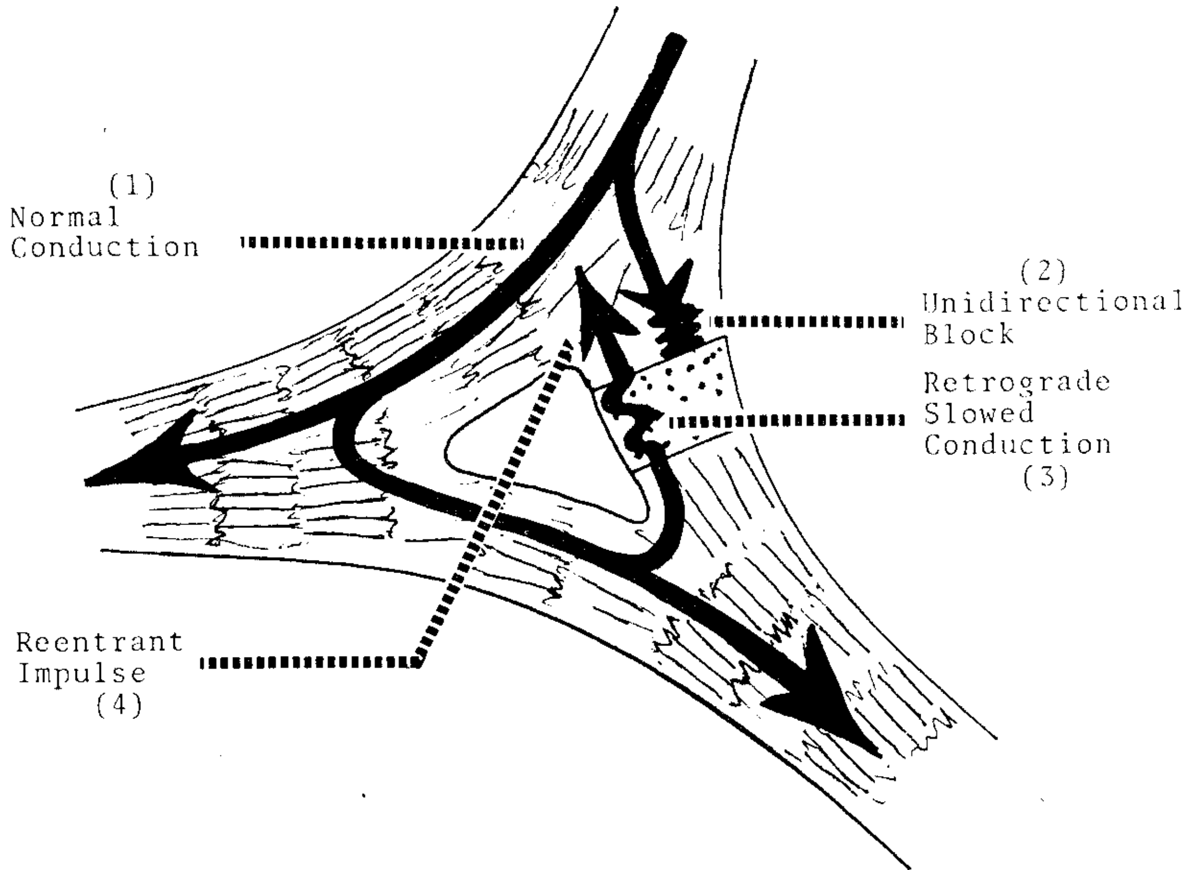


Fig. III-2 Diagram of Reentry pathway

The impulse reaches two limbs of a branch in conducting tissue, conducts normally down the left branch (1) but is blocked in the right one (2). The normally conducting impulse invades the right branch retrogradely and traverses the damaged tissue (4) which allows slow conduction in this abnormal direction. By this time the muscle is no longer refractory and is stimulated by the emerging impulse (4)

velocity in the other direction. Consider normal conduction into a branching network of myocardium with this type of abnormal electrical situation adjacent to normal myocardium. A normal impulse passes down one arm of a branch but there is no propagation in the other arm due to the presence of the area of antegrade, undirectional block. The impulse spreads distally down, then back to the affected area through branching myocardium capable of normal conduction. This is because the normal tissue has not been rendered refractory as the normal impulse has not reached it through the block. Retrograde conduction through the block is possible but at a much reduced velocity. By the time the impulse traverses the block the normally conducting muscle on the other side is no longer refractory and has recovered enough to respond to the re-entering impulse - giving rise to an extrasystole. Clearly several factors influence the functional pathology of the re-entry loop: the conduction velocity , refractory period and length of the pathway in the normal myocardium; the dimensions and conduction velocity of the abnormal area. Permutations of these factors can produce different re-entry pathways.

The explanation of the depressed conduction rests on membrane factors that result in a slow upstroke of the action potential (Arnsdorf,1977). The upstroke, and action potential, as explained in the previous section (Fig. II-1) is the result of the flow of ions (I) which depend on the electrochemical gradient (V-E) and the membrane conductance (G) for the ion (a).

$I_a = G_a \ (V_m - I_a)$ - where V_m is membrane potential.

For the total current flow across the membrane:

$$I = G_k(V_m-E_k)+G_{Na}(V_m-E_{Na})+G_{si}(V_m-E_{si})+(V_m-E_x)$$

Where K is potassium:Na is the sodium ion: si is the ion carrying the slow inward current - predominantly Ca^{2+}: and x is any other ion - for example the unknown ion carrying the transient inward current during digitalis intoxication (Lederer & Tsien, 1978).

The current concerned in the upstroke may be carried by Na^+ or Ca^{2+}, the latter normally in sino-atrial and atrio-ventricular nodes, and in partially depolarized fibres. Maximum G_{Na} may be reduced during ischaemia due to several factors. The driving force is reduced, the 'on-gate' is only partially open due to incomplete activation of the m variable, the h value is reduced and thus the 'off-gate' not completely closed. Associated with this set of factors ie.impaired steady and non-steady state reactivation,conduction velocity is reduced. A low resting membrane potential with the depressed conduction, may also be produced by a failure to repolarize due to an inability to turn I_{si} off or I_k on i.e. no regenerative repolarization takes place. Ischaemia, drugs and the potassium ion can produce the foregoing sets of circumstances (Arnsdorf, 1977 ; Cranefield & Wit, 1979)

Treatment of arrhythmia due to re-entry is aimed at the cause of ischaemia, affecting the conduction velocity of the depressed area, or converting the unidirectional block into a bidirectional one. The latter abolishes the re-entry loop and can be achieved by

suppressing the Na^+ system further (quinidine, procaine amide, propanalol, lidocaine) and by suppression of I_{si}. Electroversion may, in addition to massive depolarization of the myocardium, release intracellular K^+ into the extracellular compartment and cause further depolarization. Alteration of the refactory period of the normal myocardium and its relation to the re-entry impulse may also be an effective form of treatment. This can be achieved by delaying the reactivation of the the Na^+ system (lidocaine) or prolonging the effective refractory period (quinidine, procaine amide).

The electrophysiological changes with ischaemia or anoxia may be observed in isolated preparations but are implied in the intact heart by the character of extracellular epicardial recordings (cf Cranefield & Wit 1979 for references). Electrograms show fragmentation at which time extrasystoles can occur. This activity is an infarct area can be continuous indicating multiple re-entry pathways.

AUTOMATICITY (ALTERED AUTOMATICITY). An ectopic automatic focus, if faster than the normal focus, can drive the heart while suppressing the normal focus. This abnormal focus must, by definition, at least occasionally discharge spontaneously. The diastolic depolarization preceding spontaneous discharge of a myocardial cell depends on the balance between depolarizing Na^+ and Ca^{2+} currents and repolarizing K^+ currents. For depolarization either current may be constant while the other changes in the appropriate direction.

A slow diastolic depolarization may result in accommodation and partial inactivation of $\dot{i}_{Na}$ and the phase 0 spike is caused by $\dot{i}_{Ca}$ ($\dot{i}_{si}$).

Catecholamines increase the rate of diastolic depolarization by binding to receptors which increase adenylate cyclase activity. C-AMP thus increases to activate protein kinase which phosphorylates a site near a channel which regulatss $[Ca]_i$ distribution and hence accelerates $\dot{i}_{k2}$ deactivation (see Arnsdorf, 1977). $\dot{i}_{si}$ activation may also be enhanced by catecholamines and produces repetitive activity. However, previously injured myocardium may be hyperpolarized by catecholamines and allow $\dot{i}_{Na}$ to produce the spike depolarization rather than $\dot{i}_{si}$, and break a re-entry loop.

Hypokalaemia allows the maximum diastolic potential to approach the threshold voltage while the membrane resistance increases. This results in increased automaticity. Hyperkalaemia depolarizes the membrane, thus inactivating the Na system, and decreases membrane resistance. This increases the current necessary to attain threshold and thus automaticity is reduced. In this situation however, catecholamines could enhance $\dot{i}_{si}$ to produce slow response automaticity.

Phase 4 depolarization may be decreased by lidocaine which increases the outward K^+ current as does diphenylhydantoin. Procaine amide decreases the inward depolarizing current and quinidine has a similar action.

TRIGGERED ACTIVITY. This particular type of initiation of ischaemic arrhythmia is relevant to the arrhythimia generated by the new mechanism discussed in this thesis. A cell demonstrating triggered activity needs to be driven by an after-depolarization or a local or distant action potential. Acetylstophanthidin can cause a delayed after-depolarization in Purkinje fibres which can trigger a burst of electrical activity (Cranefield & Wit, 1979). This after-depolarization may be increased by: increasing stimulation rate, decreasing the membrane potential, increasing $[Ca]_o$, decreasing $[K]_o$. Verapamil depresses the after-depolarization but this has not proved that the $\dot{i}_{Ti}$ (Transient inward current) is carried by Ca^{2+}. Weingart et al(1977) suggest that $\dot{i}_k$ is affected by Ca^{2+} released from internal stores. Catecholamines can also cause the appearance of delayed after-depolarizations in some experimental preparations.

Triggered myocardial cells can become regularly excited without becoming repetitive. Each action potential is derived from the after-depolarization following the preceding normally attained action potential. Rhythmic activity is consequent on the trains of after-depolarizations following the non-driven action potentials derived from these after-depolarizations.

It is difficult to differentiate between re-entry and triggered activity in an ectopic focus (Cranefield, 1975). For circus movement a particular sequence is expected and interventions enhance, retard or abolish circus movement in the expected sequence. Persistent activity if the loop is cut suggests but does not

confirm triggered activity: the loops could be very small. It is not possible to differentiate between activity and re-entry in the intact heart with any confidence. A premature beat for example can begin and terminate trains of impulses, in a triggered focus and a re-entry loop.

Probably the main difficulty in attributing extrasystoles to re-entry is that the impulse must travel very slowly or around a long return path if conduction is only partly depressed. During this period, tantamount to the total refractory period, the path must be shielded from the rest of the heart in order to re-excite it at the end of this period. The time of occurence of a late after-depolarization ensures that this problem is not presented.

The mechanisms of the delayed after-depolarization are unknown. The following observations limit the number of interpretations possible.

1. They may arise from various levels of transmembrane potential.
2. They are somehow related to the previous action potential.
3. Their amplitude is enhanced by:
    a. catecholamines.
    b. Increased frequency of drive rate.
    c. increasing prematurity of the precipitating impulse.
    d. a single beat rather than a train of impulses.
4. Their amplitude is reduced by:
    a. Ca^{2+} blockers.
    b. electrogenic Na^+ extrusion) more - ve membrane P.D.
    c. increased K^+ conductance)

5. They are affected by interventions that influence contractility in parallel. (cf. 3 & 4). Thus a premature beat produces a large after-depolarization as well as a rise in force, of the next contraction.

These last observations suggest that a change in Ca_i^{2+} is implicated. The change could be via an inward Ca^{2+} current or some other cause of an increase of Ca_i^{2+}. This would decrease an outward K^+ current to cause the depolarization.

THE PHASES OF ARRHYTHMIA. Experimentally an early phase of ventricular arrhythmias begins almost immediately on coronary occlusion and lasts a few hours (Harris, 1950). It may correspond clinically to the prehospital phase. The mechanism may either be re-entry, automaticity or triggered activity. After a period of quiescence a later phase of arrhythmias appears about 8 hours after coronary occlusion and lasts for 24-48h. This phase may be related to metabolic and electrical changes in subendocardial Purkinje fibres. These fibres appear resistant to ischaemic damage perhaps due to their proximity to the ventricular cavity. As intracellular lipid accumulates the action potential takes on the characteristics of a slow response, except the duration is remarkably prolonged. (Wit & Friedman, 1975; Friedman et al, 1973). These fibres also show diastolic depolarizations and hence altered automaticity. However the prolonged depolarization provides a background for re-entrant loops when in the presence of other action potentials having a reduced duration and in the presence of depressed conduction.

A further phase of arrhythmias accom panies release of coronary occlusion (Tennant & Wiggers, 1935). The onset is almost immediately the artery is released (Stephenson 1960) and may occur without preceding venticular premature beats. The mechanism is uncertain but would presumably involve one or all of the three discussed above.

The arrhythmias found in the prehospital phase of clinical acute myocardial infarction may thus be due to the early phase of arrhythmia or conceivably a release of acute coronary spasm.

CONTRACTILE FAILURE DURING ISCHAEMIA

Systolic bulging of the ischaemic segment, reported as far back as 1935 (Tennant & Wiggers) is a consistent observation in the present studies. This ballooning is due to a reduction in myocardial segment contractility and is related to the ischaemia. Despite the understanding of the contractile process as outlined earlier, the mechanism of ischaemically induced failure of contraction remain a mystery. Clearly interruption of blood supply leads to a drastic reduction in myocardial oxygen tension (Sayan et al 1958) which halts aerobic energy production and results in intracellular acidosis. Although a fall in intracellular pH must contribute to the drop in contractility the voluminous literature will not be presented here. Instead some interesting conceps on metabolic causes of early "pump" failure will be discussed.

ATP DEPLETION AND REGULATION OF CONTRACTILITY

The cessation of oxidative phosphorylation causes an immediate reduction in phosphorcreatine content but only a small decrease in adenosine triphosphate (ATP) content - yet contractility decreases dramatically (Katz 1968). However since phosphocreatine can only transfer high energy phosphate via ATP it is possible that there is still an ATP lack which is necessary for some critical reaction. Thus despite the observation that ATP content is still high enough to saturate many of the energy consuming process concerned in the regulation of contractility, it is possible that highly local concentrations of ATP may be inadequate. There is evidence to show that ATP levels can drop in discrete compartments in ischaemic myocardium. Thus the ATP content immediately after coronary occlusion is more than 3 mols G^{-1} wet weight. If uniformly distributed the ATP concentration would be over 4 mmol L^{-1} Enough to saturate the ATP binding sites.

Phosphocreatine (PC) is formed and degraded via a reaction with ATP and creatine (C).

The relationship between these compounds is governed by equilibrium for the enzyme creatine kinase. The reaction has a mass action ratio:

$$\frac{[PC]}{[C]} \frac{[ADP]}{[ATP]} = K = 0.01 \quad \text{(Illingworth et al 1975)}$$

However in vivo under aerobic conditions the mass action ratio is

far from 0.01. This discrepancy is not due to difference in creatine kinase because under anoxic (or ischaemic) conditons, when PC decreases to low levels the ratio reaches the value predicted by the latter equation. The above finding supports the notion that ATP is compartmentalised. Further support is in the finding that cell phosphocreatine content can change independently of the rate of change of ATP content - for example during the metabolic response to changing levels of cardiac work. The observation that PC falls faster than ATP during ischaemia could be explained if only some of the cellular ATP is in equilibrium with much of the cell's PC in a discrete compartment: and the ATP in this compartment falls with ischaemia.

A reduction in ATP concentration within a critical compartment, however, cannot entirely explain the early pump failure is ischaemia. Lack of ATP for energy consuming processes in regulation of contraction would cause rigor rather than reduction in contractility (Katz 1970; 1977). For example substrate lack could inhibit the calcium pump in the sarcoplasmic reticulum, leading to rigor due to an increased sarcoplasmic calcium. Inhibition of the sodium pump would also lead to increased intracellular Ca^{2+} due to the increased Na^{+} just beneath the sarcolemma, coupled with a decreased Ca^{2+}flux(Langer, 1972). Finally mitochondrial Ca^{2+} uptake may also be inhibited.

ATP DEPLETION AND POSSIBLE MODULATORY FUNCTION

In addition to the foregoing regulation of contraction, ATP may exert a modulatory function on some myocardial processes. Thus apart from its modulation of energy metabolisum such as regulation of the enzyme phosphofructokinase, ATP can also affect passive ionic flux. For example potassium - potassium exchange in the erythrocyte which is dependent of energy mediated chemical reactions (Simmonds 1975). Similarly, in the squid axon, ATP can promote sodium - calcium exchange (Dipolo 1974; Blaustein 1976). What is interesting here is that these modulatory actions of ATP require levels of this nucleotide to be O.I to I.0 mmol - L (Dipolo 1974) which is higher than the levels mentioned previously for saturating the substrate binding sites for energy consuming reactions.

It is thus conceivable that the modulatory effects of ATP may contribute to the early pump failure observed in ischaemia. This is further supported by the observations that metabolic poisons reduce the slow channel entry of Ca^{2+} into the heart (Schneider & Sperelakis 1974; Koldardt & Kubler, 1975) and that passive Ca^{2+} efflux from the sarcoplasmic reticulum (SR) is greatly reduced in the absence of ATP (Katz et al 19). Clearly the modulatory role of ATP in the reduction of contractility with ischaemia needs further investigation.

PHOSPHATE ACCUMULATION AND ISCHAEMIA It has been suggested (Kubler & Katz 1977) that the rapid reduction of contraction with ischaemia arises from increased intracellular phosphate levels which would occur with the interruption of myocardial blood supply. The rise in phosphate would sequester calcium making it unavailable for excitation-contraction coupling. Inorganic phosphate can cause a relatively small fall in high energy phosphate to have a large influence on other metabolic influences (Kubler & Katz 1977): a type of amplification. An (estimated) eight fold rise in phosphate in the cytosol to about 20 mmol L^{-1} could have the following effects in ischaemic mycordium: a stimulation of glycolytic flux (Neely & Morgan, 1974). and a Ca^{2+} precipitation, particularly in the sarcoplasmic reticulum. Here the pH is about 7.0, phosphate exists as HPO_4^{2-} and there are relatively large amounts of Ca^{2+} - a 3000 Ca^{2+} concentration ratio across the S.R. membrane. Thus a calcium phosphate precipitate forms where ever Ca^{2+} attains a level of about 88 mM and phosphate, which readily diffuses across membranes, reaches it. Calcium could be trapped in the mitochondria as well, thus the Ca^{2+} availability for contraction would be markedly reduced and its distribution changed while the total Ca^{2+} content of the myocardium remains the same.

It thus appears that ATP depletion cannot explain the rapid contractile failure during ischaemia on the basis of the reactions requiring energy consumption, for two reasons: First the ATP affinity of these reactions is high and the total content of ATP is enough to saturate the substrate binding sites. Secondly, the lack of chemical energy for contractile protein and ion pumps would promote rigor rather than reduced contractility. It seems reasonable to suggest that if ATP has an effect it is via its modulating action which requires a higher concentration of ATP and would reduce both Ca^{2+} entry into the cell as well as Ca^{2+} efflux from the S.R. The interesting possibility also exists that raised intracellularly phosphate would precipitate contractile dependent Ca^{2+} and thus also reduce contractility. One's own feeling from the available evidence is that contractile dependent Ca^{2+} is the final cause of the rapid decline in contraction with ischaemia: either because its precipitously reduced or because its action is blocked.

CHAPTER IV

STUDIES IN ISOLATED STRIPS OF MUSCLE

THE DEMONSTRATION OF CONTRACTION EXCITATION FEEDBACK IN CAT PAPILLARY MUSCLE

METHOD

Experimental preparation

Male and female adult cats weighing 1.5-2 Kg. were sacrificed while under light ether anaesthesia. Thehearts were rapidly excised and transferred to a chamber containing oxygenated Tyrode solution. After opening the right ventricle along the anterior border of the ventricular septum papillary muscles of about 6 to 10 mm. long and 0.6mm. in diameter were dissected including a large part of their attachemnt to the ventricular wall. The muscles selected were uniformly cylindrical without any branching.

The Tyrode solution used in these experiments had the following composition (mM): NaCl 136; KCl 2.68: $NaHCO_3$ 11.9; $CaCl_2$ 2.5; NaH_2PO_4 4.2; glucose 10.0; EDTA 2×10^{-5}; $MgCl_2$ 1.0. EdTA was added to form a complex with traces of heavy metal such as Cu^{2+} or Zn^{2+} which may have a deleterious effect on the long term contractile performance, even if present in sub-ppm concentrations. The Tyrode solution was bubbled with carbogen (95% O_2 + 5% CO_2); temperature was kept constant at 31° C and pH at 7.39 ± 0.01.

The need for simultaneous recording of both mechanical and electrical events from small cardiac muscle preparations excludes the use of experimental arrangements usully designed for the analysis of muscle mechanics. As the microelectrodes must be freely suspended in order to follow displacements of the contracting muscle, the preparation has to be horizontal. Sources of additional mechanical vibration such as the switching of mechanical stops, loading the isotonic lever by airstream and manipulation in close proximity to the muscle chamber must be minimised. Therefore to meet these requirements special recording systems had to be constructed.

Muscle Chamber for microelectrode recordings.

The first type of muscle chamber was an open acrylic plastic one measuring 35 mm long, 8 mm deep, and 6 mm wide. The chamber was perfused with oxygenated Tyrode solution at 31° C and the rate of perfusion adjusted to be about 15 ml min^{-1}. Twice threshold stimuli were applied via Ag/AgCl electrodes parallel to the muscle.Micro electrodes (tip diameter less than 1 μ) were filled with 3-molar KCl solution and freely suspended on the end of a thin silver wire which was attached via a connector to the lever of a micromanipulator. The resistance of the microelectrodes used was in the range of 10-30 MOhm. It was empirically found that a particular relationship between the weight of the microelectrodes and the free length of the suspending wire gave the best results as far as artefacts produced by mechanical oscillations of the microelectrode-wire system were

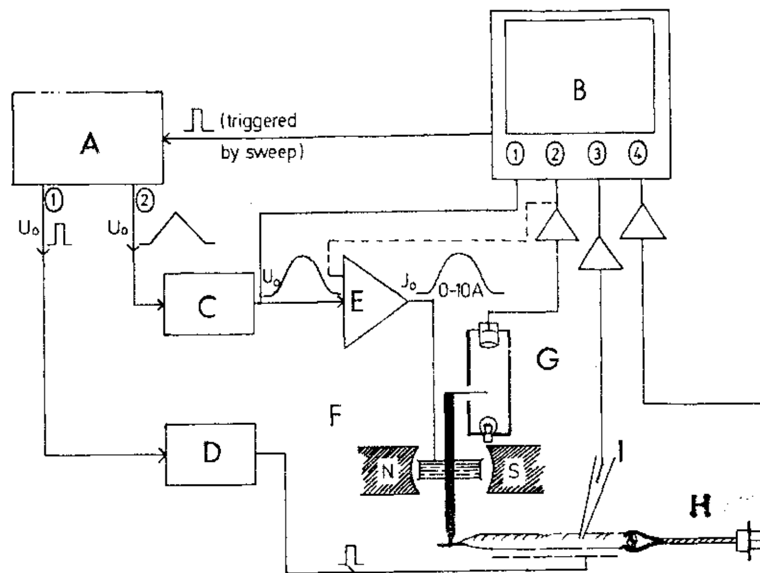


Figure IV-1

Diagram of mechanical and electrical recording and control system.

(See text for further explanation).

A. Double pulse generator

B. Oscilloscope

C. Function generator

D. Stimulator

E. Current amplifier

F. Galvanometer

G. Length transducer

H. Force transducer

I. Microelectrode.

concerned. Even then the ratio between impalements and successful recordings was about 60:1. Conventional high impedance input preamplifier (electrometer valves or FETs) were used for the potential recordings.

Force Transducers.

During the first part of the experiments tension measurements were made with RCA 5734 transducer valves. The transducer pin was extended by a 10 mm long stainless steel tube to which a thin piece of glass tubing was glued, in parallel. The proximal part of the glass tube was connected to a suction pump. The lower end of the papillary muscle was thus fixed to the distal orifice of the glass tube by the negative pressure. The compliance at the tip of the extension lever was about 0.02 mmg^{-1}. The transducer tube was mounted so that the tip of the extension reached the middle of the chamber without obstructing the field of view or the microelectrode.

In some of the investigations, when stretch experiments were performed, the displacement of the extended transducer pin contributed excessively to the series elasticity of the system. A new force transducer was thus contructed using a pressure sensitive transistor -('Pitran'). For our purposes this transducer had no mechanical displacement, yet had the advantage of deliving an output signal of about 0.5 V/g.

Isotonic Lever Displacement Transducer and Load Generator. (Fig.IV-1)

This system was made of a coil-type galvanometer in which the reset spring was detached and the indicator replaced by an aluminium lever about 25 mm. long. The tip of this lever consisted of a flattened forceps-like shaft 8 mm. long which was directed downwards at right angles to the moving plane of the lever. This shaft was immersed in the perfusion chamber and clamped the tendinous end of the preparation. A controlled d.c current flowing through the coil of the galvanometer generated a force of up to 10 g at the tip of the lever. This force was seen by the muscle as a load. Adjustable screws allowed us to predetermine the initial and final muscle lengths. The displacement of a 15 mm. rear extension of the lever varied the amount of light falling on a photo-diode and provided an electrical signal proportional to length. The output of the system was linear over about 3.5 mm movement at the tip of the lever. The equivalent mass of the movable system was about 200 mg.

Load Control Unit

With the device described above changes in load such as quick-stretch or quick-release, constant or varied afterloading, and controlled stretch could be imposed by means of a load control unit. This unit consisted of a d.c. amplifier with an output of 0-6 amps through the 1 Ohm resistance of the coil. The output of the amplifier was manually controlled by a potentiometer calibrated in grams. A voltage signal was fed into the unit to control the time course of the load seen by the muscle. It was also possible to fix the

initial muscle length using negative feedback from the length transducer to the load control unit. The muscle length could be electrically adjusted by the balance potentiometer in the differential pre-amplifier of the unit. Here the force transducer could be omitted since the force developed by the muscle was represented by the time course of the load generating current and could be taken as the isometric tension curve. This method was a mechanical analogue of the voltage clamp technique used by electrophysiologists.

In the muscle chamber the initial muscle length was adjusted by a standard preload of 0.007 Nmm^{-2}. The average muscle length attained with this preload corresponded to the length at which maximum tension was developed (L_{max}) in most cases. However, since L_{max} was not determined in each preparation, the initial muscle length was denoted by L_i.

The muscles were equilibrated in the chamber for at least one hour. During this time they were stimulated at 24 min^{-1} and allowed to shorten isotonically against the small load (0.3 g mm^{-2}) Only those preparations which did not show spontaneous activity and reached an isometric peak tension of at least 3 g mm^{-2} were used.

Muscle chamber for Sucrose gap recordings (Fig. IV-2)

The second system for recording potentials was a sucrose gap method which necessitated a different chamber and mechanical recording system. This muscle chamber basically consisted of two compartments separated by a thin paraffin

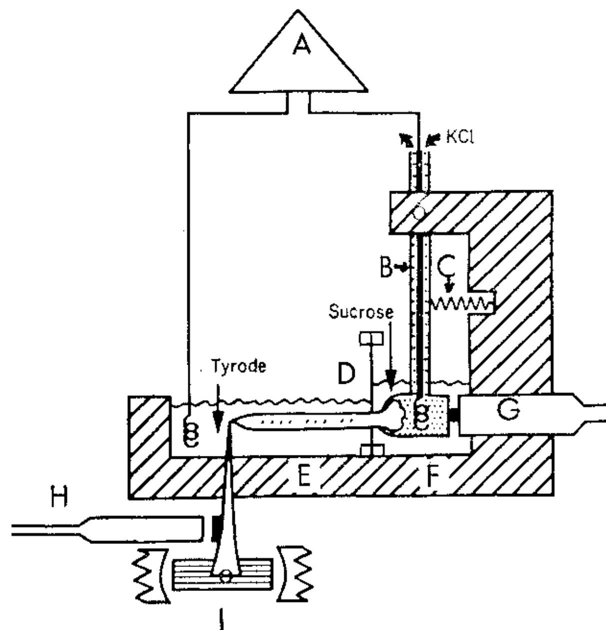


Figure IV-2

Schematic representation of the sucrose gap recording chamber.

A. Differential amplifier

B. Isometric lever (KCl perfusing through)

C. Spring

D. Parafin film separating chamber into 2 compartments:-

E. Left compartment (with Tyrodes)

F. Right compartment (with Sucrose)

G. Force transducer

H. Length transducer

I. Isotonic lever (part of galvanometer)

membrane. Whilst the left compartment - similar in shape to the bath described above-was perfused with Tyrode solution a flow of isotonic sucrose solution was maintained through the right compartment. The end of an isometric lever, holding the muscle, consisted of a small cylindrical acrylic chamber through which isotonic KCl was perfused. After dissection of the muscle it was mounted by pulling it from the inside of the KCl chamber through a tightly fitting hole until the excised portion of the ventricular wall was "wedged" in the hole, providing a firm attachment for the muscle. The lever supporting the muscle was then submerged in the sucrose compartment and pressed against the surface of a force transducer (Statham MP15) by means of an adjustable spring. The free end of the muscle was pulled through a second hole in the paraffin membrane separating the sucrose from the Tyrode compartment. This arrangement allowed an average muscle 6 to 8 mm. long to be perfused with Tyrode solution over about 90% of its length whilst a sucrose flow extended over a gap region of about 0.5 to 1.0 mm. Voltage reference was at the excised ventricular wall in the KCl compartment. The tendinous end of the muscle was fixed to the isotonic lever. This lever system was similar to the galvanometer modification above and a controlled d.c. current through the coil generated a force up to 0.1 Nmm^{-2}. The membrane potentials measured by the sucrose gap were 70 to 90 mV, and a stable action potential (AP) could be recorded for periods of up to 2 h. Whilst this technique allowed the recording of the integrated electrical activity of the muscle continuously without the disadvantages encountered with the micropipette technique. some fidelity was lost in the mechanical recordings: the sucrose gap,unavoidably,

introduced a series elasticity by the inactivated part of the muscle exposed to the sucrose solution. True isometric conditions were thus unobtainable. In consequence the sucrose gap was kept as small as was necessary to obtain AP recordings of at least 80 mV in amplitude.

Displacement transducer for use with "gap" recordings.

In this sucrose gap system muscle displacement was sensed by an inductive displacement transducer (Hugo Sachs Electronics,Hugstetten, Germany) and consisted of a small coil around a ferromagnetic core. This coil (inductance of about 670 μH) formed part of an oscillator circuit (250 kHz). Changes of coil inductance were brought about by a small aluminium disc attached to the electromagnetic lever system. Displacement-induced changes of the oscillator frequency were converted into voltage changes which were electrically linear within 1% over more than 4 mm displacement at the tip of the isotonic lever.

RESULTS.

The ACTION potential duration as dependent on the mode of contraction.

An experiment similar to that performed by Parmley, Brutsaert, and Sonnenblick (1969) is shown in Fig. IV-3. This figure contains consecutive traces of the mechanical activity of an isolated papillary muscle stimulated at 24/min. In part (A) the muscle first shortened isotonically against a load of 0.5 g mm^{-2} and is subsequently made to contract isometrically at the same initial muscle length. During

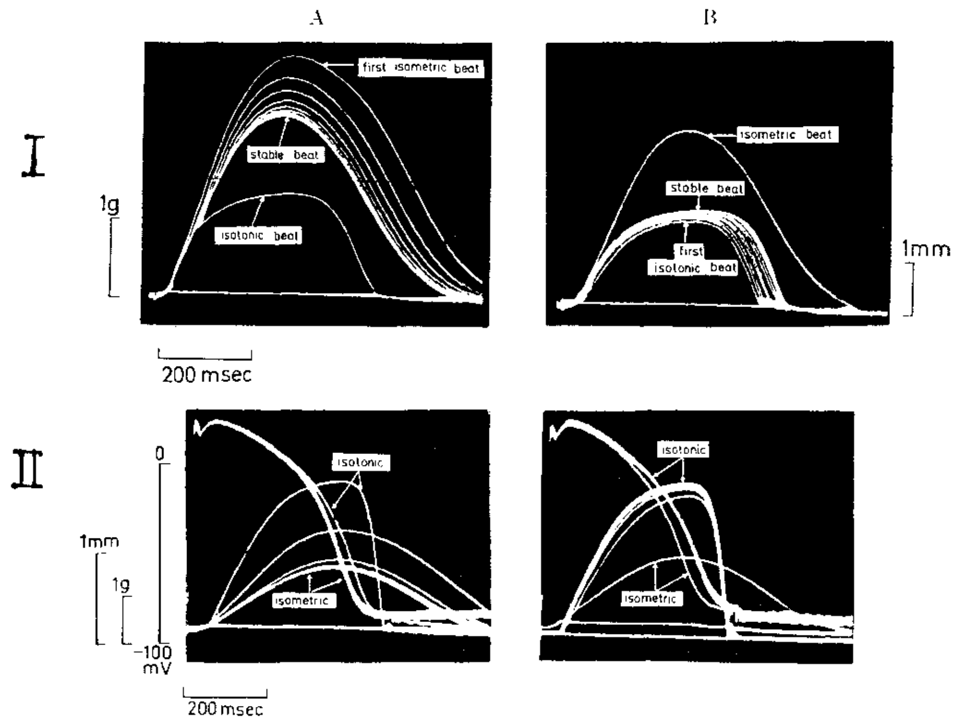


Fig.IV-3. Mechanical transients following a sudden change in the mode of contraction. In Part I A the first beat is isotonic against a small load of $0.3g/mm^2$. The second and subsequent isometric beats show peaks of decreasing tension stabilizing at a new level after 6-8 beats. The procedure is reversed in part I B with the first contraction isometric and the following 6-8 isotonic beats showing a positive staircase. The same experiments are shown in part II with simultaneously recorded intra-cellular action potentials. The action potential is shortened by passing from isotonic to isometric contractions and is again lengthened by the reverse operation. The mechanical transients are thought to be partly due to the alterations in the duration of the action potential.

the following isometric contractions the peak tension and the rate of tension development declines in a staircase like manner reaching a new steady state after about 8 - 10 beats. In part (B) the sequence is reversed; the muscle first contracts isometrically and then is allowed to shorten. In this type of experiment the subsequent 8 - 10 isotonic contractions show an increase in the amplitude and the velocity of shortening forming a positive "staircase".

In part (A) the duration of the action potential is immediately reduced when the mode of contraction is changed from isotonic to isometric conditions. This abbreviation is first detectable about one third the way through the plateau and becomes more prominent during further repolarization. Switching back from isometric to isotonic conditions part (B) leads to an immediate broadening of the action potential to the time course observed at the beginning of the experiment.

In contrast to the changes in the electrical phenomena which are immediate and persist as long as the particular mode of contraction does, the changes in contractility show characteristic mechanical "transient" taking place over several subsequent beats. Under given experimental conditions the amount of prolongation or shortening of the action potential varies considerably between the different preparations ranging from only a few msecs. to 60 msecs.

The question now arises as to whether the alterations in action potential duration and the mechanical transients are independent

or interrelated phenomena. The arguments in favour of the mechanical transients being a result of the electrical changes are as follows. The duration and intensity of the contractile activity in cardiac muscle is strongly dependent on the duration of the action potential (Antoni,Jacob and Kaufmann, 1969; Wood, Heppner and Weidmann, 1969). Further clamping of the membrane potential for different durations Beeler and Reuter (1970) showed that the inward Ca^{++} current and tension developed was greater the longer the clamping period. It has also been recognised from several investigations(Wood,Heppner and Weidmann 1968: Antoni, Jacob, and Kaufmann, 1969: Beeler and Reuter, 1970; Braveny and Sumbera, 1967)that sudden changes in the duration of action potential produced by electrical polarization, voltage clamping or sudden temperature changes also typically result in a staircase like mechanical response developing over 5 - 6 subsequent beats.

Therefore it is reasonable that in this series of experiments the changes in the duration of the action potential may reflect in the subsequent contractile activity of the muscle. There is some relationship between the amount of action potential shortening, measured at 80% repolarization, and the degree of the negative mechanical transient following a sudden change from isotonic to isometric conditions. (Kaufmann, Lab, Hennekes & Krauze 1971). These tensions transients, however, are not entirely attributable to the changes in AP duration since tension transients may be observed with no accompanying AP change (Hennekes, Kaufmann, Lab and Steiner 1977).

ACTION POTENTIAL DURATION AND QUICK RELEASES.

It appears thus far that there exists a feedback link between contraction and excitation in that the action potential duration is abbreviated when the shortening decreases and the corresponding tension development increases and, conversely, the duration is increased when the muscle shortens with a light load. If this is true, then the duration of the action potential should be increased to a maximum when the muscle contracts against zero load. An attempt is thus made to make the shortening velocity faster than in the isotonic contractions at the smallest preload ($0.3 g/mm^2$). This may be achieved during quick-release experiments. In all parts of Fig. IV-4 the muscle is stimulated at 24/min and contracts isotonically against a preload of $0.3 \ g/mm^2$. Thereafter, in each part of the figure, the first subsequent beat after the isotonic steady state begins isometrically. Then, at progressively longer times after the onset of the isometric beat the muscle is released with an exponential decay of tension and the contraction continues isotonically. The exponential decay is used in order to approach a uniform shortening velocity during the release. (The shortening velocity in these experiments can be adjusted by choosing the appropriate time constant of tension decay). In the experiments shown in Fig. 4 the time constant governing the exponential decay of tension is set to 20 msec resulting in a shortening velocity, which is higher than that during the preceding isotonic beats at $0.3 \ g \ mm^{-2}$ ($1.9 \ L_i S^{-1}$ as compared with a value of $1.1 \ L_i S^{-1}$. Thus each part of Fig 4 contains two super-imposed action potentials, one associated with the isotonic contraction and one accompanying the subsequent released isometric beat. Comparing the time course of each pair of action potentials one can differentiate two effects; the first is an initial abbreviation of the action potential associated

with switching from isotonic to isometric conditions (eg.Fig IV 3). However, in these experiments the muscle is not allowed to complete its isometric contraction but is released at different points during the isometric contraction. As a consequence of this release the action potential is prolonged, as expected from the proceding results, but is now longer than that of the normal isotonic beat. This is seen in panel A & B of Fig IV 4 where the release is 100 and 170 msec after the onset of the isometric contraction. The action potential associated with these releases crossed over the "isotonic" one about half way down the repolarization phase.

The release experiments shown in Fig IV 4 demonstrate 3 additional features of this contraction-excitation feedback system. First the prolongation of the action potential induced by quick-releases appears to be due to a substantial depolarizing current with its own time course. This is clearly seen when the release is made after the action potential is virtually complete (panels C & D). A new wave of depolarization ranging from about 12 - 20mV in amplitude and 150 - 200 ms in duration appears. Second, and of particular importance here, is that occasionally this new depolarization reaches threshold with the initiation of a propogated action potential (panel F). The third additional feature seen in this figure is that the "latent period" between mechanical and electrical events is relatively short. The time between the beginning of the release to the point where the change of shape of the action potential is detected, is about 1o msec.

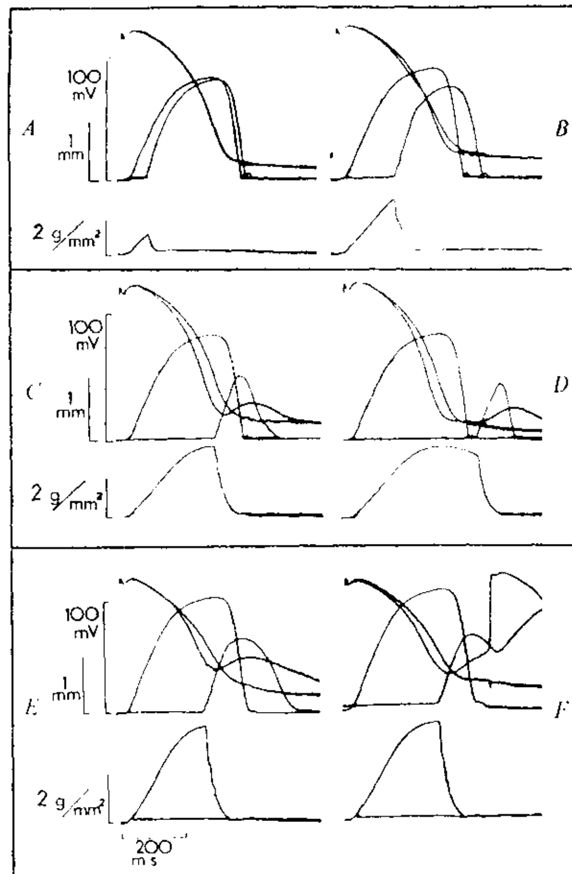


Figure IV-4

Quick release experiments, performed on cat papillary muscle preparations. In each set of recordings, that is, A to F, the muscle action potential, length and tension appear from above downwards at zero time. In all recordings two consecutive beats are shown; the first beat is wholly isotonic, the second beginning isometrically with releases to a standard light load (0.3 g/mm^2) at four progressively longer durations of isometric contraction (A to D). The corresponding action potential recordings exhibit two different effects. During the isometric period the action potential is shortened, but a few ms after the start of the release a depolarizing current interrupts this trend, producing a further prolongation (B) or a new wave of depolarization (C and D). In (F) an experiment is shown where this depolarization reached threshold and initiated a new propagated action potential although, in (E), it did not do so. One preparation produced A to D; another, E and F.

ACTION POTENTIAL DURATION AND STRETCHES.

The question arizes as to whether an equivalent effect on the action potential can be produced with a quick stretch ie. an abbreviation of the action potential. In the following series of experiments an isometrically contracting muscle was stretched at different times after the onset of contraction for 500 ms to a preselected new length, until a steady state was attained. Then the application of stretches was terminated and tension transients, developing on the subsequent undisturbed isometric contractions, were observed. This intervention did not produce any detectable changes in AP duration (Figure IV 5,a-c) upper traces of panels). However, a tension transient was observed (middle traces) which was most prominent with stretches applied before or early during the rising phase of contraction (Figure IV 5a, but declined in amplitude if applied later during contraction (Figure IV 5c)

SUCROSE GAP STUDIES: RELEASES AND RELEASE-RESTRETCH CYCLES.

The microelectrode recordings have shown that a release during isometric contraction can result either in a prolongation of the AP (if applied early enough) or in a new depolarization wave (if applied after full repolarization). These experiments were difficult to perform systematically and quantitatively, for quick releases very often dislodged the micropipette, requiring a new impalement of another cell. Such recordings must be cautiously interpreted, for the possibility of artefacts and electrical inhomogeneities from cell to cell cannot unequivocally be excluded. The sucrose gap technique with field

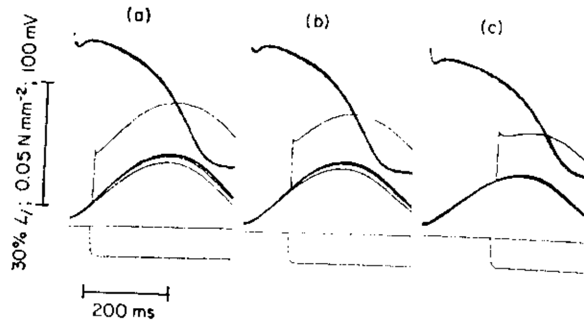


Fig. IV 5. Effect of stretch in action potential and tension. From a stable state reached in stretching the muscle for 500 ms from 90% to 100% L_i at different times (50,100 and 175 ms, respectively) after the onset of contraction, a return to isometric contraction of 90% L_i does not induce changes of AP duration (upper traces). However, tension transients (middle traces) develop, the amplitudes of which are larger the earlier during contraction the stretch is applied (sucrose gap techniques).

stimulation, however, allows integrated electrical sampling of large parts of the active preparation and, therefore, might be more suitable to relate parameters of the releases applied, to the effects on AP duration.

It would be useful at this stage to get some indication of the type of mechanical change that is mostly implicated in generating the electrical change. Clearly stretch has no effect but a change in the other direction ie.,shortening of the muscle does. To resolve the point as to whether it is the release of the muscle, and its length change, that causes the transient depolarization per se, the muscle was restretched immediately following the release ie., a quick-release-stretch manoeuvre. The sucrose gap arrangement was used for these experiments but before proceeding it was considered worth doing a simple release only experiment in a modified set-up. This was to establish with certainty that the electrical effect was not an artefact produced by alteration of current flow in and around the insulation gap.

After recording the prolongation with a release (Figure IV 7A) all the compartments including KCl and sucrose, were filled with the normal perfusing solution. The resultant electrical recording the extracellular electrogram (Figure IV 7B) shows that most, if not all,

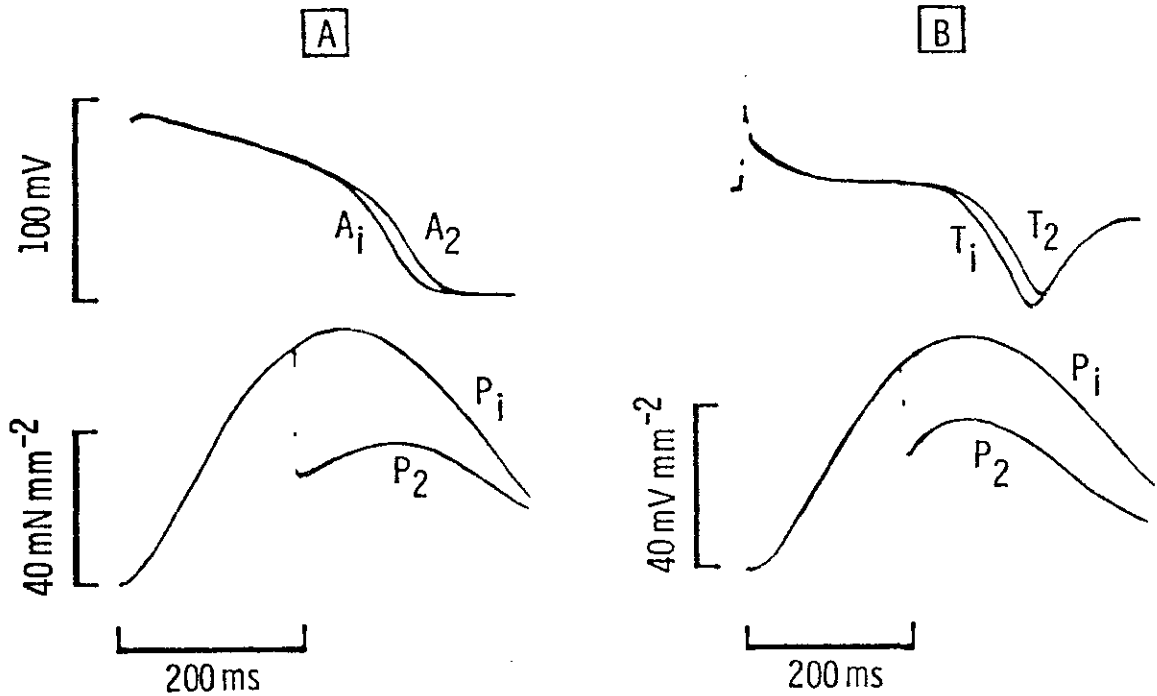


Figure IV 7

Effect of release on electrical recordings from cat papillary muscle. A The action potential, recorded via a sucrose gap, is prolonged (A_2) (after depolarization) when compared with the action potential (A_i) of an undisturbed isometric contraction (P_i) following a release of the muscle. The release is accompanied by a reduced tension development (P_2). B The extracellular electrogram shows a prolonged Q-T interval, (QRS-T_2) following a similar mechanical manoeuvre as in Part A, when compared with the undisturbed contraction i.e QRS-T_1

the cells in the preparation could have their electrical activity altered by the quick release which prolonged the Q-T interval of the electrogram: an observation in keeping with part A of this figure. However to resolve the point above concerning the length change, quick-release-stretch manoeuvres were used in the subsequent investigations . By varying the amplitude of this mechanical perturbation at a given time the degree of muscle deactivation was varied and the extent to which transient depolarizations developed could be observed. The recordings in Figure IV 8A / show that the greater the drop in redeveloped tension (deactivation) after the mechanical intervention, the greater the amplitude of the afterdepolarization. The correlation was linear ($r = 0.88$) and statistically significant ($P < 0.001$, $N = 13$). However, Figure IV 6 showed an alternative way of using release cycles to vary the degree of deactivation; by altering the timing of the intervention rather than its amplitude. Superimposed examples of such recordings are shown in Figure IV 8B. Clearly no linear relationship between deactivation and afterdepolarization is immediately evident. The late intervention produced a large deactivation but the amplitude of the afterdepolarization was small. By comparison the early intervention produced a small deactivation but a larger afterdepolarization. The membrane response to the mechanical perturbation appeared to be related to some factor(s) which became less effective as the muscle repolarized. This can be taken into consideration by dividing the amplitude of the afterdepolarization by the amplitude of the undisturbed action potential

Figure IV 8

Effect on action potential of transient releases of cat papillary muscle.
A An isometrically contracting muscle, P_i contracting at L_i is transiently released, to different lengths L_2 - L_5 for 50 ms each. This procedure deactivation the muscle, despite the restretch, to produce the reduced tensions, P_2 - P_5. Associated with the mechanical change, transient depolarizations are produced A3 - A5: the greater the deactivation the greater the depolarization
B An isometrically contracting muscle (P_i) is transiently released to the same length for the same duration, but at different times, 2,3,4, & 5. Increasing deactivations, P_2 - P_5, are produced and are associated with depolarizations A_3-A_5. No apparent relationship between deactivation, voltage of time emerges.
C The degree of deactivation, and amplitude of depolarization are expressed as ratios of the control measurements (see insert) and plotted in this graph. A linear relationship emerges (see text) despite the wide scatter. The scatter is a consequence of deriving the voltage ratios from several different preparations using both micro-electrode and sucrose gap recordings; the latter method also introduced a series elasticity in the preparation which influenced the tension ratio. INSERT. The voltage and tension ratios, following transient releases of varying amplitude the time t are derived as follows. At the end of the intervention, control measurements T and V are made. The maximum amplitude of the after depolarization ΔV is then obtained, to produce the ratio $\Delta V/_V$. The maximum reduction is redeveloped tension, or deactivation ΔT, is obtained for the ratio, $\Delta T/_T$. Maximum ΔT may be found just after the tension overshoot on restretch, e.g. P_2 in A, or some time later, e.g. P_2 in B.

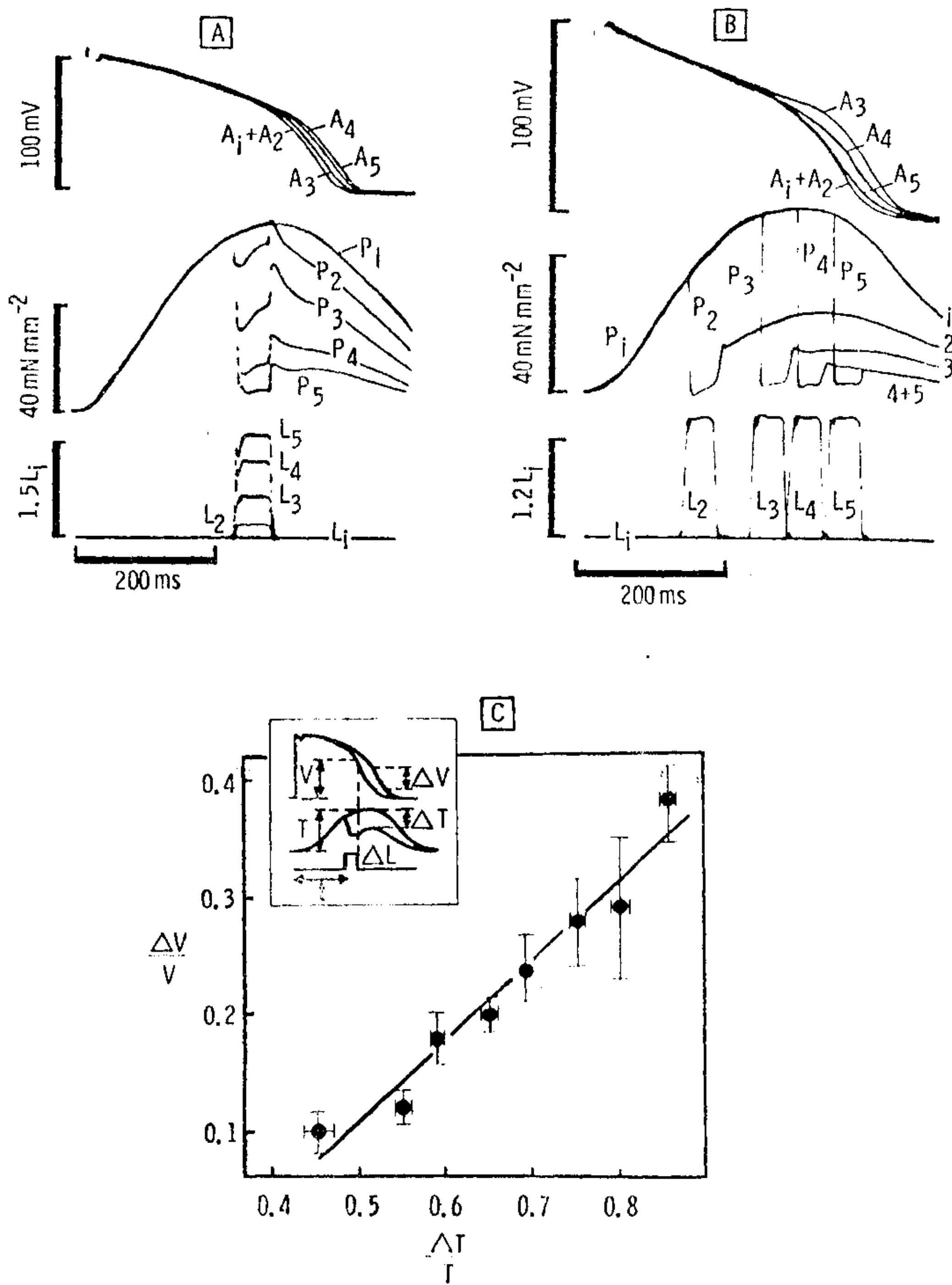


Figure IV 8

at the time of the intervention. If this ratio is plotted against the ratio, reduction in redeveloped tension: maximum undisturbed isometric tension, the relationship shown in Figure IV 8C emerges. This composite graph includes measurements from several different preparations using microelectrodes as well as the insulation gap technique, and was derived from mechanical perturbations that varied in amplitude and/or time. The correlation coefficient (r) of the 80 points used was 0.63 with P = 0.001. A higher correlation coefficient was obtained when the data was used from one preparation or when one recording technique was used.

The discussion of these results is incorporated in the final chapter.

CHAPTER $\underline{V}$

STUDIES ON INTACT FROG VENTRICLES

MECHANICALLY DEPENDENT CHANGES IN ACTION POTENTIALS RECORDED FROM INTACT FROG VENTRICLE

METHOD (Fig. $\underline{V}$ 1 - A)

The experiments used frog hearts in which the ventricular chamber was cannulated and perfused via the aorta. The cannula was connected to a tap constructed to allow isovolumic or free-loaded contraction, the continuous recording of intraventricular pressure, and rapid externally imposed changes of ventricular volume.

Perfusion System

The ventricles of frogs (Xenopus laevis) were cannulated via the aorta and perfused with continually oxygenated Ringer's solution (NaCl 111.3mM, KCl 1.8mM, $CaCl_2$ 1.08mM, Na HCO_3 2.4mM) at room temperature which varied between 20-22°C. The cannula was in 2 sections; a small cannulating section which connected the ventricle to a piston tap, and a second vertical section glued to the other end of the tap. A pressure head of about 2-3 cm. of Ringer in the cannula provided adequate diastolic filling of the ventricle. The diameter of the ventricle was adjusted by varying the pressure head against which it worked, to be approximately the same as that observed in the intact animal with the chest open. The volume moving up and down the single perfusion cannula with each beat was between 0.1 and 0.3 ml. The contractions were thus free-loaded unless the cannula was closed by the tap, as described below, in which case the contractions were isovolumic. The atria were usually left

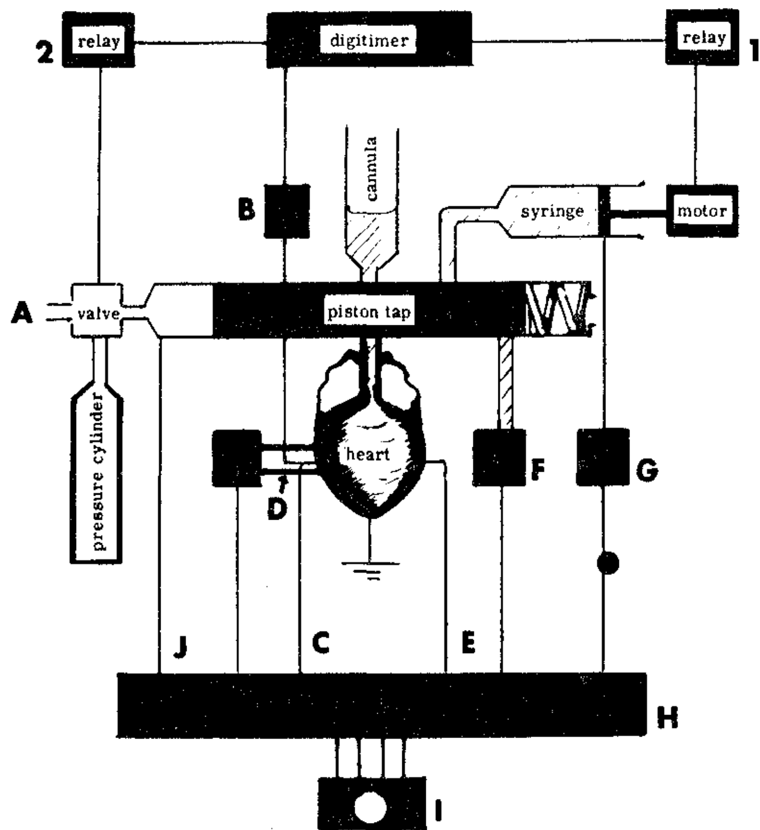


Fig. VI - A Diagram of experimental arrangement. The fr g ventricle is cannulated and attached to the cylinder of the piston. The motor driven s ringe is operated by Relay and a "Digitimer". A 3-way solenoid valve is connected to a pressure cylinder operated by Relay 2. A, Vacuum; B Schmidt-trigger; C suction electrode; D caliper; E electrocardiogram; F pressure transducer; G photo-electric circuit to monitor plunger of syringe; H multichannel pen recorder; 1 storage oscilloscope; J Marker.

intact and the heart allowed to beat spontaneously at between 20-30 beats/min. but in some cases the atria were tied off and the ventricles electrically stimulated at 24 beats per min.

Piston Tap

Construction. The piston tap consisted of an outer cylinder with a piston inside. The cannula from the ventricle was inserted into a sealing ring in the wall of the outer cylinder. There were holes running through the piston in different directions so that the position of the piston could determine with which of the resevoirs of fluid the ventricle was connected. There were three basic positions of the tap: for free-loaded (auxotonic) contraction, for isovolumic contraction and for rapid, externally imposed changes in ventricular volume. Ventricular pressure could be monitored at any of the positions.

Operation of the tap. The relevant position of the piston and thus alignment of the appropriate holes was chosen by setting a mechanical stop onto which the piston was driven by gas under pressure. The three way solenoid valve releasing the gas under pressure was opened by the closing of a gated relay$_2$, controlled by a "Digitimer". (Devices). The piston was spring-loaded and returned, aided by vacuum, when the valve switched back. The "Digitimer" was triggered by a Schmitt Trigger (B) into which the action potential was fed.

Injecting System

Intraventricular injections and withdrawals were accomplished by a motor driven syringe which was operated when another relay$_1$ closed at times preset by the "Digitimer". The relay allowed current to flow first in one then in the other direction to drive the motor forward then in reverse.

Recording System.

Measurements of segment length. During these investigations on the ventricles of intact frog hearts (see also Lab, 1971) it became necessary to record qualitatively the movement of two points on either side of an electrode on the epicardium. The usual methods, of suturing mercury in rubber and wire strain gauges to the epicardium were unsuitable. There was also additional difficulty of working with a small heart as well as having to record from different parts of the heart at different times. A light-weight calliper was therefore developed which could be fixed to the tissue surface by vacuum.

The perspex calliper (Fig. V1B) is shaped like an elongated letter H with a double crosspiece (g) near the lower end. The cross-piece allows one of the arms of the calliper to pivot. To minimize inertia, the greater part of this pivoting arm is of balsa wood (f) which is made to incline towards the stable arm. A graded density film (d) is glued to its upper extremity. The non-pivoting arm (e) is cranked so that a photo-electric transducer assembly (c) can be mounted to allow the film (d) to move between a light source and photo-transistor. The circuit of the photo-electric system has been previously described (Lewis, 1969). An additional focusing lens is included in front of the lamp and the circuitry is remote from lamp and photo-transistor, except from the 1 K resistor which is glued to the assembly to reduce the number of leads from it.

Longitudinal holes (j) at the short ends of the calliper allow it to adhere with suction to the surface of the heart by vacumm from a

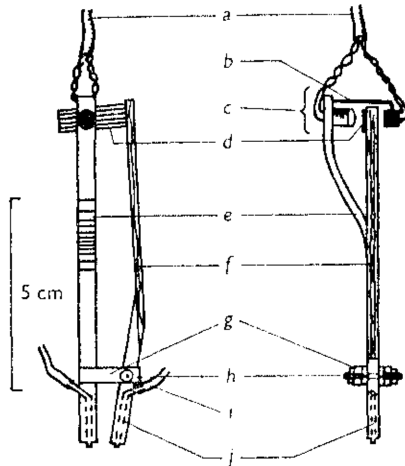


Fig. V 1B. The calliper is made mostly from 1 x 3 mm.Perspex. One of the arms (e) has a small double cross-piece (g) fixed to it so that the other arm (f), weighing less than 200 mg, is gripped by and can pivot between, this cross-piece. The pivot (h) consists of two sharp-pointed screws in tapped holes in the Perspex in each branch of the cross-piece. They are directed inwards to a small hole in the pivoting arm. When the screws are adjusted in the hole and secured by lock nuts the pivoting arm can turn freely. The ends of the calliper nearest the heart have 1 mm diameter holes (j) drilled longitudinally to meet a length of hypodermic needle of the same diameter pushed into the Perspex near the pivot. This location reduces the drag due to the flexible polyethylene tubing, 1 mm i.d. (i) which is pushed over the needles. The tubing is then connected to a vacuum of 40-60 cm Hg. The lamp and a lens of short focal length is held by a small cylinder near the end of the non-pivoting arm. The photo-transistor, onto which the lens focuses, is also fixed to this arm by a piece of stiff, bent wire (b). The movement of the pivoting arm is converted to an electric signal by the photo-sensing assembly (c). (a)=Leads; (d)= graded density film.

conventional water pump. The instrument is held by the flexible leads (a) and, with the vacuum tubing suitably draped, drag is minimized.

If the calliper tips are close (2mm - 5mm apart) the readings of tissue surface movement are reasonably quantitative for large hearts or for relatively flat surfaces. For hearts, e.g. frog, with small radii of curvature, however, the discrepancy between the chord length, which the device actually measures, and the arc length becomes greater. The measurements are thus now mainly qualitative.

Monophasic action potentials from the epicardium were recorded by means of suction electrodes [Fig. V IA (C)] after Hoffman et al 1959. These authors have drawn attention to some of the pitfalls in the interpretation of the records obtained by this method of recording. However, interest in the present studies is centred on the slower time course changes, for example the repolarization phase of the monophasic action potential which is not qualitatively different from the repolarization phase found in microelectrode action potentials. The suction electrode recording was fed into one input of a high input impedance differential amplifier. The other input was a wick electrode on the ventricle. The precise location of the wick did not affect the nature of the recording obtained.

The relative movements of two points on either side of the electrode were monitored by the photo-electric calliper (D) placed either

longitudinally or tranversely. The construction of the calliper did not allow for 'shearing' between the points of attachment of the calliper to the epicardium. A bipolar electrocardiogram (E Fig. 1) was recorded from two epicardial wick electrodes, soaked in Ringer, or with one electrode in the intraventricular perfusing fluid. Intraventricular pressure was monitored with pressure transducer (F) and the volumes injected into the ventricle monitored by means of a graded density film attached to the plunger of the motor driven syringe. The film interrupted a light source to a photo-transistor (G) Lewis 1969a. The signals from transducers and electrodes were fed into a multi-channel pen recorder (H) (Devices) and storage oscilloscope (I) (Tektronix).

Confirmatory Records in Frog Ventricular Strips.

Intracellular recordings. A ring of muscle was cut form the base of the ventricles of Rana Pipiens, connective and atrial tissue were cut away and the ring was converted into a strip about 1 mm^2 and 4 - 8mm long. The muscle was clamped horizontally in a perfusion chamber and a conventional microelectrode recording system for cardiac muscle used (Allen et al 1976). The electrodes were floating free from earth and their resistances were not less than 20M ohm. Records of all the attempted impalements were stored on magnetic tape so that those which were successful could be analysed later. The number of successful impalements was rather low because frog ventricular cells are small and the muscle was changing length. Tension was measured by R.C.A. transducer valve.

Insulated Gap Recordings. The method described by Niedergerker (1956) was used for these recordings. The strip of ventricle, prepared as above, was vertically mounted and clamped near one end in a canal 0.5 mm long and 0.5 mm diameter. The canal was formed by two sliding plates of acrylic plastic which created a partition between two chambers. Silicone grease in and around the canal completed the separation between the two chambers. The muscle traversed the gap and lay in the chambers; the first chamber containing Ringer and the second isotonic KCL or 0.5% procaine in 7% sucrose. Action potentials recorded across the insulating gap, between the first and second chambers, were 20 mV to 60mV in amplitude (Lab, 1971). Tension and length were recorded by a modified strain gauge lever (Lewis 1969 a, b; Lab, 1971).

RESULTS

Mechanical and electrical changes on converting free-loaded contraction to isovolumic contraction.

When the frog ventricle was allowed to contract auxotonically (free-loaded), the action potential displayed the normal configuration except for the reduced amplitude associated with the suction electrode method of recording (first two action potentials on the left side of Fig. V3). The same segment of epicardium showed shortening (upward deflection) and relaxation, as expected. The recorded intraventricular pressure fluctuated little and this was due to the muscle moving a small pressure head of Ringer solution in the perfusing cannula.

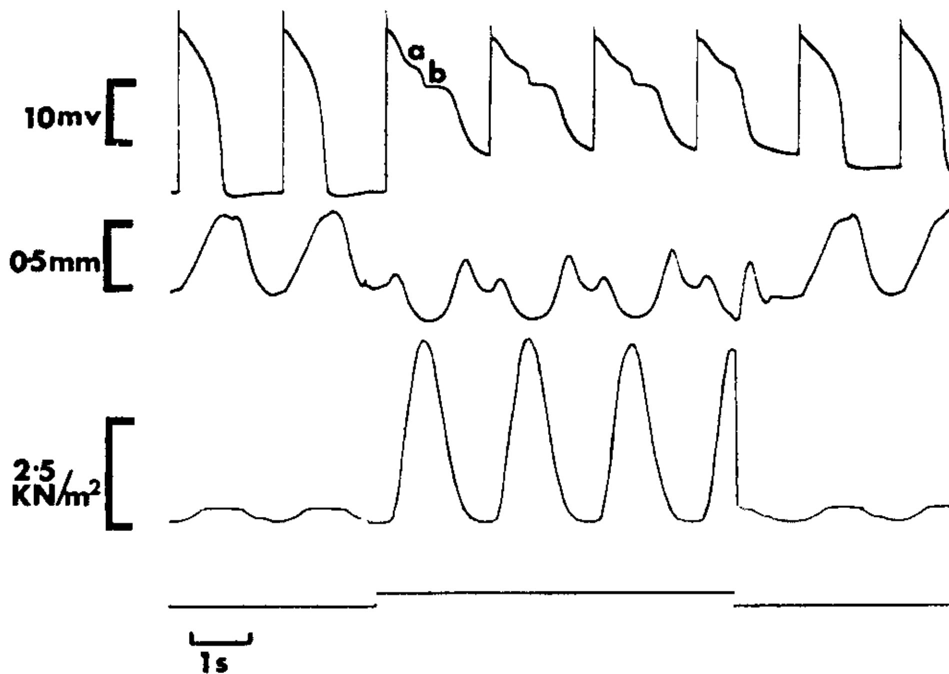


Fig. V 2 Action potential changes (top trace) and movements of an epicardial segment (middle trace) with cyclic intraventricular pressure changes (bottom trace) due to isovolumic contraction of a frog ventricle indicated by the deflection of the marker (horizontal line). The first two beats show "normal" configuration of action potentials and segment shortening (upward deflection). At point (a) of this record, after the onset of isovolumic contraction, the first part of the repolarization phase of the action potential is accelerated while the pressure is rising, and the segment lengthens. Note that the segment length during most of the isovolumic contraction is greater than that of the relaxed isotonically contracting ventricle (trough of recording, before isovolumic contraction). During the relaxation phase, when intraventricular pressure declines, the rapid repolarization phase is interrupted (at b) to form a depolarizing after potential which does not allow the action potential to return to base line before the next action potential. The ensueing spikes have a reduced amplitude. On resumption of isotonic contraction, the changes are completely reversed.

But when the ventricle was made to contract isovolumically at its end-diastolic volume (marker, horizontal trace) changes were seen in both the action potentials and in the pattern of contraction of segments of the epicardium. On closing the aortic outflow at the appropriate time (diastole) the action potential exhibited several phenomena when compared with the auxotonic contraction. It had a faster initial repolarization followed by a delay in recovery which took on the appearance of hump-like after potentials. The potential did not reach the same base line as the pre-isovolumic action potential and the next action potential had a reduced spike amplitude.

The changes in epicardial segment length were distinct from those found during a free-loaded, auxotonic beat. In Fig.V2 the fibres of the epicardial segment actually lengthened when they were expected to shorten, that is during ventricular contraction per se. This lengthening coincides with the steepening of the plateau phase. Thereafter the segment shortened but again at a time when these muscle fibres should "normally" lengthen - when the ventricle relaxes as a whole. The segment length throughout the isovolumic cardiac cycle was greater at any given time than during a free-loaded contraction. Study of segmental movement on the epicardium of dog ventricle has been carried out previously (Fisher et al 1966, Dieudonne & Jean, 1969) and since the time relationship between the mechanical and electrical events of any one segment was the centre of interest in this study, the recordings were obtained with the calliper on a convenient surface usually orientated along the longitudinal axis of the surface of the ventricle, anteriorly.

In order to quantitate some of the results, the amplitudes of the action potentials were taken at mid-plateau (at t_1 inset Fig. $\overline{\underline{V}}$ 3) and compared before and after aortic closure; the difference was obtained, (ΔV_1), and expressed as a percentage of a control height. (This form of expression was desirable, for the action potential amplitude obtained by suction electrode varied from preparation to preparation). The control base-line was established using action potentials before and after aortic closure, and the measurements were made from this line drawn through the greatest diastolic potentials. The peak height of the afterpotential produced on closing the aorta was measured at t_2 (inset Fig. $\overline{\underline{V}}$ 3) and the percentage difference obtained (ΔV_2), again expressed as a percentage of control plateau height. The time t_2 corresponded in most cases with the middle of the fall in intraventricular pressure, and where the peak of the after potential was not clear, t_2 was put at this mid-fall point. Changes in segment length were expressed in strains $\frac{\Delta L}{L}$ at t_1 and t_2. (Where, for example, ΔL is the difference in length at t_1 between pre and post aortic closure (LE-LC) and L is the control segment length at t_1 (LC). Correlation coefficients (r) between potentials and length at t_1 and t_2 were 0.52 and 0.235 respectively, with $p < 0.001$ and between 0.05 - .01 respectively.

In one experiment the ventricle was emptied and refilled in 0.05 ml steps to its end diastolic volume. At each volume the same measurements as above were made but at each volume the figures obtained were related to the control values in the empty ventricle.

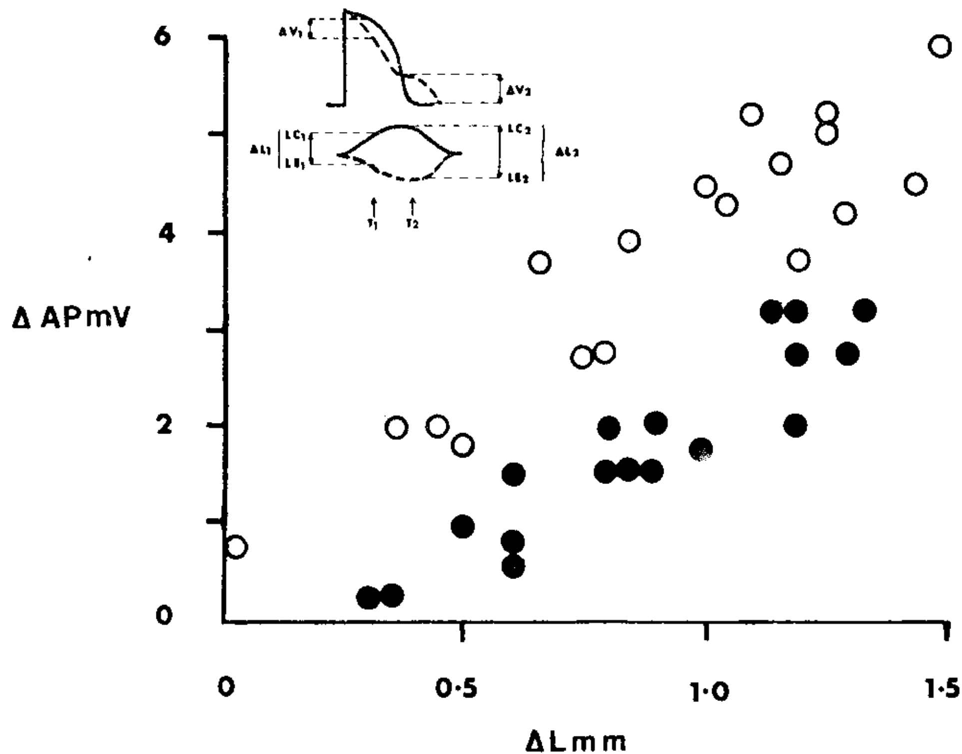


Fig. V3. Relationship between changes in segment length and changes in action potential (open circles) and after potential (filled circles). An isovolumic contracting empty ventricle was progressively filled, to normal end-diastolic volume with 0.05 ml increments. Changes in plateau and after potential voltages were measured and plotted against segment length changes. (initial length was 3.5 mm).

Inset (see text) Control action potential and segment length (solid lines) superimposed on experimental records (dashed lines). Changes in potential, V, and segment length, L, were taken at times t_1 and t_2 e.g. L_2 is the change in segment length at t_2 obtained from LC_2, the control length, and LE_2 the 'experimental' length.

The results are plotted in (Fig. V 3). Clearly the greater the length change the greater the change in potential. For a given length change the change in the plateau was greater than the change in after potential. Not all the changes need be present at the same time. Fig. V4 shows an unaltered plateau phase with the beginnings of a normal (shortening) contraction pattern of the segment of epicardial muscle. Thereafter the segment shows an exaggerated lengthening compared with the same period during isotonic contraction. Associated with the latter, the hump-like after-potential and the rise in base line is observed. Although action potential changes were occasionally seen without segment length changes only once was a significant length change observed with little accompanying action potential change. These last two observations could be explained if epicardial contraction is inhomogeneous with the electrode on a segment having a different mechanical behaviour to the 'calliper' segment. Fisher et al 1966 in fact found no distinct regular pattern of epicardial length changes in their experiments. It is likely that epicardial segments can lengthen in a direction not monitored by the calliper, no length change being apparent, but with the action potential still changing. The reverse is less likely but possible. Hence the isolated observation of a length change, with the electrical recording presumably form a relatively unstrained bit of myocardium, showing no change in potential.

Electrical effects related to the recorded changes in potential

Threshold depolarization

If the depolarizing after potential observed during isovolumic contraction is non-artefactual, it may be expected to be capable

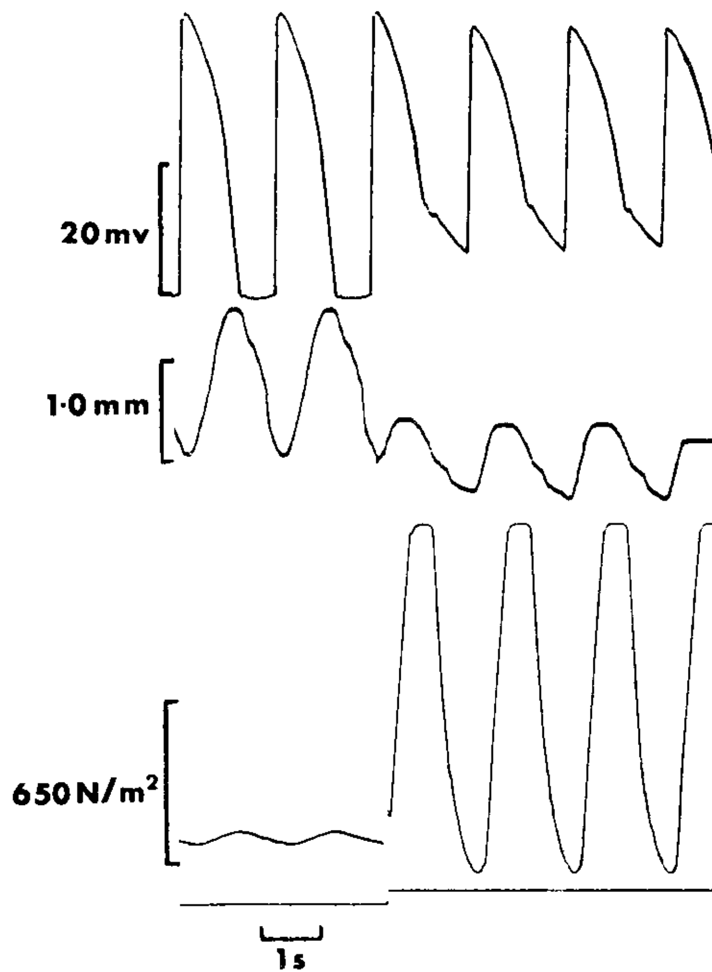


Fig V4. The same type of experiment as in Fig. V2 but with no obvious accelerated repolarization of the plateau phase of the action potential (top trace) when the ventricle is made isovolumic (marker). In this case the predominant lengthening of the epicardial segment (middle trace) takes place during the decline of intraventricular pressure (bottom trace) and the length attained is greater than the isotonic diastolic length - left hand side of picture. At roughly the same time as the segment lengthens, during the isovolumic contraction, the rapid repolarization phase of the action potential is interrupted by a depolarizing after potential.

of reaching threshold to initiate a propagated action potential. Fig V5A again shows the steepening of the plateau phase with whole ventricle contraction and the depolarizating after potential with whole ventricle relaxation. (As designated by the decline in intraventricular pressure). But in Fig V5B, this depolarizing potential is seen to reach threshold and to produce an action potential capable of initiating a contraction. This may be regarded as being a point in favour of the depolarizing potential not being an artefact.

Although the after potentials or the points at which threshold are reached in Fig. V5B are somewhat larger than in Fig. V5A the last potential, d, was the largest of all. Yet there was no action potential propagated. The explanation for the last observation, made in several preparations, is speculative at present. It is possible, first, that ionic fluxes accompanying mechanically induced multiple extrasystoles alter the immediate extracellular environment and thus produce conditions which change the threshold. Secondly, the cells that actually reached threshold may have been distant from the electrode and may have shown, at the time of recording potential, an actual reduced amplitude potential.

Microelectrode recordings

The action potentials obtained by suction electrodes are proportional to intracellular potentials but are reliable in following slow changes in potential (Hoffman et al 1959). However, during the changes in length of the epicardial segments there is inevitably distortion at the tissue-electrode interface. Thus the current flowing into

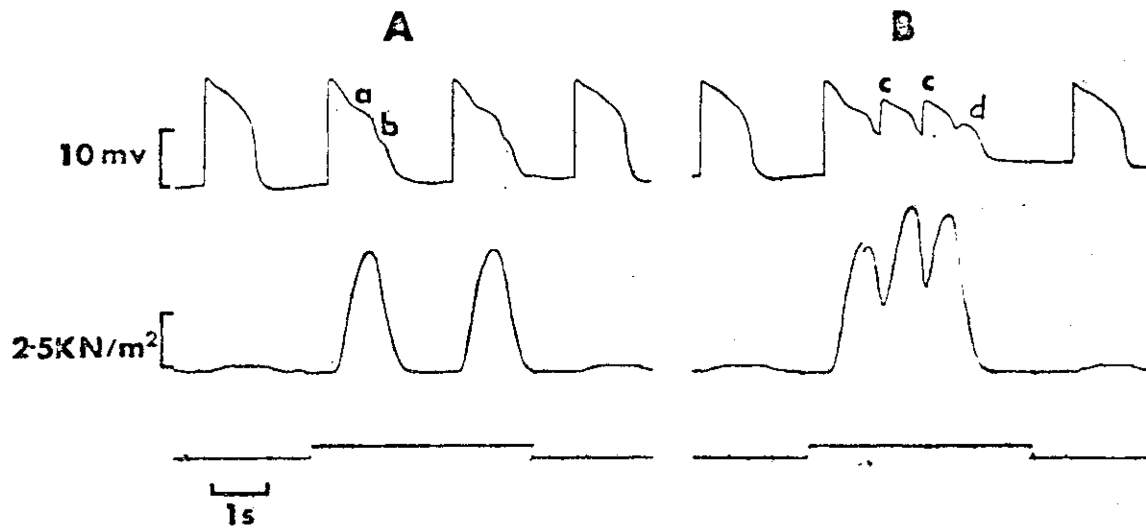


Fig. V5 Action potentials (top trace) and intraventricular pressures (middle trace) of frog ventricule during isovolumic contraction (upward deflection of the marker on the bottom trace). In part A isovolumic contraction resulted in an increased intraventricular pressure, and the accelerated repolarization (a) followed by depolarizing after potential (b). In part B of this figure (the same preparation) the after potential is accompanied by new propagated action potentials (C).

the electrode may vary either by a reduction in the number of cells contributing to the current or by a change in the intracellular shunting resistance. Some confirmatory studies were therefore thought necessary and were done on isolated strips of myocardium using microelectrodes.

It was technically difficult to keep an electrode impaled in a single cell and record reliably while imposing rapid mechanical changes on the muscle. A success rate of 1 in 60 attempts was quoted for cat papillary muscle with larger cells in Chapter IV. However, the measurement of the duration of action potentials were attempted in these experiments for even if the full resting potential was not recorded, the duration of the action potential at 80% repolarization was identical with an action potential duration obtained with a good impalement. Action potential durations could thus reliably to compared at two different lengths: at slack lengths and at lengths at which near maximum tension was produced (L_{Max}). Using several different impalements, making 16 observations in two preparations, action potential duration at a slack length was 864 ms and at near L_{Max} 790 ms (SD 106 and 140 respectively with $P < 0.001$ Students t test).

A good example of a change in action potential duration when extending a muscle from a length at which little active tension is produced to a length less than L_{max} is seen in Fig. V6a. The two action potentials are consecutive and super-imposed, being obtained from the same impalement and the action potential duration is clearly reduced with stretch.

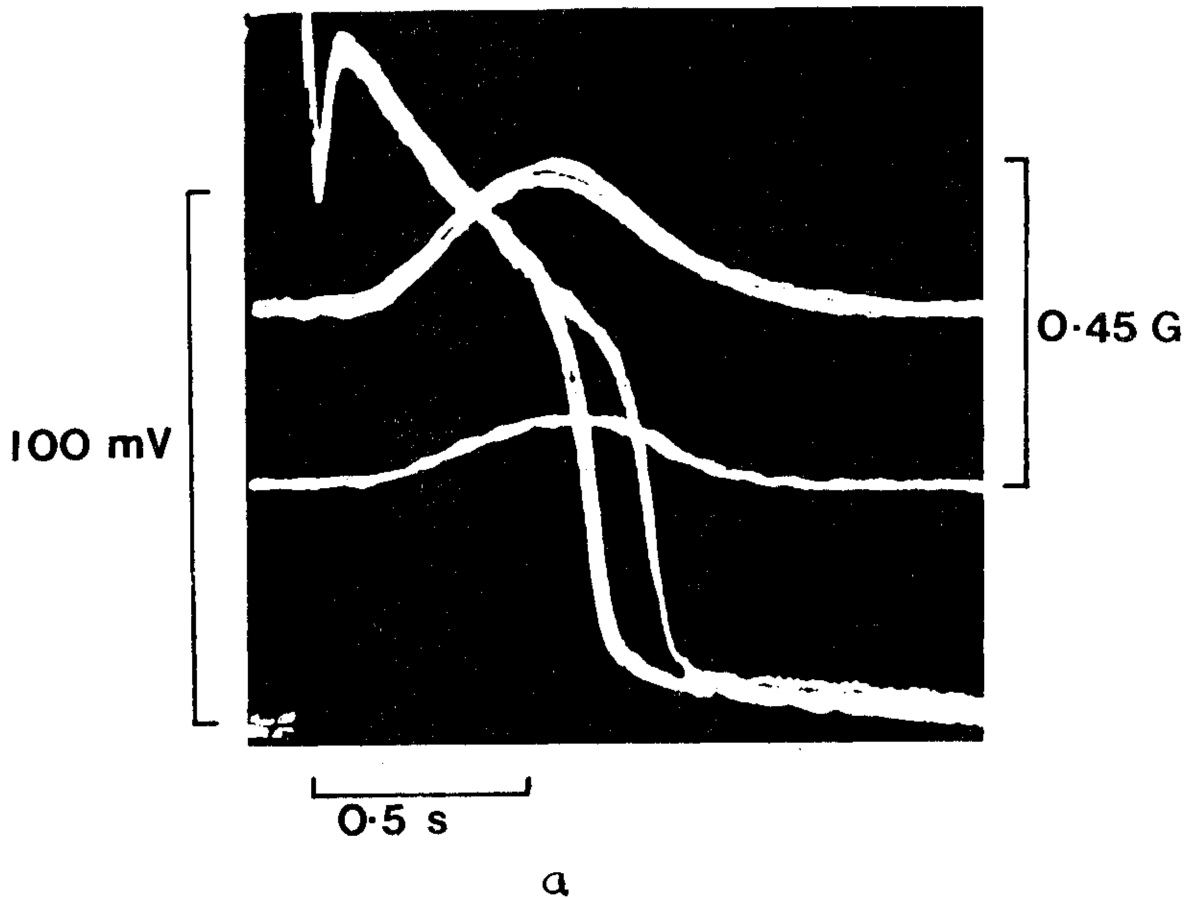


Fig. V6a Superimposed traces of action potentials, obtained with micro-electrodes, and tensions (two middle traces) during stretch. Same impalement in an isolated strip of frog ventricle. The muscle first contracted (1) at a short length, producing a small tension, and was associated with the action potential with the long duration. On stretching before the second contraction (2) the muscle passive and active tension increased and the action potential duration simultaneously shortened. Stimulation frequency, 30/min; Temperature 18°C; muscle slack length, 2.0 mm. These records were obtained in Dr. B.R. Jewell's laboratory at University College London, with the assistance of Dr. M. Boyett.

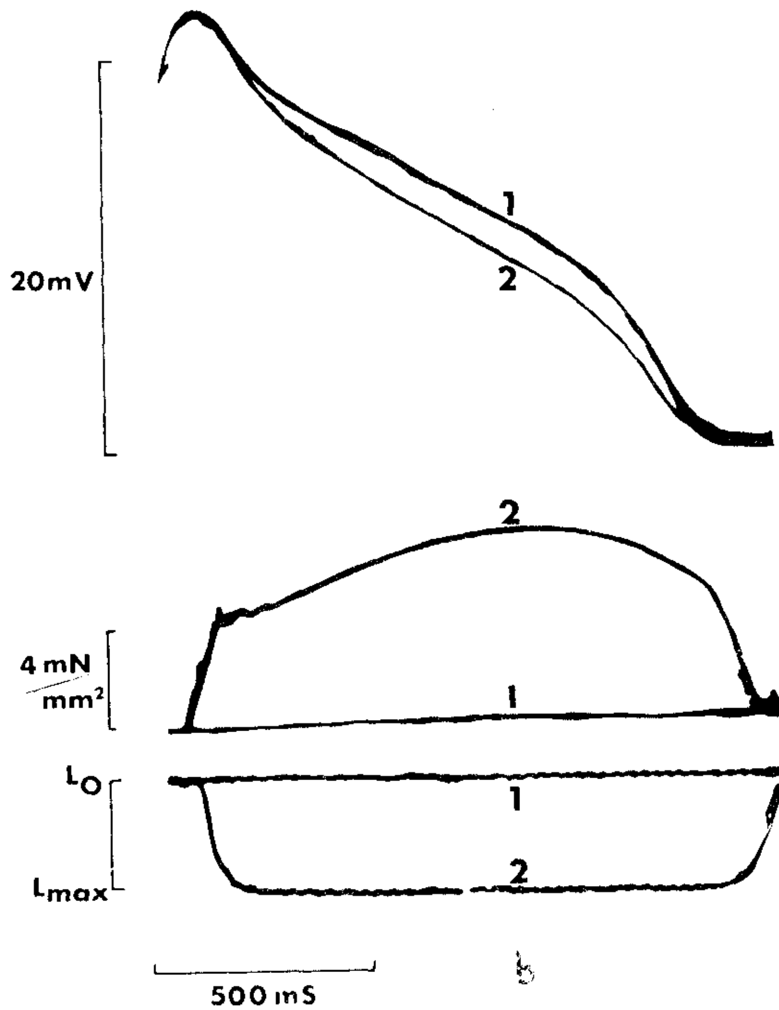


Fig.V6b Monophasic action potentials, obtained with an insulated gap technique (upper traces, calibration bar, 20 mV), tension (middle traces, calibration bar, $4mN/mm^2$) and length (lower traces, calibration bar, downward deflection, Lo to Lmax) recorded from an isolated strip of frog ventricle. IN the first contraction (1) at the short length the muscle produced almost no active tension and was associated with the longer action potential. During the second contraction (2) stretching the muscle to L_{max} resulted in a larger tension and the shorter action potential. Stimulation frequency 30/min; Temperature 20°C; muscle slack length 5mm.

Although changes in plateau of action potential, with alteration in length, were clearly demonstrable, consistent changes in resting potential, or the production of after potentials following mechanical changes, were less obvious. It was extremely difficult to stretch a muscle at rest and keep the electrode impaled so that when releasing the muscle, the control, full, resting potential was re-established. With resting potentials more negative than about - 40 mV, a depolarization was the predominant response with a transient stretch (Fig. V 7). This response could be explained by the electrode coming out and re-impaling the cell. However, even with short duration stretches confined to less than L_{Max} a new propagated action potential, producing an active tension, could be generated (Fig. V7) which is in keeping with a depolarization being real and reaching threshold to activate contraction. A stretch induced "extra-systole" has been seen in every isolated strip in which it was sought ($N > 40$).

Insulated gap recordings

In view of the limitations in interpreting the records with suction electrode and some of the microelectrode recordings, particularly the depolarization on stretch, a third method of recording was used (Niedergerke 1956). In this method, one end of the muscle was firmly held in an insulation gap while the mechanical changes were obtained via the other end. The electrical recordings were transgap potentials. Changes in action potential durations, recorded across the insulated gap, (inset Fig. V6) were again confirmed with length changes of the same magnitude as used with the microelectrode studies. With this technique of recording several transient stretches could

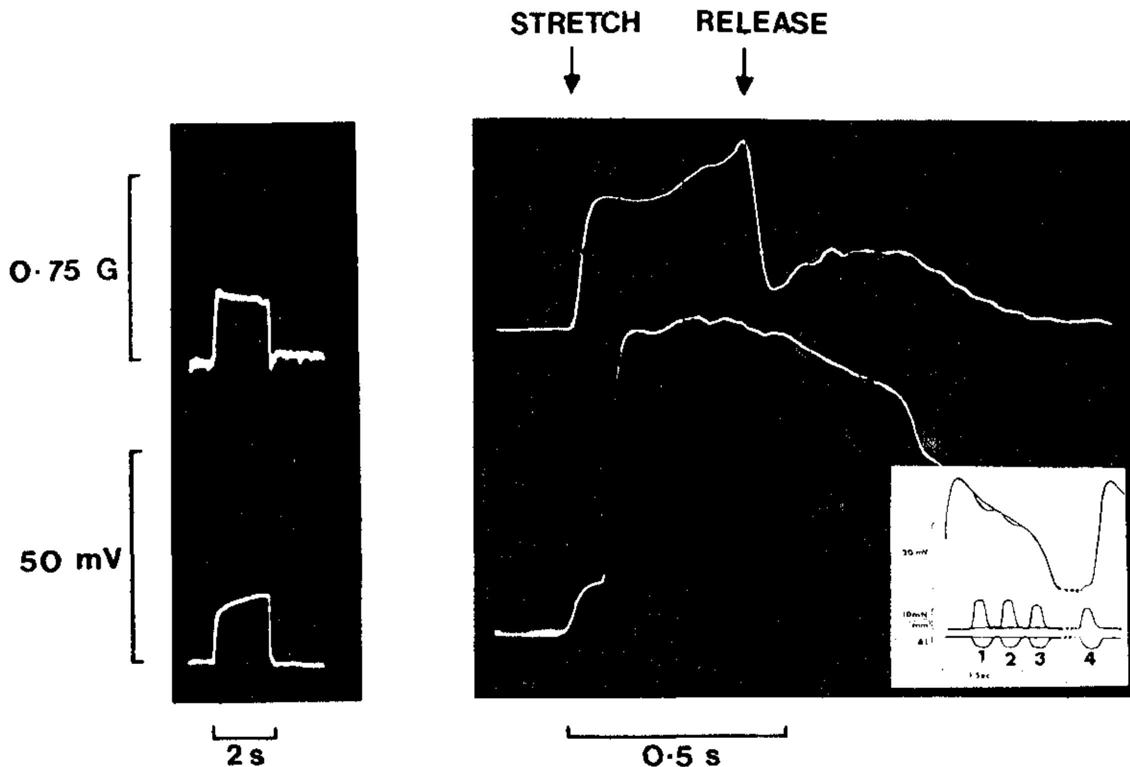


Fig.V7 Effects on tension, and potential recorded with microelectrodes, of stretching frog ventricular strip at rest.

Left hand side. A small transient stretch, to about 80% L_{max}, produced an increase in passive tension (upper trace) and an apparent depolarisation (lower trace) both reversible and of the same duration as the stretch.

Right hand side. The apparent depolarisation (lower trace) was accompanied by a stretch-induced action potential which activated the muscle (upper trace). The stretch, to L_{max}, invariably disturbed the impalement even if the muscle was not released during the induced contraction. Stimulation frequency, 30/min; Temperature 19°C; muscle slack length, 2.5 mm; wt. 3.2 mg. (These records were obtained in Dr B.R. Jewell's laboratory at University College London, with the assistance of Dr M. Boyett).

Inset. Effect of transient stretches at different times on recorded potentials.

Monophasic action potentials, obtained with an insulated gap technique (upper traces calibration bar, 20 mV) tension (middle traces calibration bar, 10 mN/mm^2) and length (lower traces calibration bar, Lo to L_{max}) recorded from an isolated strip of frog ventricle. The recordings are taken over 5 consecutive beats. Stretches 1 and 2 during the early and middle of the plateau phase of the action potential at all. Stretch 4, however, during the resting phase produced an apparent depolarisation that was accompanied by a new action potential

be given at different times in consecutive action potentials. Brief repolarizations were seen during the action potential and transient depolarizations were again produced with stretch which could also be accompanied by new action potentials as if the depolarizations reached threshold (inset Fig. V7). There was a period during activity in which a stretch produced no electrical effect.

Changes in ECG in intact ventricle.

The Q - T interval and T-wave of the electracardiogram represent the extracellular voltage manifestations of the repolarization wave through the ventricle wall as a whole, and, as has been seen, mechanical changes are associated with alterations in cellular repolarization.

If therefore different segments of ventricular wall have different mechanical behaviour during isovolumic contraction compared with isotonic, then contraction under the two conditions should produce different repolarizing rates and vectors. That is, the time course of the repolarization wave through the intact heart (Q - T interval) may be altered. The localized changes in action potential should therefore be reflected in the electrocardiogram and Fig. V8 shows an example of this. The superimposed tracings of the action potentials shows both the steepening of plateau (accelerated initial repolarization) and depolarizing after potential previously described with isovolumic contraction. The associated electrocardiogram has a shorter Q - T interval and reduction in T-wave amplitude compared with auxotonic contraction.

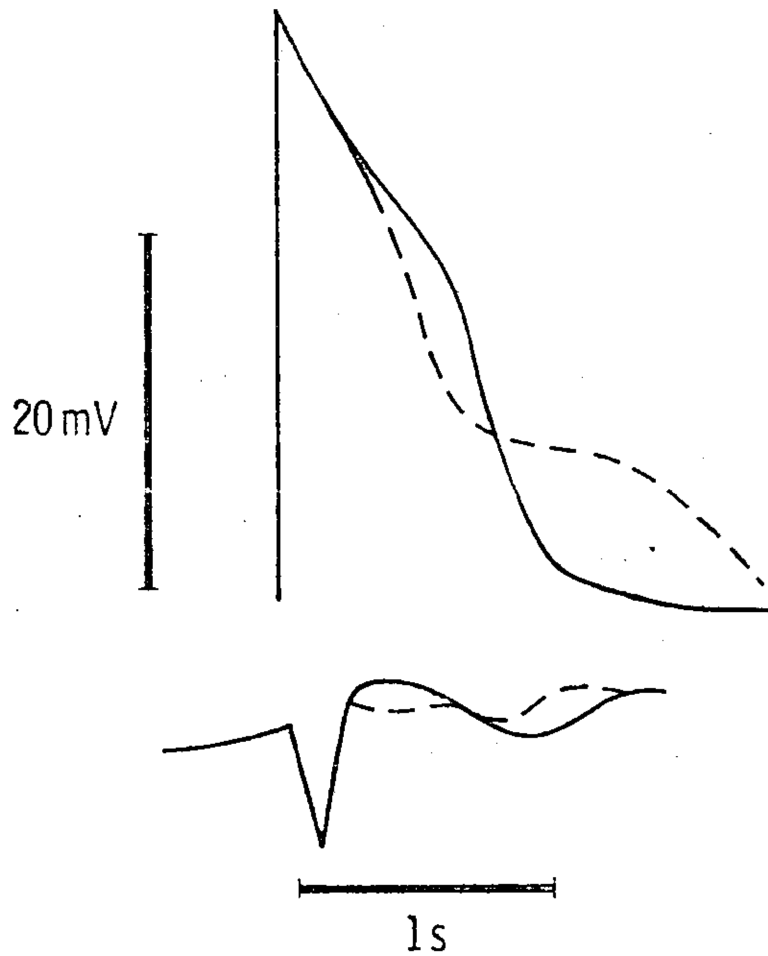


Fig. V8. Superimposed traces of electrocardiogram (bottom trace) and action potential (top trace) during isotonic contraction (solid lines) and isovolumic contraction (dotted lines). The action potential shows the previously observed initial acceleration of the plateau phase followed by the depolarizing potential. The E.C.G. of the isovolumically contracting ventricle has a shorter Q-T interval and a lower amplitude T-wave than the auxotónically contracting ventricle.

Simulated mechanical and electrical changes

(Intraventricular injections)

The changes in segment length seen in osovolumic contraction do appear to be associated with alterations in the shape of the action potential. Further, imposed changes in length of an isolated strip of myocardium affect the recorded potentials. It is possible therefore that similar electrical effects might be generated if some of the mechanical changes in the epicardium were produced artificially. An experiment was therefore performed in the intact ventricle analogous to that in the isolated strip (see inset Fig. V7). This was done by injecting small volumes of Ringer into a ventricle contracting isovolumically at a small diastolic volume. The ventricle was not distended beyond its normal diastolic volume and the injections, followed by withdrawals, were given at different times during the cardiac cycle. The results are seen in Fig. V9. In all the sections of this figure the first contraction took place at a small, unchanged intraventricular volume during which time the epicardial segment contracted, the intraventricular pressure rose and the action potential had a normal configuration. When the injection and withdrawal was given early in the cycle, V_2 in Fig. V9, stretching the segment to L_2 and increasing the intraventricular pressure to P_2, there was a transient decrease in the amplitude of the plateau of the action potential (A_2). If the injection was given near completion of repolarization, the potential change had the opposite polarity producing a transient depolarization (Fig. V9). It is clear that a period should exist during the action potential when an injection has no effect. A moment of transition, as it were, between the early repolarizing and later depolarizing effects of the injection. Fig. V9

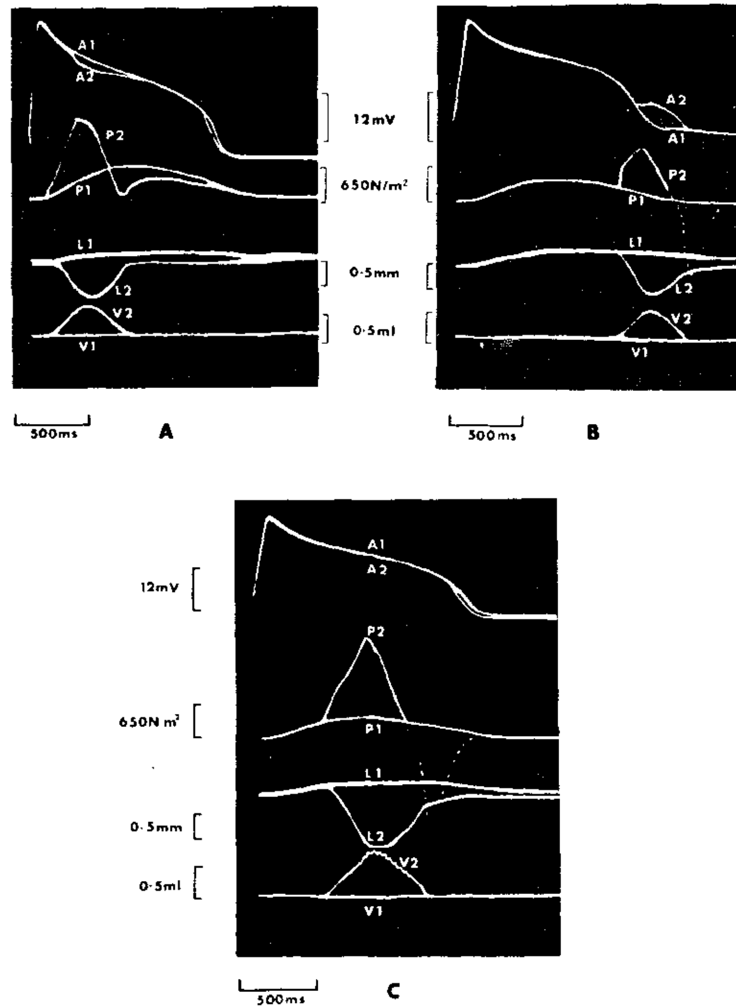


Fig.V9. Parts A, B and C. Effect of transient increases of intraventricular volume at various times in the cardiac cycle during normal isovolumic contraction. In each part of the figure, V_1 is a small intraventricular volume (end systolic); L_1 the change in length of the epicardial segment (upward deflection is shortening); P_1 intraventricular pressure; A_1 action potential with normal time course. Again in each part, a transient rapid increase in intraventricular volume, V_2 produces the mechanical effect of lengthening the epicardial segment L_2, and increasing the intraventricular pressur, P_2. But although the mechanical changes in each part of the figure have the same direction, the effects on the action potential differ: transient repolarization when the injection is given early in plateau phase, (A_2 in part A); and the opposite, namely transient partial depolarization when the injection is given near completion of repolarization, (A_2 in part B). Between these times an even larger injection has no effect (A_2 in part C). The dotted line in B and C on the pressure trace is an artefact due to undershoot of the pressure transducer on rapidly withdrawing from the ventricular chamber.

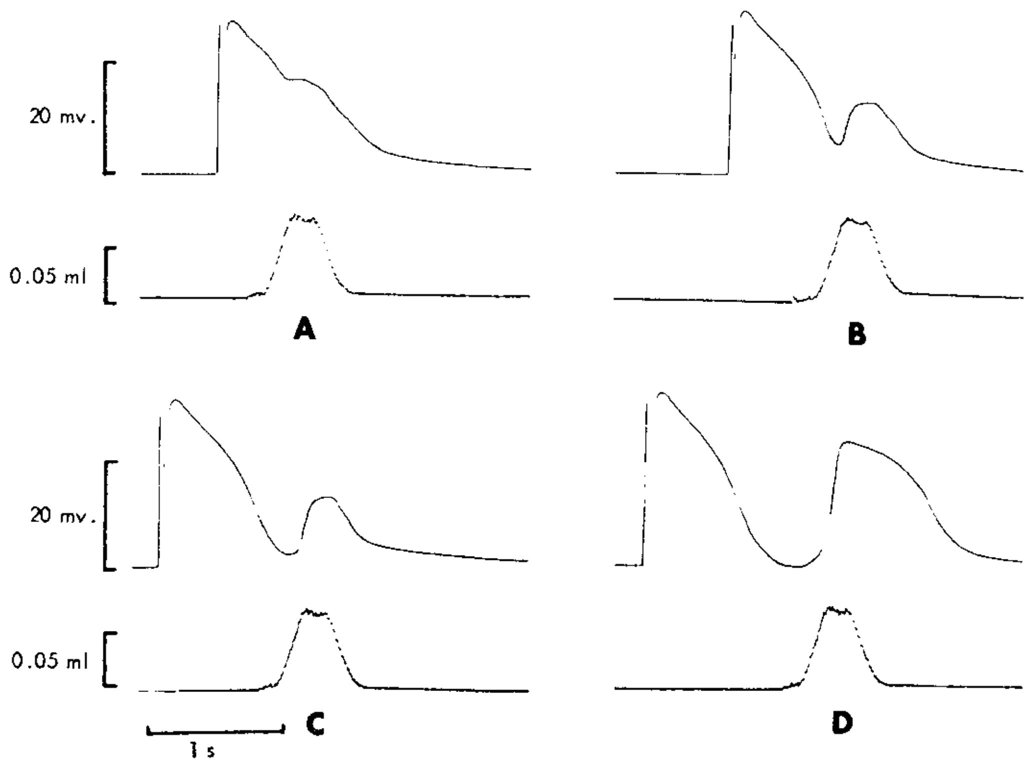


Fig.V10. Effect of a transient increase in intraventricular volume (lower traces in ABCD) at four different times during the action potential (top traces). The depolarizing potential becomes progressively more distinct as the increase in volume takes place later during the repolarization phase (Parts A, B and C). In part D, however, the depolarization is immediately followed by a new action potential.

demonstrates a period during which even a larger injection than in Parts A and B has no effect on the plateau of the action potential. The depolarizing potential seen in Fig. V9 may also be associated with the generation of a new action potential, mechanically induced, as was also observed with the isolated strip (Fig. V 7) This is demonstrated in Fig. V10. The earlier of the intraventricular injections (Part A) is associated with a relatively small depolarizing potential as compared with Parts B and C where the injections were given later in the cycle. However the mechanically induced depolarization in Part D is accompanied by a new action potential. The epicardial and isolated strip studies thus largely compliment each other.

The investigations centre on the paradoxical movements of epicardial segments during contraction. Spontaneous lengthenings during isovolumic contraction are observed, mimicked by externally imposed increases in volume, and are accompanied by changes in action potential. The changes during the plateau phase, per se, are directional and a longer or lengthening segment has a repolarizing tendency compared with a segment that shortens, which has a longer duration action potential (polarizing tendency). In Chapter IV a cat papillary muscle shows a prolonged action potential when the muscle is allowed to shorten and, when the muscle contracts isometrically, an acceleration of repolarization. To make a more direct comparison between the former results and the present investigation a ventricle was made to contract isovolumically at normal end diastolic volume (Fig. VII). During the next action potential the ventricle was emptied and immediately refilled on completion of repolarization. The duration

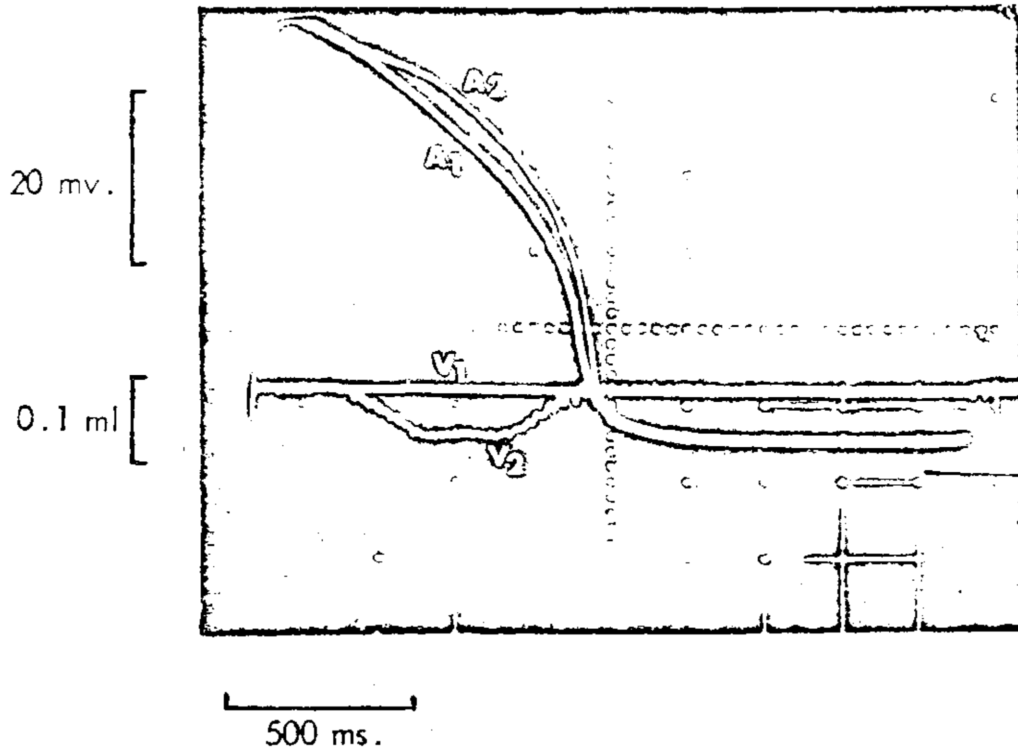


Fig. V11. Effect on action potential of reducing ventricular volume. The ventricle was made to contract isovolumically at its normal end-diastolic volume. (V_1). The action potential at this volume was A_1. During the next contraction the ventricle was rapidly emptied to V_2 and then rapidly refilled. The action potential duration increased to A_2 during the change in volume.

of the action potential associated with the empty ventricle is prolonged as compared with the action potential of the ventricle with the greater volume, where repolarization is accelerated.

DISCUSSION

It is suggested, for reasons outlined below, that the observed changes in recorded potential, following the mechanical changes, are the result of changes in ionic currents. The changes in duration of action potential are probably a true reflection of cellular events, for first, suction electrodes are a reliable method of following the time course of the repolarization phase of the cardiac action potential, and secondly both the insulated gap and intracellular recordings also show changes in duration. In addition the electrocardiogram shows changes in Q-T interval and T-Wave, both manifestations of altered ventricular repolarization as a whole (see also Stauch 1960 cited in Stauch 1966) These ECG changes cannot be simply explained by a shift in direction of the repolarization vector in relation to the recording leads, for example by the rotation of the heart during contraction, for the T-Wave and Q-T interval changes are always in the same direction, i.e. smaller on isovolumic contraction at normal end diastolic volume. Furthermore, the Q-T interval per se is a chronological measurement and is unlikely to be significantly influenced by a change in amplitude or direction of the repolarization vector. The changes are therefore more likely to be due to a true alteration in the time course of the ventricular repolarization gradient.

The depolarization is more difficult to interpret, for although it was seen using three different recording techniques, each depolarization could be explained by recording artefact. The depolarization seen with the suction electrode could be a manifestation of a distortion at the tissue-electrode interface and altered current flow between the recording and indifferent electrodes. The changes in resting potential observed with microelectrodes could be the electrode leaving and re-impaling the cell. In fact stable resting potentials have been observed despite changes in length (e.g. Fig. V6). To explain the last observation one would have to postulate that a significantly larger extension of the muscle is needed to alter the resting potential than to change the action potential duration. This possibility is qualitatively in keeping with the graph in Fig. V3, and the relatively large extention required to depolarise the muscle to threshold (inset Fig. V7). Finally the mechanical distrubances, using the insulating gap technique, may produce changes in current flow in and around the gap and thus simulate depolarization.

However, the implications of stretch induced depolarizations in ventricular muscle are important. Therefore in view of the above pitfalls in interpretation additional points in favour of the depolarization not being artefact are worth summarising even though these points should be treated with caution. To begin with the depolarizations observed with stretch are in keeping with other observations in Purkinje muscle (Deck 1964; Kaufmann and Theophile 1967) and in papillary muscle (Kaufmann and Theophile 1967) using microelectrodes. Stretch also produced depolarization capable of reaching threshold in ventricular muscle (Kaufmann and Theophile 1967) (the last two works were not concerned with action potential durations per se).

Furthermore, predictions on the expected behaviour of the myocardium, based on the results being a true reflection of changes in resting membrane potential, are verified by two observations in these present experiments. First it is significant that the depolarizing effect, observed using all three recording techniques when the muscle elongated during relaxation, can apparantly reach threshold and be followed by an "extrasystole". Secondly, this new action potential often has a reduced amplitude, as is expected when the membrane is partially depolarized. One cannot exclude the possibility however that "stretch-sensitive" cells of the pacemaker type may conceivably be found in frog ventricle. If one allows that the length of the segment of muscle fibres are adjusted to be near the physiological optimum (L_{Max}), then the final length reached when the segment was stretched, for threshold depolarization, agrees with that found in the isolated ventricular strip.

The above observations and arguments taken together with previous experimental work strongly suggest that the recorded electrical changes between isovolumic and free-loaded contractions are a true reflection of altered current flow across membranes and, considering the electrocardiographic changes, between groups of cells in the intact frog ventricle. Furthermore the epicardial movements under these two conditions show that the mechanical behaviour between groups of cells also differs. This is simply because epicardial fibre lengthening in one section of the mycocardium, when taken together with a rise in intraventricular pressure, necessitates shortening and tension development in other sections of the myocardium. These experiments are not as far removed from the physiological situation

as they appear. In fact comprehensive studies of epicardial strains had been carried out by Fischer et al (1966) and Dieudonne and Jean (1969) who also found positive strains (lengthening) during the normal isovolumic phase of contraction of intact dog ventricle. The segments thereafter showed some shortening during isovolumic relaxation.

Having demonstrated that both mechanical and electrical behaviour in a particular segment alters at the same time, the question arises as to whether they are casually related and if so, what mechanisms are involved. It is clear from the stretches in isolated strips (Fig. V 6,7) as well as the intraventricular injections in Figs. V9 & 10 and other experimental situations (Chapter IV) that the mechanical change precedes and thus is somehow related to the electrical change. This observed time relationship together with the evidence described above, are compatible with the existence of a "mechano-electric coupling" in ventricular cardiac muscle. Some "contraction-excitation-contraction" coupling has been introduced in Chapter IV,-see also Kaufmann et al (1971). Keeping cat papillary muscle at a longer (isometric) length results in a abreviated action potential duration as compared with a muscle that is allowed to shorten (isotonic contraction). The direction of electrical change is identical to the present studies where isovolumic contraction is accompanied by a shorter action potential than an auxotonic contraction. The switch from isometric to isotonic contraction in the papillary muscle studies resulted in a small positive mechanical staircase. This mechanical transient may in part be attributable to the same mechanism that causes the transients when the action potential is electrically prolonged (Antoni et al 1969).

Small pressure or segment length staircases were occasionally observed in the intact frog when altering the mechanical conditions of contraction. But to determine whether these small mechanical changes are related to ionic fluxes across the membrane or to the visco elastic properties of cardiac muscle needs further investigation. In this context it is worth noting that an electrical prolongation of the action potential in frog only affects the immediately accompanying contraction in frog myocardium and not the subsequent beats (Antoni et al 1969) i.e. no tension transients follow. It may therefore be difficult to ascribe the transients that were occasionally seen in these experiments to changes in action potential per se.

For the sake of completing this section it is appropriate at this stage to begin speculation as to what architectural alteration, cellular or molecular, could take place during the imposed mechanical disturbance to influence the membrane potential. It is significant in this context that both the active state (tension development) and membrane conductance, are altered by changes in membrane potential. The calcium ion implicated in both active state and membrane phenomena (Langer 1973, Morad and Goldman, 1973) could play a role and the time course of repolarization influenced by change in membrane properties or internal calcium ion kinetics as a result of the mechanical changes. This change in active state and internal calcium would in turn affect ionic flux across the membrane either directly or indirectly. There is in fact considerable evidence that calcium release in muscle may be mechanically dependent or length dependent (see Jewell 1978 for a review) and that changes in internal calcium can alter the potassium outward current (Bassingthwaight et al 1976.) One could speculate further that the calcium ion could be related to the depolarization

observed when stretching the muscle at rest; again either by direct or indirect implication. Gordon and Ridgeway 1976 have found in the single barnacle muscle fibre a length dependent electromechanical coupling in which a depolarization produced by stretch is calcium mediated. It is thus conceivable that mechanical stress or strain in the membranes of the ventricular muscle could alter the ionic permeability of the membranes thus changing membrane potentials.

Alternative hypotheses to the preceding one are more speculative and will be only briefly recounted as they are discussed in more detail elsewhere in the final Chapter. First, bearing in mind the possible involvement of the active state mentioned above, a link between a mechanically induced change in the muscle and membrane phenomena, could be "energetically" mediated either by cyclic AMP, or ATP. Secondly, the immediate extracellular spaces and/or SR may be distorted such that an altered diffusion or accumulation of some ion therein could influence the membrane potential to produce potentials analagous to those described under certain conditions with skeletal muscle.

Further discussion concerning the mechanism of "mechano-electric coupling" must await more direct experimental evidence. However, if there is sufficient evidence at this stage to support the hypothesis that some forms of "mechano electric coupling" or mechanically dependent potential changes are to be found in cardiac ventricular muscle, then it is required of the hypothesis to fit or explain existing observations. This is dealt with in considerable detail in the final chapter but it is

relevant here to outline the possible role and context of this phenomenon in cardiac ventricular muscle. First, it has been suggested that there is a localised control system, operating on a beat-to-beat basis, in which the length/tension relations of cardiac ventricular muscle may influence the duration of the immediate and ensuing action potentials and active state (Kaufmann et al, 1971; Chapter IV). This mechanism is probably closely related and in fact works in the appropriate direction to be in keeping with the mechanical changes described by Jewell and Rovell (1973). Secondly, the observation that different degrees of shortening or tension development of ventricular muscle have varying rates of repolarization, as indicated by the duration of action potentials, may be one of the determinants of the ventricular repolarization gradient. This repolarization vector (T-Wave) is normally in the same direction as the QRS vector and thus deflects in the same directions in the electrocardiogram. The T-wave direction indicates, in part, that the epicardial fibres repolarize faster than the endocardial fibres and it has been suggested (Lab 1971) that this observation is related to the fact that the epicardial fibres shorten less than the endocardial fibres (Rushmer 1970). This point cannot be more than a suggestion at this stage, for no systematic study has been done on the time course of these action potential changes following mechanical changes, over a prolonged period of time.

Finally, mechano-electric coupling may have important clinical relevance. It is now well established that there can be exaggerated inhomogeneity or ventricular wall contraction after a myocardial infarct. (Tennant

and Wiggers 1935). It is feasible that the ventricular dysynergy which can follow such an episode results in an appropriately timed deforming stretch of the surrounding viable myocardial tissue to produce ectopic beats or extrasystoles, thus facilitating ventricular arrhythmia (Sherf and Schott 1973).

CHAPTER VI

STUDIES OF NORMAL AND ISCHAEMIC SEGMENTS OF INTACT PIG VENTRICLE

MONOPHASIC ACTION POTENTIALS, ELECTROCARDIOGRAMS AND MECHANICAL PERFORMANCE IN NORMAL AND ISCHAEMIC EPICARDIAL SEGMENTS OF THE PIG VENTRICLE IN SITU.

INTRODUCTION

There are serious difficulties in trying to measure mechanical performance in the intact heart and in making comparisons between isolated and intact preparations (Sonnenblick, 1974; Reichel, 1976). Some attempt has been made to bridge the gap between the above types of studies, and mechanical measurements have been obtained from epicardial segments by the use of strain gauges sutured to the heart (Forrester et al 1974; Tyberg et al 1974), or ultransonic crystals implanted in the heart (Bugge-Aspheim et al 1969; Theroux et al 1974). These methods have two disadvantages. Firstly, they are permanently placed, and secondly they usually provide an indication of strain in one direction only. Levers, from potentiometers fixed above the heart, which are not permanently sutured to, but stab the heart, have been used to record segment lengths (Linden and Mitchell, 1960). However these also show undirectional length changes at any one time. For measurements in three directions on the heart's surface a tripodal device has been previously developed (Dieudonne et al 1972) but the feet of this instrument have to be stabbed into the heart and may reach a variable depth. Great care also has to be taken to ensure that the instrument does not fall

off during vigorous contraction of the heart. With the appropriate calculations an indication of the strains in the tissue to which it is attached can be obtained. The electrical changes in epeicardial segments can be obtained extracellularly by the use of wick electrodes on the ventricular surface. Ideally, for measuring electrical events in tissues, microelectrode recordings are desirable. However the technical difficulties of realiably and consistently recording transmembrane potentials in the intact beating ventricle in situ are almost prohibitive (Toyoshima et al 1965; Downer et al 1976b). Further, this technique frequently necessitates the partial immobilisation of the area to be studied which will affect mechano-electric interrelationships (Ch. IV & V; Kaufmann et al 1971; Hennekes et al 1977; Lab 1978). An instrument partly described previously (Lab and Price 1977) was thus constructed for electromechanical correlation in epicardial segments, that overcame some of the above difficulties. The object of this study is therefore, first, to develop a relatively simple but reliable method of monitoring action potentials and epicardial ECG's with mechanical performance of small areas of epicardium and, secondly, use this system to record electrophysiological and mechanical changes in normal and ischaemic myocardium.

METHOD

Experimental preparation

Landrace pigs weighing 20-30 Kg were anaesthetised with intravenous sodium methohexitone (10 mg/kg) after premedication with 1% halothane after pre-medication with intramuscular azaparone (8 mg/kg). Using a ventilator anaesthesia

was maintained with a 1:1 mixture of nitrous oxide and oxygen during the operative phase. The heart was exposed by removing the front of the chest and a pericardial cradle formed to support the heart. Intraventricular pressure was measured with a Statham[R] P.37 transducer via. a silastic catheter (15 cm. long, I.D. 1.2mm) through the apex of the ventricle. Aortic pressure was similarly measured through the carotid artery. A catgut snare was placed around a small branch of the left anterior descending coronary artery and, when tightened, produced a cyanotic area about 1.5 cm x 3.0 cm. These small ischaemic segments produced no change in heart rate, peak dp/dt or ventricular pressure in most cases. During some of the experiments halothane was discontinued and a 1% solution of chloralase administered as necessary. Arterial blood gases were monitored throughout the experiments and ventilation adjusted to maintain them within normal limits.

Construction of tripodal device.

A tripodal device modified from Lab and Price (1977) is attached by vacuum through the legs to the area of the epicardium to be made ischaemic. Each leg of the tripod (Fig. VI-1) consists of a thin strip of phosphobronze shim (5mm x 18mm) with the lower end bent horizontally forming a $5mm^2$ foot. A strain gauge (micro-measurements -09-03 IDE - 120) is glued to the upright part of the leg and the collar of a 5mm length of 'perspex' tube, [I.D.,I. 5mm; O.D., 3mm] is inserted through a hole inthe horizontal part. A short piece of flexible vinyl tubing (I.D. 0.75mm. O.D. 1.5mm) is then placed over this collar. Each of these three pieces of flexible tubing are glued to a vacuum manifold which consists of a 10mm. hollow perspex cylinder (I.D. 2mm O.D. 4mm) closed at both ends.

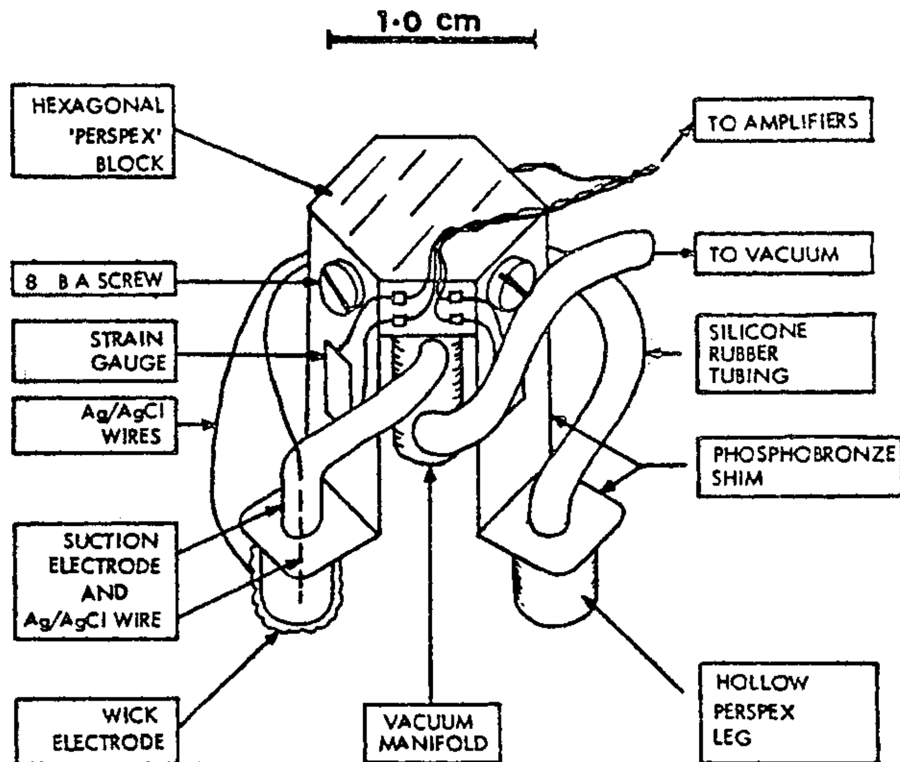


Figure V-1

Diagram of tripodal device used in mechano-electrical studies. The three legs (third one obscured by vacuum manifold in the diagram) are attached to the surface of the heart by vacuum. The movement of the legs deform strain gauges giving three different outputs. One of the legs is both a suction electrode for monophasic action potentials, and a wick electrode for epicardial ECG's.

The cylinder is connected to vacuum (approx. 400 mmHg = 50-60 K Pa), via a long piece of flexible tubing. One end of this cylinder is glued to the underside of a hexagonal perspex plate (diameter 11mm, thickness 3mm). The three legs of the tripod are screwed at the top to alternate edges of this plate, overlapping it by three millimetres. This leaves 10mm of the vertical section of the leg to bend in response to movement of its base and to thereby deform the strain gauge. The remaining three faces of the hexagonal plate have solder tags glued to them for attachment of the wires from the gauges and amplifiers. The arrangement above leaves the feet of the tripod on a 16 mm pitch circle. The instruments weigh 2.0 - 2.5 G.

Calibration of tripod.

Movement of a foot, relative to the baseplate, produces an output from the strain gauge which can be calibrated in units of length as shown below. The horizontal distance from the centre of the baseplate to each of the feet is 7 mm and the unstrained output of the strain gauge is allocated this value. The calibration of the instrument is then carried out by moving each leg in turn along its axis of movement (from the foot to the central point of the baseplate) in 0.5mm steps over the range of values encountered experimentally (6.5 to 8.5 mm). During this procedure, the central point of the baseplate is fixed and the feet are attached in turn, to a calibrated precision screw. The device output is linear over this range to with $\pm$ 5%.

Some performance characteristics.

The compliance of each leg is tested by applying forces along the axis capable of producing the length changes described above, and is

between 0.1 - 0.2 mm G^{-1}. The compliance perpendicular to the axis is unobtainable with the forces used and is less than .0004mm G^{-1}. With any given force, therefore, the output of a gauge should vary with the cosine of the angle between the direction of the force and the axis in which the leg moves (Fig. VI - 2A). To test this the tripod is placed with the feet in the same vertical plane and a weight hung from one of them while the device is rotated (Fig. VI - 2A). The actual putput (Fig. VI - 2B) varies as predicted.

Detailed geometric analysis of the signals from the tripod in use could indicate the true magnitude and direction (vectors) of the length changes (Dutetre et al 1974) however our interest is not in these absolute values, but rather in detecting changes in overall contractile behaviour. We have arithmetically summed the three leg outputs continuously, via summing amplifiers, to indicate this overall behaviour and accepted an inherent error. We could reduce this error by merely rotating the tripod on the heart until we obtained a maximum displacement from one of the legs, which would ensure that the axis of maximum shortening was parallel to that leg. But in practice it is better to place the tripod to match the shape of the infarct, and to avoid any underlying vessels.

In order to display the length changes in relation to the phases of the cardiac cycle, we have constructed pressure-length loops (Tyberg et al 1974) which are in some respects analgous to the pressure-volume loops used to describe ventricular function.

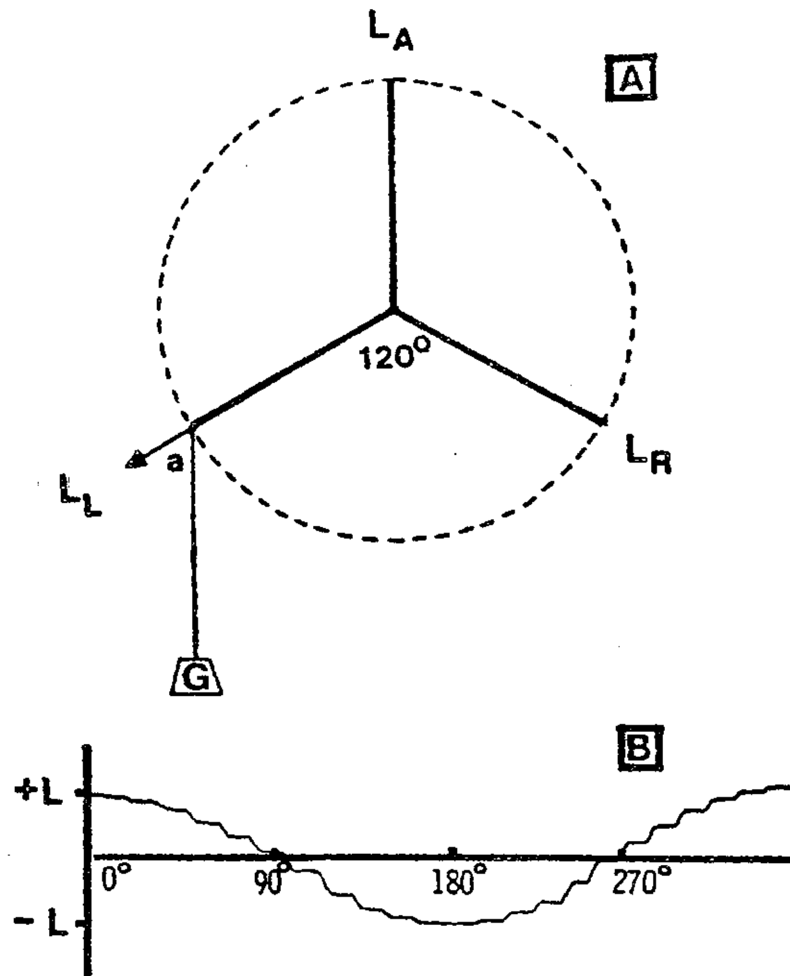


Figure VI-2

Method used for determining some of the performance characteristics of the device.

Part A. The device is rotated while a force (G) is applied to one of the legs(L_L). Since the angle between the legs is fixed at 120°, angle a varies between 0° and 360°, and the resultant force vector along the axis L_L varies as the cosine of this angle.

Part B. The actual voltage output (force vector) during the procedure in Part A. The output is a cosine wave with maximum output when the force is along the axis of the leg movement and zero output when perpendicular to it. Shortening is upward.

Using a moving coil driven by a signal generator and amplifier the frequency response of the tripodal device is found to be flat up to 50 Hz. Occasionally oscillations approaching this frequency, are obtained from the tripod in use. They are likely to be artefacts produced by wobbling of the device during rapid movements of the whole heart.

Suction electrode and epicardial ECG.

The suction electrode consists of one of the legs of the tripod, or a separate similar hollow perspex tube. A thin Ag/Ag Cl wire passes into a leg through the wall of the flexible tubing and makes contact with the epicardium directly (Churney and Oshima 1964) or by KCl injected into the leg before suction is applied (Szjekeres and Szurgent 1974). This wire forms one input to a differential amplifier while the other input is derived from a second, external, Ag/Ag Cl wire electrode in contact with cottonwool glued around the outside wall of the suction electrode. The two inputs are thus never more than 1.0 mm apart. This cottonwool also provides the epicardial ECG electrode which forms a stable contact with the epicardium. The petechiae from the suction are not a source of ectopic impulse formation and nor do they affect the mechanical performance of the segments. Using the suction and wick electrode we are able to monitor monophasic action potentials and epicardial electrocardiograms from virtually the same areas as the mechanical records are obtained. In practice, these electrical records have the configuration expected of them and the injury produced by the suction has little effect on the electrocardiogram.

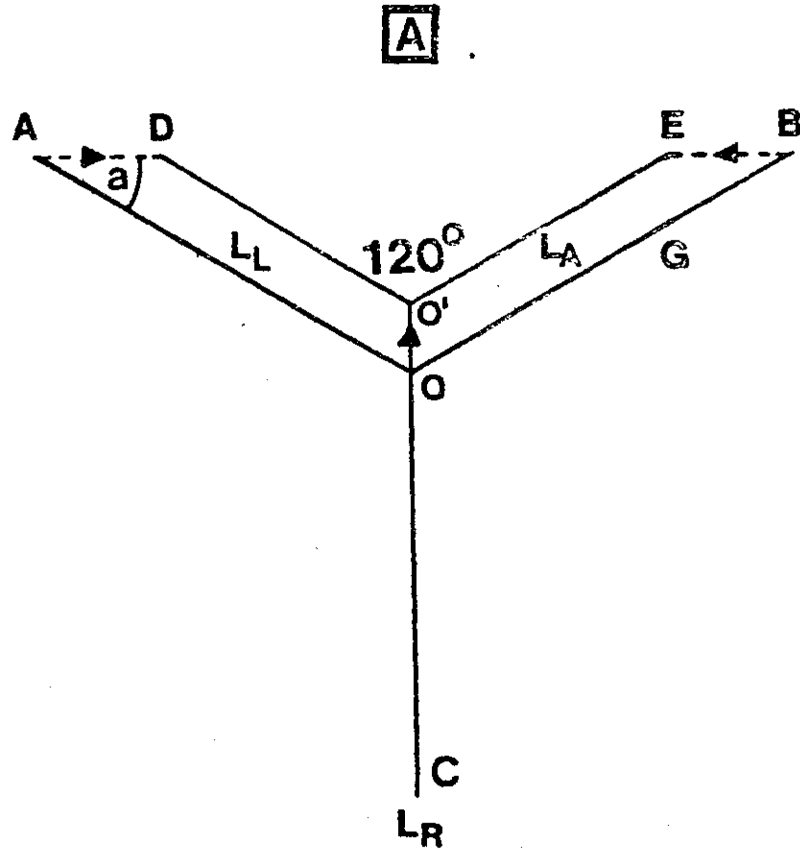


Figure VI-3

Part A Analysis of the behaviour of the tripod when epicardial shortening (horizontal arrows) occurs at 30° (angle a) to two of the axes. Because the angles between the legs are fixed, a change in the direction AB, of (AD + BE (2Y in the text)) results in the position of the tripod moving from ABC to DEC with the centre 0 moving vertically to 0'. Simple calculation shows that L_A and L_L produce an output less than if the length change were along the axes. However L_R will show an artefactual lengthening. Summing the outputs will produce an over-all small underestimate.

RESULTS

Recording of normal wall motion.

The legs of the tripod are orientated at 120° to each other and for practical purposes, can only move along these axes. Whatever the change in shape of the underlying epicardium, the legs can move along their axes to adopt that shape. However, in doing so, the central point of the tripod may be required to shift in relation to the epicardium, thereby partly invalidating the output of the individual gauges as a true measure of the epicardial segment length in the direction of the leg movement. This shift of the central point will not occur if there is symmetrical expansion and contraction of the epicardium, but will occur if the underlying length change is predominantly in one direction and is not parallel to any of the tripod axes. Such a situation is illustrated in Fig VI - 3A where the length change is a shortening entirely in one direction at 30° to two of the axes. The tripod moves from its resting position ABC to its new position DEC - with the angles maintained at 120°. If the true length change in the direction AB is 2Y then L_A and L_L will both show a reduction in length of Y/cos 30° and L_R will show an increase in length of Y tan 30°. The combined outputs of the three legs will therefore show a change of ($2Y/\cos 30^{\circ} - Y \tan 30^{\circ}$) which is approximately 1.75 Y ie. an underestimation by 12½% of the true figure. From these theoretical considerations we would expect to observe a variation in the summed length change of not more than 10-15% when rotating the tripod through 30° on an area of epicardium which did vary in length largely in one direction. This experiment is demonstrated in Fig. VI - 3B . In Fig. VI 3B and C. the individual outputs are displayed in the conventional manner showing marked variations in the three "directions", at the three

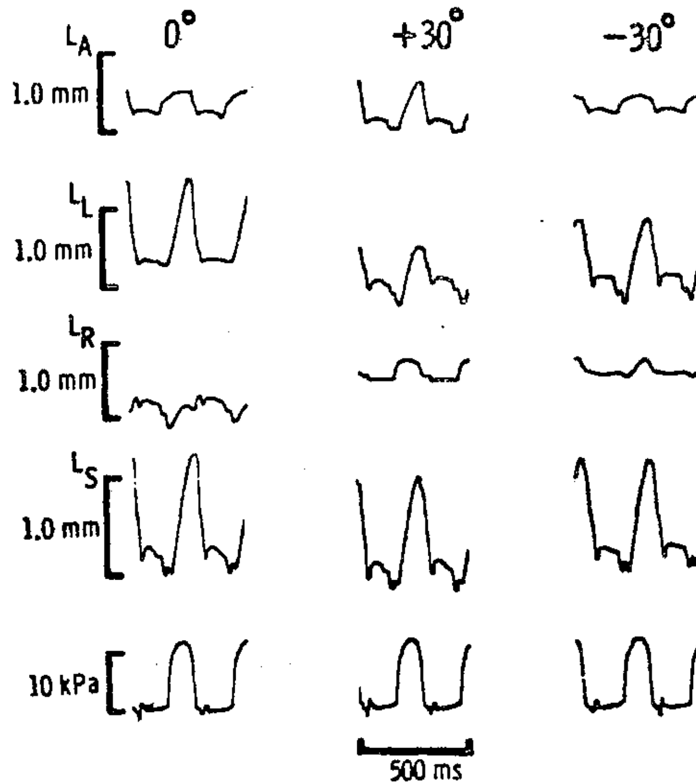


Figure VI-3

Part B Mechanical outputs of the tripod at 0°, + 30° and -30° over an area of epicardium with different contraction patterns along different directions.

Shortening (upward) of the individual segment lengths, and their sum, at the three positions. Despite the differences in the outputs along the three axes, L_A, L_L and L_R, summed lengths L_S produce length changes with the small errors that are within the limits expected of the device. Lowest trace is the intraventricular pressure.

positions, but the summed outputs are remarkably similar. In Fig. VI 3C we have displayed pressure-length loops (intraventricular pressure plotted against summed outputs) derived from such a situation to show the phase relationships. Clearly the loops at the three positions are similar. Artifactual recordings due to inertial forces moving the centre of the tripod (inset Fig. VI 3C) would account for the "wobble" during diastole just prior to the rise in pressure. This artifact does not affect the area within the loop which may be taken as an index of segment "work" (Tyberg et al, 1974). Thus selecting the ejection period (when myocardial forces are large) to be reliable for measurement of length changes, then the overall ejection shortening varies from 1.0 mm. at the zero position to 0.9 mm at $+30^{\circ}$ and 0.9 mm at -30°.

Recording of ischaemic wall motion.

The question arises as to whether the method of mechanical recording has any advantage over previous methods. In Fig. VI - 4 we have displayed length changes recorded from the legs during a five minute period of ischaemia on the anterior surface of the left ventricle. The ejection period has been visualised by reference to the aortic pressure trace. During the control period, the greatest length changes occur in L_A (ejection shortening of 0.6 mm) and this leg is therefore likely to be closest to the axis of maximum epicardial length change. The 'paradoxical' lengthening of L_L during the ejection period could then be explained as being artefactual by the mechanism described above. On occluding a small branch of the left coronary artery, the major length change (ejection shortening 0.25 mm) now occurs in L_L, while L_A and L_P both show some lengthening

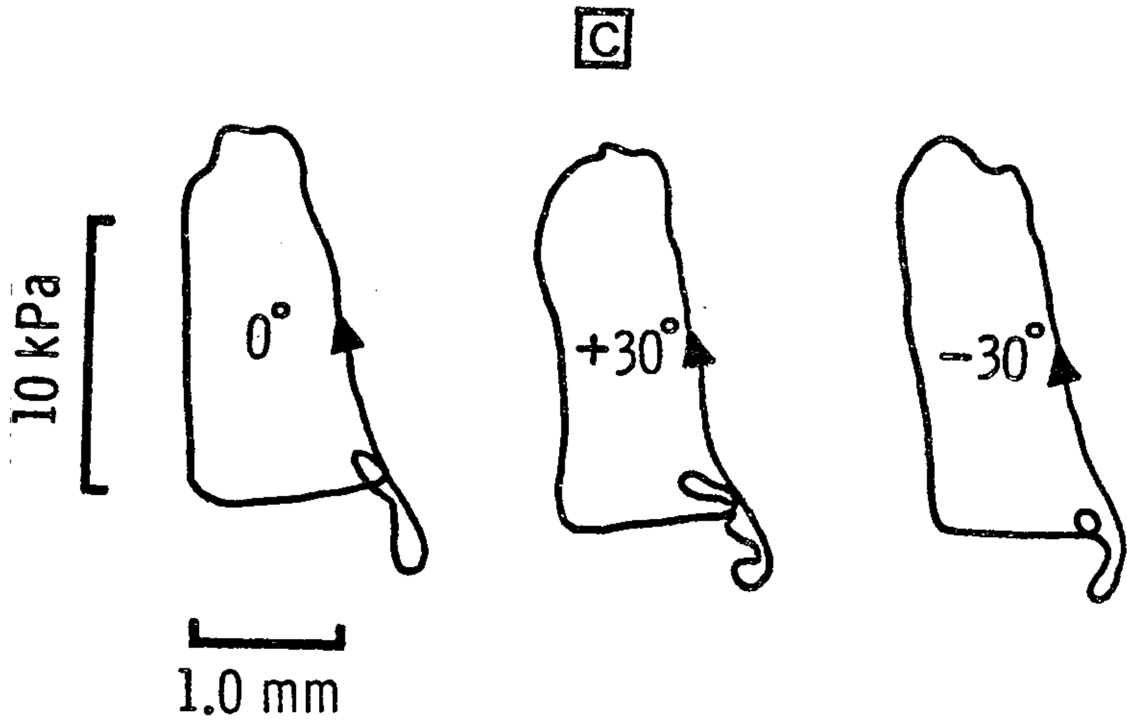


Figure VI-3

Part C Pressure-length loops using the summed lengths, showing very similar phase relations at the three positions.

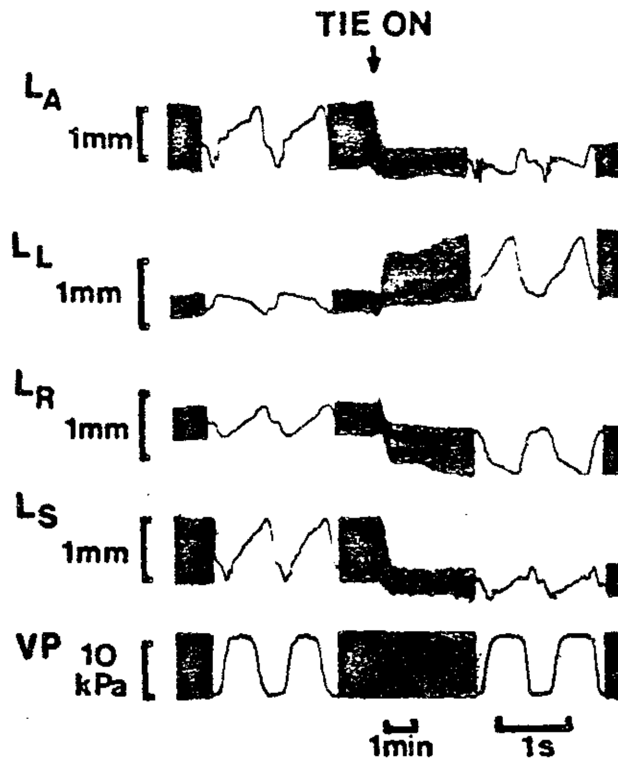


Figure VI -4

Length changes recorded by the device during occlusion of a small coronary artery. The records L_A, L_L and L_R show that there are directional as well as quantitative changes in motion in the epicardial segment. Monitoring in the direction of any of the axes in isolation would not have provided a true record of the overall mechanical behaviour of the underlying myocardium. The summed length records (L_S) however, reveal the major length change during the onset of ischaemia. Despite the loss of directionality and the underestimate in recording the length changes, the summed lengths detect the qualitative change in mechanical behaviour. VP is the intraventricular pressure.

during the ejection period. The three outputs, L_A, L_R, and L_L are summed in the lower part of Fig. Vl - 4 (L s). Although the directionality is lost this record clearly shows some ejection shortening during ischaemia which would not have been detected if length changes only in the direction of maximum normal shortening (L_A) were used for the study. The summed leg recording shows ejection period shortening of 0.8 mm during normal perfusion and this falls to about 30% (0.25mm) of that value 4 minutes after coronary occlusion.

Monophasic action potential and S-T segment recording.

The epicardial ECG is recorded from an area immediately surrounding the point of suction which produces a small hemisphere of injury. Using the multiple dipole theory all the electrical vectors radiate outwards from the hemisphere and mostly cancel each other except for those opposite the point of suction. The resultant vector should be small. To facilitate visualising just how small this potential should be, the solid angle theory is used (Holland & Arnsdorf 1977) Fig. Vl - 5A The ECG electrode is at point P which is outside the injury produced by the suction. The potential at each of an infinite number of points surrounding the injury may be described by the relationship: $E = K(V_m - V_{in}) \Omega /4$, where K is interstitial conductivity, V_m is the normal membrane potential V_{in} is the transmembrane potential at the site of injury produced by the suction and Ω is the solid angle. This angle is given by an area which is cut off the surface of a unit sphere surrounding each of the points P. The area is determined by lines drawn from the points P to every point on the "boundary of interest" (not the injured tissue mass) having current flow due to $V_m - V_{in}$. The various values in the above equation remain constant on a beat to beat basis

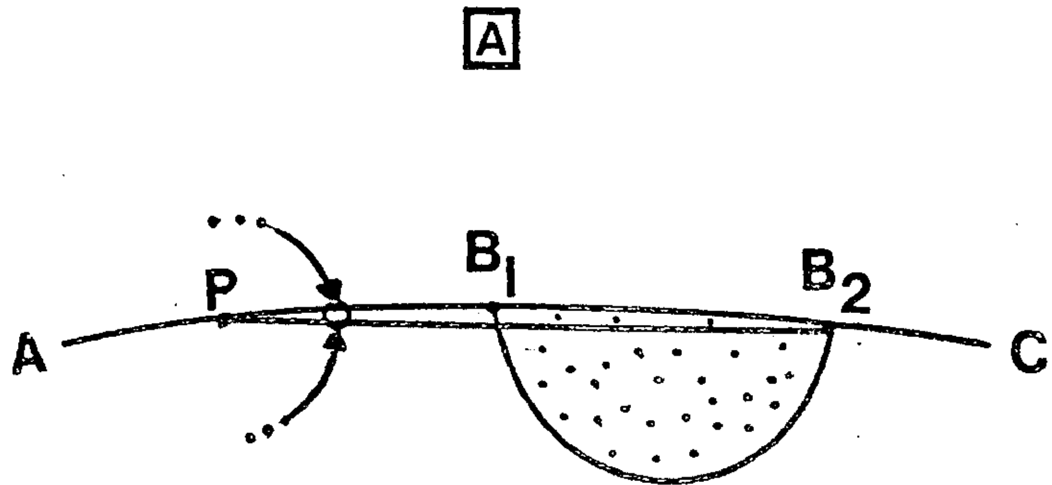


Figure VI - 5

Part A Diagrammatic representation of method used to obtain the solid angle and thus the potential at point P. Curvature A to C represents the epicardium and the stipled area is the injury produced by the suction electrode. Point P is the epicardial ECG surrounded by the partially dotted unit sphere. The solid angle, indicated by the two arrows, is small; being formed by lines from the boundary of interest B_1 and B_2 to point P. (In practice, the distance between B_1 and B_2 is 2-3mm and P is lmm away from B_1. The radius of curvature of the arc AC is about 5 cm. The potential at point P is thus very small).

and as P and the boundary of interest are close and both lie on the surface of a sphere with a relatively large radius of curvature. E is small because Ω must be very small. These predictions may be put to the test by a simple experiment, showing that the electrical records obtained by the epicardial electrode have the configuration expected of them and the injury produced by the suction has little effect on this ECG. While recording the epicardial ECG (Fig. VI 5B) vacuum is applied and the monophasic action potential obtained with relatively little distortion of the ECG. There is a small reduction in the R-wave and a small elevation of the S-T segment both of which are predictable and these observations are consistent in all the records obtained using this method.

The action potential has a normal configuration with a reduced amplitude and rise time as compared with transmenbrane action potentials. The durations of repolarization, however, are comparable (Hoffman et al 1959). Thus, although some qualitative interpretations of changes in amplitude and base line can be made occasionally, change in duration of action potential is the measurement which can be most reliably used. In a simple experiment we tested the validity of this assumption and altered the temperature of the area from which the electrical recordings are made since microelectrode recordings have previously shown changes in duration of action potentials under similar circumstances (Toyoshima et al 1965). Local cooling of the myocardium resulted in a prolongation of the action potential whereas warming the area abbreviated it. (Fig.Vl - 6A). Simultaneous epicardial ECGs showed that cooling lengthened the Q - T interval and made the T-wave more negative. Warming the area produced the

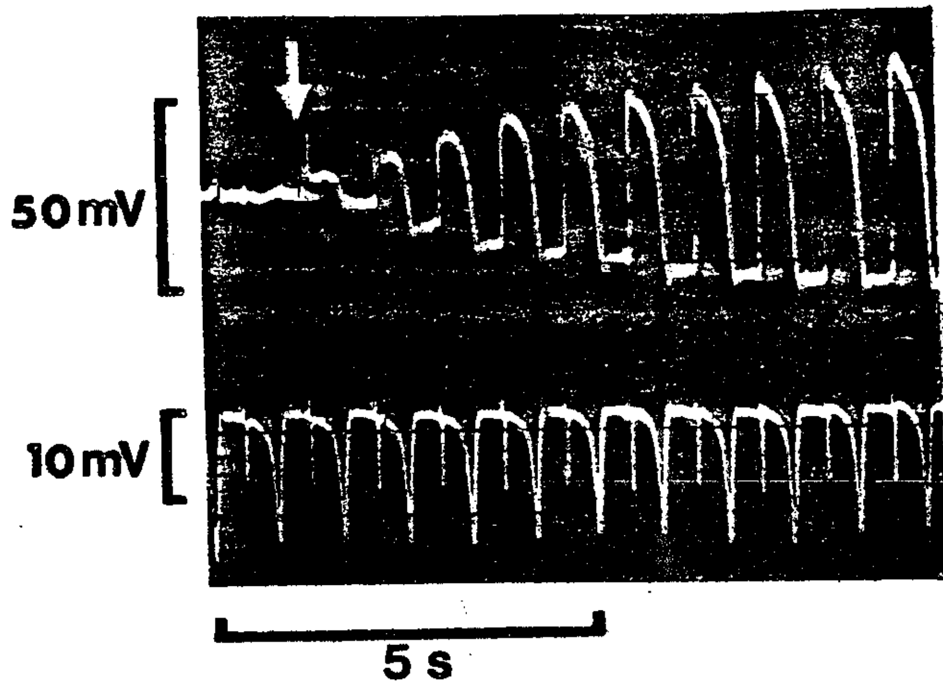


Figure VI-5

Part B Simultaneous recordings of monophasic action potentials (upper trace) and epicardial electrocardiograms (lower trace) while suction is applied for obtaining the monophasic action potential. The injury produced by the suction causes little distortion of the epicardial electrocardiogram despite the close proximity of the two recording electrodes. (These particular records were obtained from electrodes which were not part of the tripod).

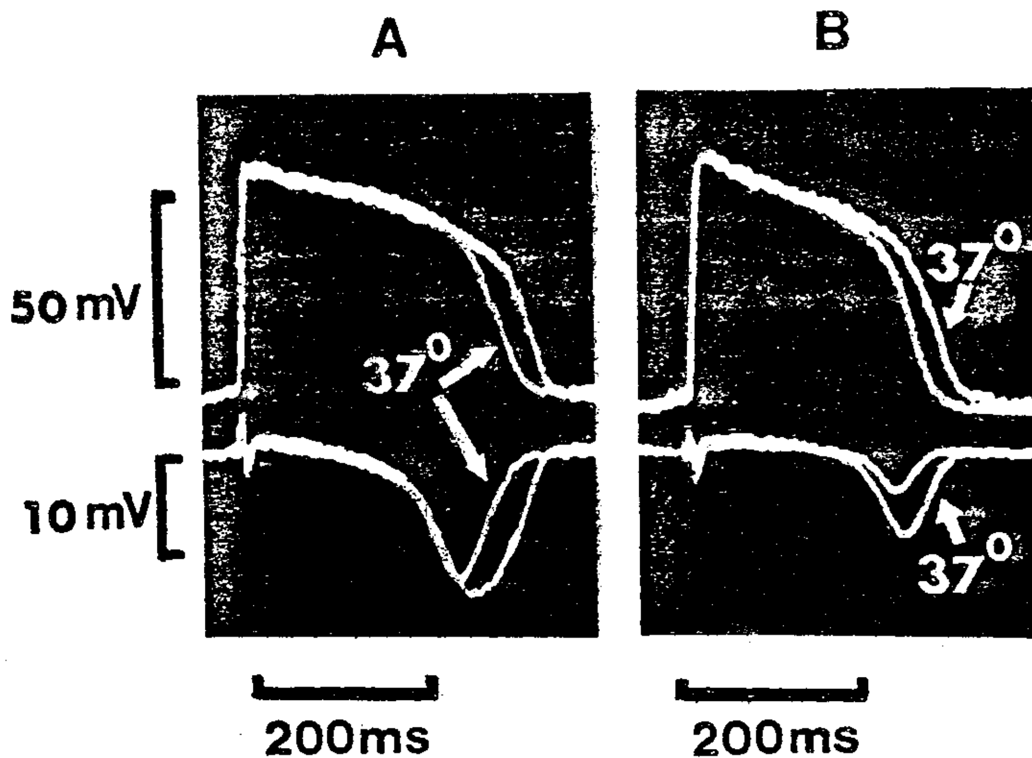


Figure VI - 6

Part A Superimposed on control (37°) action potential (upper trace) and epicardial ECG (lower trace) are an action potential and ECG recorded on lowering the temperature of the epicardium locally with 3 drops of saline at 10° C at the recording site. The action potential is prolonged and the T wave of the ECG deepens.

Part B In this part saline heated to 55°C abreviated the action potential and reduced the amplitude of the T wave compared to the control records at 37°C.

expected and opposite effects on the action potential and ECG. These observations are almost identical to those of the last authors.

The results just described suggests that the electrical recording system used here may be a simple and useful alternative to microelectrodes where mechanical changes need to be followed as well. We therefore used this technique to record electrical changes during myocardial ischaemia in our experimental preparation.

Electromechanical records during ischaemia.

Following the occlusion of a small coronary artery the action potential duration reduces (Fig.Vl - 7A) and the S-T segment elevates. The T-wave also changes direction. There is a clear difference in the onset of the deflection of ECG and action potential (inset Fig.VI-7A). During prolonged ischaemia the electrocardiogram becomes completely monophasic whereas the action potential shows very little or no evidence of electrical activity (Fig.Vl -7B). Action potentials during ischaemia may also be observed that have two components, a spike and a plateau separated by a notch and (Fig Vl -7C) is a prominent example.

The results thus far indicate that the mechanical records are similar to those obtained by other techniques (Forrester et al 1974; Tyberg et al 1974; Theroux et al 1974) and in fact provides useful information that might otherwise be missed. Further, the action potentials recorded with the suction electrode are in keeping with microelectrode recordings previously obtained. (Samson & Sher 1960; Toyoshima et al 1965; Downer et al 1977 A & B). If these monophasic action potentials are from very localised areas of

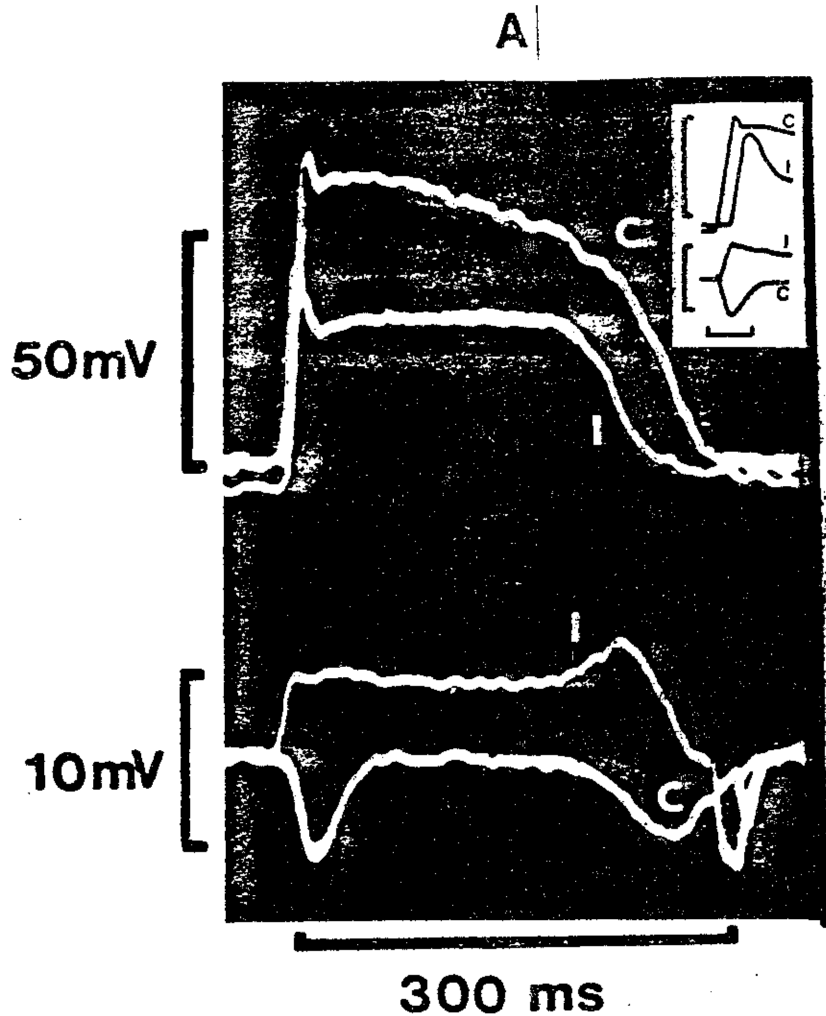


Figure VI·7

Effects of ischaemia on monophasic action potentials (upper traces in each part) and epicardial electrocardiograms (lower traces in each part).

Part A Compared to the control situation (C), mild ischaemia (I) produces a reduction in duration of action potentials and a raised S-T segment.

The base-lines of the epicardial ECG are superimposed. There is in fact significant T-Q depression. The sharp downward deflection at the end of the T-waves are conducted atrial pacing spikes. They are not detected in the action potentia

Inset Begining of electrical activity on expanded time base. The action potential is delayed compared with the epicardial ECG. Action potential calibration, 50 mV, ECG calibration 10mV, horizontal calibration 50 ms

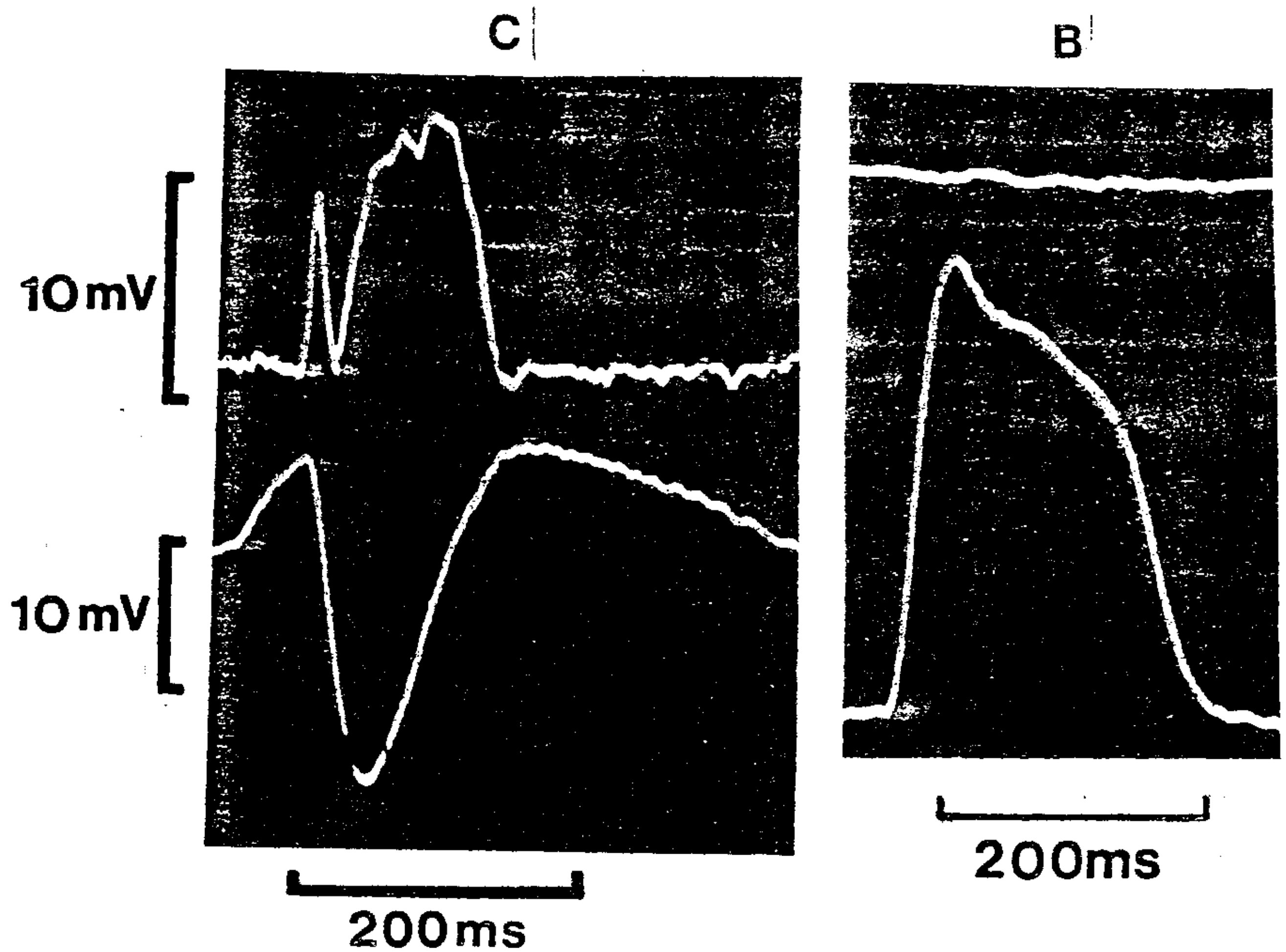


Figure VI - 7

Part B Marked ischaemia causes a monophasic type of response in the epicardial ECG (lower trace) while no electrical activity is recorded by the suction electrode (upper trace).

Part C Prominent example of a notched action potential frequently observed when recording from ischaemic areas.

myocardium and are relatively unaffected by nearby changes in current flow (Fig.Vl - 7B .) they should prove valuable in the study of the electrical behaviour of ischaemic muscle. In Fig.Vl - 8 are continuously displayed simultaneous mechanical and electrical recordings during the onset of a period of ischaemia. The summed mechanical output of the device and the action potential as well as the epicardial ECG show normal configurations before the coronary tie. On tightening the coronary snare the epicardial segment rapidly becomes dyskinetic and the onset of the action potential is delayed (inset) with its duration at 70% repolarization a little shortened. There is an upward shift in baseline of the monophasic action potential and its amplitude and rise time are reduced. (The latter two observations could be explained by a true depolarization of the resting membrane). Approximately 1 min after the tie, occasional action potentials become even more delayed and markedly reduced in amplitude and duration. As the duration of ischaemia progresses these action potentials become smaller until sometimes no action potential is seen between beats and an apparent two to one conduction block manifests. A run of these may be observed in Fig.Vl - 8 preceding ventricular tachycardia which in this case progressed to ventricular fibrillation.

DISCUSSION

Advantages and limitations of the mechanical recordings.

All methods of measuring epicardial segment-length changes as an indicator of regional myocardial performance have some inherent disadvantages. However, the results we have obtained by measuring length changes from strain gauges attached to the legs of a suction

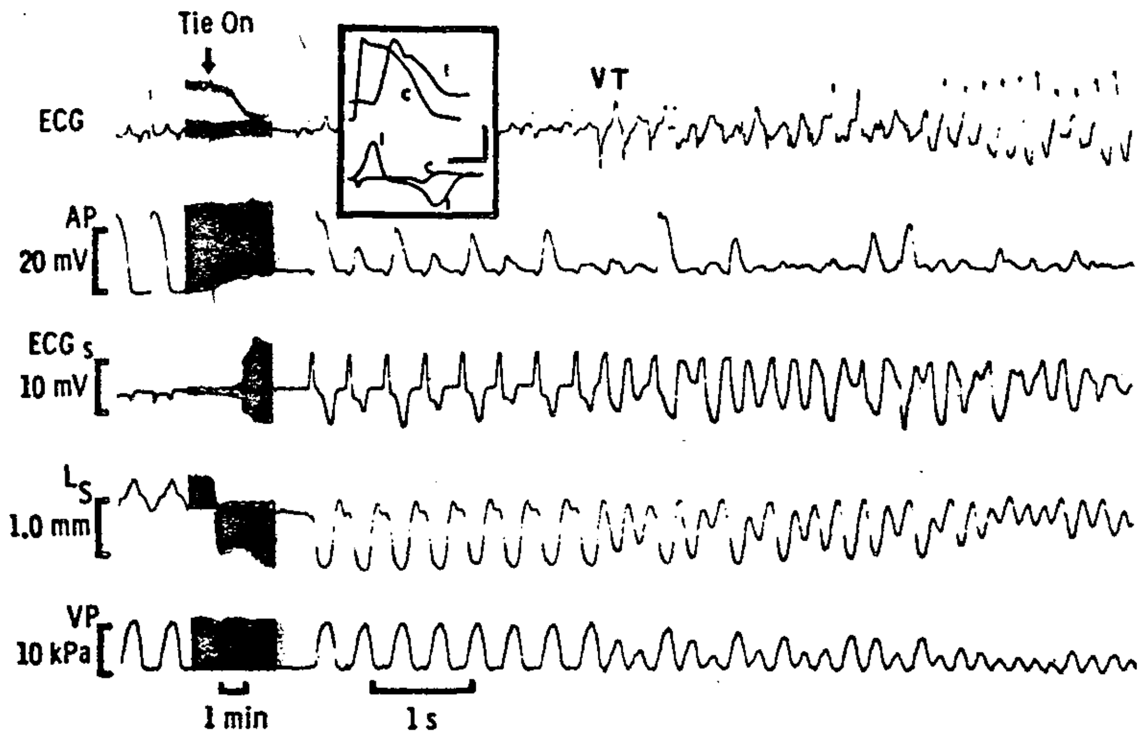


Figure VI 8

Simultaneous and continuous recordings of action potentials (AP), epicardial ECG, (ECG_S), segment length (L_s) and intraventricular pressure (VP) during the onset of ischaemia. Top trace is conventional ECG. On the left hand side is demonstrated the control recording. On occluding the artery the action potential duration and amplitude reduces while the segment becomes dyskinetic. The changes in the action potentials thereafter indicate conduction delay and partial to complete block in the underlying epicardium. (In the inset compare the time relationship between the epicardial ECG (lower trace) and the onset of the action potential (upper trace) in the control and ischaemic periods, Horizontal calibration bar 100 ms; vertrical calibration bars 10 mV for action potential, 5 mV for ECG). The uninterrupted recording shows the onset of ventricular tachycardia (VT) which progressed to fibrillation (not shown). Variation in mechanical and electrical behaviour is clearly evident.

applied tripod, suggest that it may be an improvement on previous methods and the loss of accuracy of mechanical recording obtained with more sophisticated techniques is made up for by the simplicity of its use. The advantage of suction application is self-evident, in that it allows full manoeuvrability of the device around the anterior and lateral surfaces of the exposed heart.

By allowing the legs of the tripod to move only along their axis at 120° to each other, we have simplified the analysis of the outputs from the strain gauges (cf Dieudonne et al 1972) without constraining the epicardial movement significantly. In particular, we have not seen the major iso-volumic lengthening during normal perfusion which has been suggested to be an artifact (Bugge-Aspheim, 1969) in some recordings from mercury in rubber strain gauges.

The major iso-volumic lengthening displayed by ischaemic tissue is unlikely to be artifactual, as it is unchanged by reapplication during the ischaemia, to allow the tripod to revert to its resting length. As we have shown however, the restriction of the movement of the legs of the tripod to these axes produces outputs from the individual legs, which, if examined in isolation, are not necessarily related to epicardial length changes in that direction. This is because of movement of the central point of the tripod, either through forces transmitted via the legs or directly by inertial forces, in relation to the underlying epicardium. We could possibly have obtained the 'true' direction and magnitude of the length changes in the underlying epicardium by vector analysis of the three leg signals, taking into account any estimated shift in the central

point;or we could have used the variation in the calculated area enclosed by the feet of the tripod as our indicator of local contractile behaviour. However, in theory and apparently in practice, simple arithmetical summing of the three leg outputs produces a value for epicardial length change which has a maximum error of about 12% under-estimate. Analysis by the more complicated methods suggested before (Dutetre et al,1974) would have severely restricted our use of the tripod and we have accepted the small possible error inherent in the simpler method. This under-estimate depends upon the angle between the direction of epicardial length change and the nearest leg axis. The error is therefore not a constant factor for a single position of the tripod if some intervention, such as a coronary occlusion, changes the direction of the major epicardial length change. The area within the loop described when plotting segment length against interventricular pressure has been used as an index of segment work (Tyberg et al, 1975). The artifact produced by inertial movements of the centre of the tripod during diastole does not affect the loop area (Fig.V1 - 3C) particularly if 'work' during the injection period is used.

Our demonstration of a change in the axis of major length change induced by ischaemia illustrates another possible advantage of our method of recording these length changes. Gauges measuring in only one fixed direction, decided upon before coronary occlusion, could fail to detect the epicardial shortening occurring close to the edge of the infarct which presumably is contributing to the ejection of blood from the ventricle. From our study shown in (Fig V1 - 3) where the tripod is rotated and reapplied over a

small area of epicardium that had its major length change in one direction, it is apparent that the summed leg output would always detect any shortening even if there was a significant direction change. It is clear from the above that it is neither necessary nor particularly desirable to spend time orientating the tripod, to ensure that one of the legs lies parallel to the axis of maximum shortening of the epicardium.

The change in direction of contraction induced in epicardium by ischaemia, is of some interest. It might be that the fibres in the direction of L_L in Fig.Vl - 4 are unable to shorten during normal ejection because of the geometry of fibre orientation. With ischaemia, these fibres, being close to the edge of the ischaemic area may maintain their tension bearing ability, and therefore shorten against the reduced resistance of the more ischaemic muscle. This mechanism would be similar to that described by Tyberg et al(1969) in their tandem muscle experiments. A second possibility is that the fibres showing an increase in contraction during ischaemia may have moved along their length-active tension curves. Finally, if gauge L_L is, in this case, recording transverse to the bulk of the muscle fibres, it could have been measuring their increase in diameter during normal contraction and their reduction in diameter when the fibres are stretched during ischaemia. The last two explanations, although they may partially contribute, could not on their own account for the magnitude of the changes in direction of contraction.

Advantages and limitations of the electrical recordings.

The recording of transmembrane potentials from the intact beating ventricle in situ is difficult, sometimes necessitating the suturing of a ring onto the area of the ventricle to be studies to limit epicardial movements, and even then, recordings may be subject to difficulties in interpretation (Downer et al 1977). Since we are interested in simultaneously monitoring electrical and mechanical events during ischaemia, such restraints placed on the myocardium defeat the object of the exercise. Interfering with mechanical behaviour directly affects recorded potentials,(Chapter lV,V; Kaufmann et al 1971; Hennekes et al 1977; Lab 1978). This would defeat some of the objects of the exercises. We have therefore accepted the limitations of the monophasic action potential and, according to our results which are in keeping with many microelectrode recordings obtained from ischaemic myocardium, we feel that much useful information may be obtained from the records, particularly the repolarization process, at a qualitative level. The close proximity of the epicardial ECG to the suction electrode is also a valuable adjunct in that comparisons between these ECGs and the monophasic action potential may be made. The suction has no significant effect on the recorded epicardial ECG, and in fact, stabilizes the contact between the wick and epicardium to provide more consistent recordings.

Several of our observations using the monophasic action potential and wick electrodes confirm those of Toyoshima et al(1965) and Downer et al(1977a). The latter authors used microelectrodes recordings in almost an identical experimental preparation to ours. As with

the microelectrodes it may be difficult to interpret the absolute amplitude of action potential and resting potential using the monophasic action potential. With the latter, however it may be possible to comment on them in a qualitative manner, which is impossible with a disturbed impalement using microelectrodes. Finally, a two-component type of action potential similar to ours was also observed in their intact heart studies and has been described in detail in an earlier study (Downer et al 1977b). Our observations strongly suggest that the monophasic action potential we use provides a highly localised record of electrophysiological events which reflect changes at the membrane level in response to ischaemia. Thus the action potential duration is reduced with mild ischaemia and no action potentials are recorded in prolonged or severe ischaemia. We have observed conduction delays and two to one response of the action potential. The action potential changes in Fig.Vl - 8 thus indicate alterations in myocardial conduction and excitability which precede the ventricular fibrillation and are in keeping with the re-entry mechanism of ventricular arrhythmia. Although we can not measure differences in action potential in cells separated by a mm or so we can often find two to one blocks or a type of electrical alternans by simply exploring the ischaemic area with the suction electrode. This type of exploration may also provide a better method of timing the arrival of the impulse in ischaemic myocardium than epicardial ECGs.

DESIGN AND CONSTRUCTION OF AN AUTOMATIC CARDIAC ACTION POTENTIAL DURATION METER.

The measurement of the duration of action potentials (AP) obtained from cardiac muscle is important in the research and diagnosis of the electrophysiological problems of the heart. This measurement is most reliably done on transmembrane action potentials recorded with glass micropipettes impaling a single cell in an isolated piece of living tissue, (Draper & Weidman 1951, Woodbury et al 1951). The recording thus obtained from normal ventricular muscle is, diagrammatically shown in the inset Fig. VI 9. In a good recording system there is remarkably little variation in the positive overshoot E 1 and the resting potential E 2 . The amplitude E is in the order of 100-120 mV. The time taken for the action potential to return from E 1 to 80% of E , i.e. 80% repolarization (T 80), is commonly 150-400 ms. However, this measurement is difficult to obtain reliably by eye because, first, the tailend (phase 3) of the action potential is asymptotic and second, repetitive manual measurement to an accuray of, say, 5% is very tedious.

The above difficulties have resulted in the design and construction of several instruments to measure action potential durations electronically (Miller et al 1971; Meyerberg et al 1970; Wasman et al 1976). These instruments were designed to be used in conjunction with microelectrode recordings from isolated pieces of heart muscle. However, the technical difficulties of continuous recording with microelectrodes from more physiological preparations,

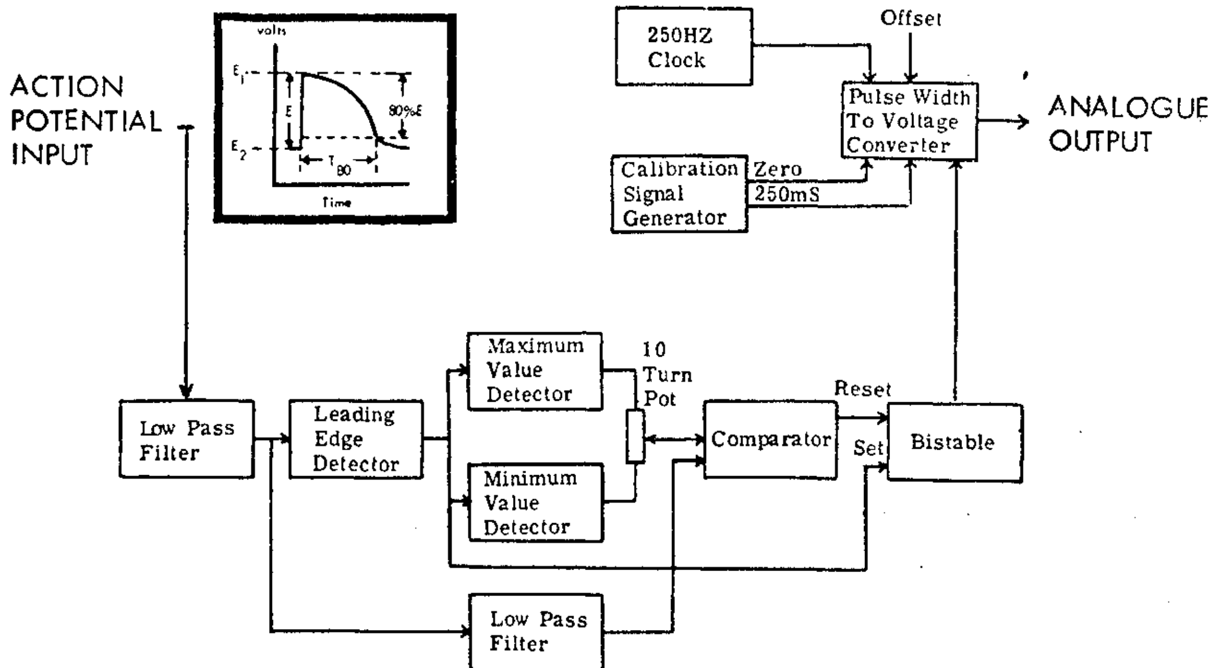


Figure VI.9 Block diagram of the circuit. The 250Hz clock driving the counting circuitry in the Pulse Width to Voltage Converter gives a total measurement span of 0 to 1 second and a resolution of 4 milliseconds. The resolution may be increased by either reducing the overall span or increasing the number of bits in the counter.

Inset Diagram of the cardiac action potential showing the definition of the term T_{80}, the time taken for the signal to reach 80% repolarisation. E_1 is the maximum positivity, E_2 is the 'resting' potential, E= amplitude of action potential.

such as the intact heart in situ, are prohibitive. Suction electrodes of thin polythene tubing (or variations thereof) have been developed (Hoffmann et al 1959;Churney and Oshima 1964; Szekees & Szurgent 1974; Lab & Woollard 1978), and this method of recording results in action potential signals of the same configuration as those obtained with microelectrodes, e.g. as in Fig. VI-9 (inset). There are, however, some drawbacks in these recordings. First, E_1 and E_2 vary, with E being between 20 and 80 mV, and second, the action potential steadily gets smaller and deteriorates with time. Repeated brief applications of the electrode are therefore necessary if the action potentials are to be monitored over some time. In contrast to the amplitude, the duration of the action potential recorded with this technique is reasonably constant and compares well with intracellular recordings. Thus changes in duration due to the influence of drugs, or the lack of oxygen (Lab & Woollard 1978) for example, can be readily detected and meaningfully used.

The suction electrode can therefore provide a means of getting important information from intact beating hearts in experimental preparations (Lab, 1978) and in human subjects (Cotoi & Dragulescu 1975; Brorson et al 1976). The instruments mentioned above, however, are not designed to cope with changes in E_1 and E_2 (Fig. VI 9 and so a new instrument has been designed primarily to compensate for these changes and track an action potential signal having a wandering base line. It can also be used for measuring the duration of action potentials obtained from microelectrodes.

The instrument has been used for monitoring the action potential durations in a variety of situations including experimental myocardial infarction in anaesthetized preparations.

Principal of Operation.

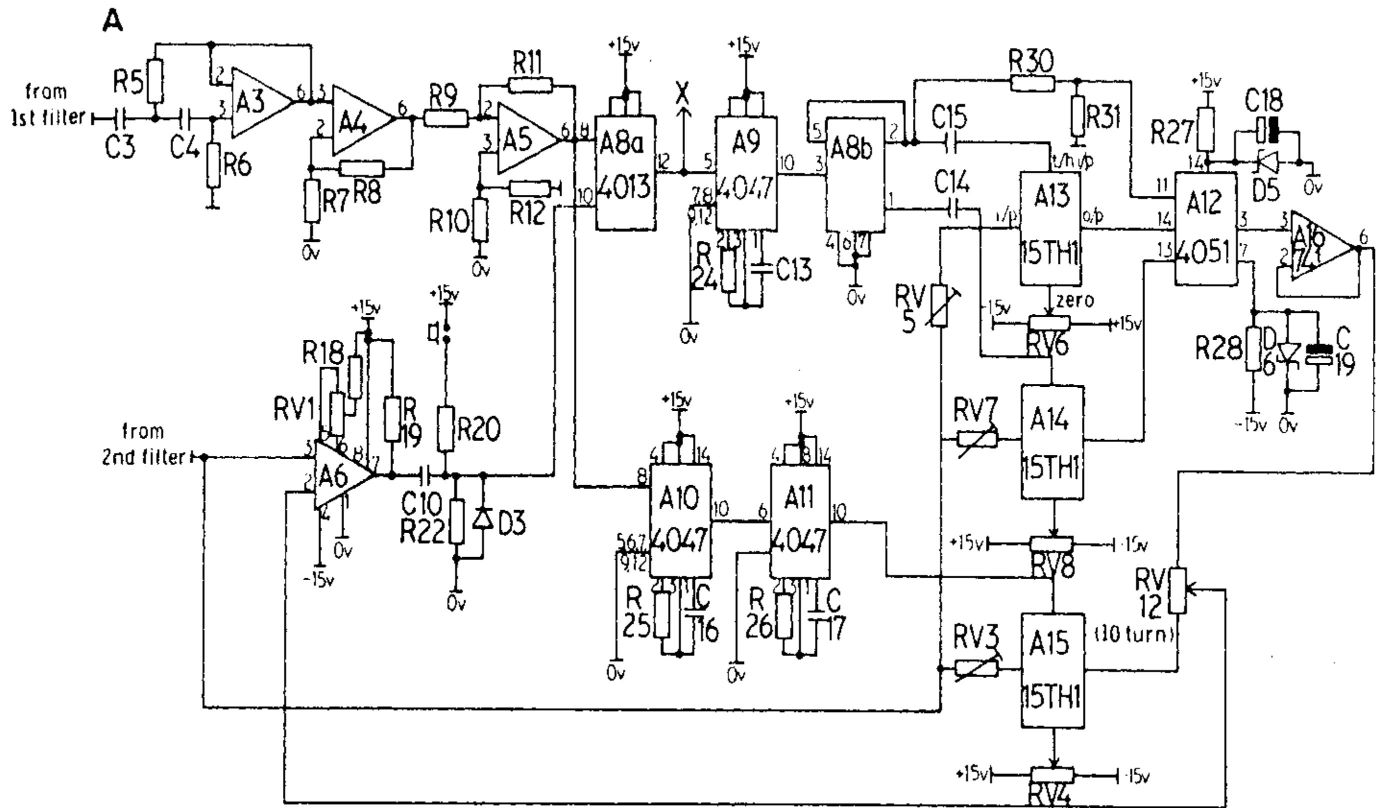
The instrument is required to measure the value of the base line voltage just before the onset of the action potential (E_2) , and an intermediate level (e.g., 80% repolarization of T_{80}). To do this the action potential is first filtered by a low pass filter (Fig.V19) with a cut off of about 75 Hz, to remove high frequency noise. A leading edge detector, which consists of a high pass filter and a Schmitt trigger, produces a pulse at the onset of the action potential, which is used to set a bistable latch. The pulse is also used to trigger a minimum value detector, which holds the value of the signal 12msbefore the leading edge (E_2), and a maximum value detector, which holds the value of the signal 10-50ms (depending on experimental requirements) after the leading edge E 1. These two voltages are applied across a potentiometer which is used to select an intermediate value usually the 80% repolarization value. A comparator is then used to detect when the action potential crosses this value, and the resulting pulse resets the bistable latch. The output of the latch is therefore a square pulse of duration equal to the duration of the action potential. It is then a simple matter to convert this signal into a voltage for display on a chart recorder. The resolution of the pulse width to voltage converter is 4 ms and calibration signals of 0 and 250 ms are provided to calibrate the recordings.

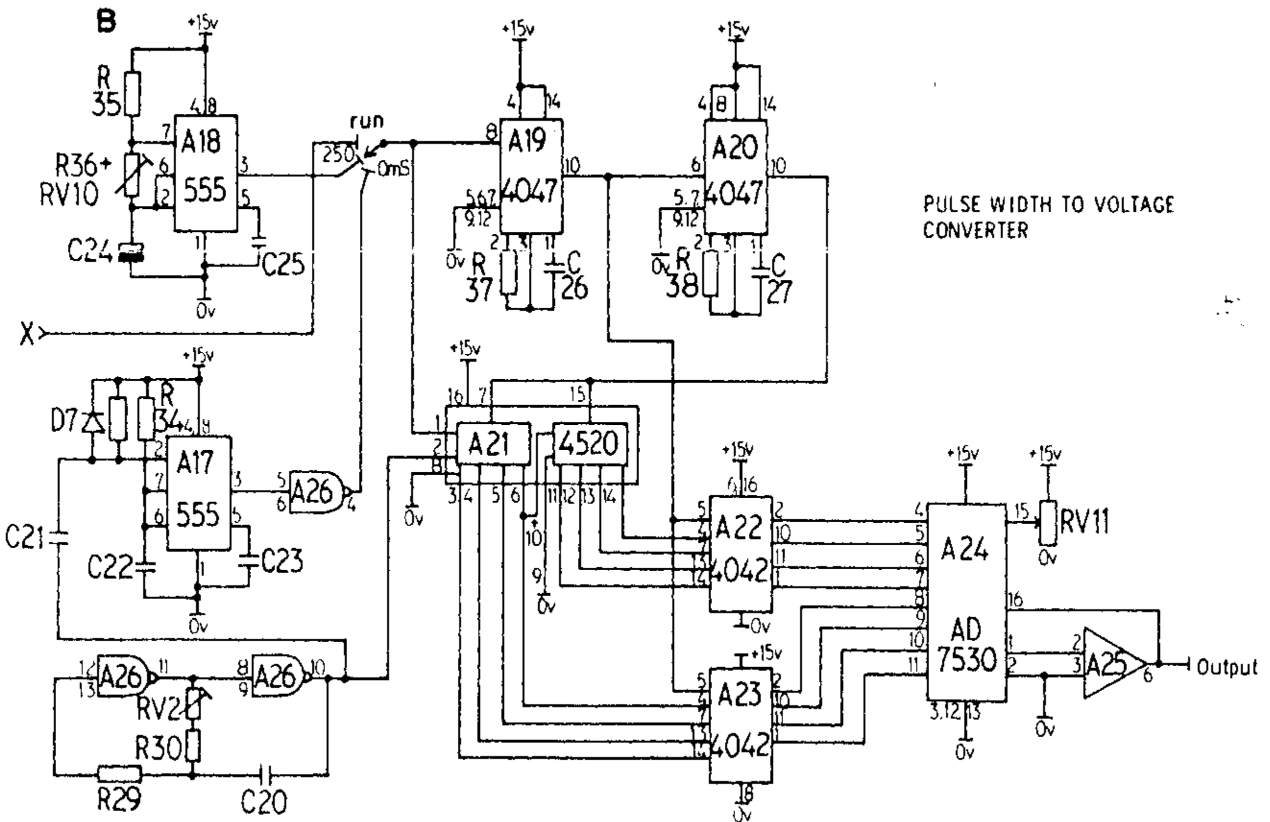
Circuit description.

AP duration to pulse width conversion (Fig. VI 10A) To obtain the minimum value of the action potential, the signal is filtered by two low pass Sallen and Key type filters (A1 and A2, not shown). The signal from the first stage, is differentiated by A3, A4, and A5; the positive edge from the output of A5, which occurs at the onset of the action potential, is used to set an R-S flip-flop, A8a. The output of this flip-flop inhibits the free running oscillator A9. This oscillator controls two track-hold amplifiers A13 and A14 whose inputs are the filtered action potential signal. The amplifiers are normally in the "hold" state but each is switched to the "track" state momentarily on alternate clock pulses from the oscillator. When the oscillator is stopped by A8a, the voltage held in the amplifier that was penultimately switched, is gated by A12 to one end of a 10-turn potentiometer via a noninverting buffer amplifier, A16. This voltage is the "minimum value" of the AP.

A10 and A11 produce a short pulse about 50 ms after the onset of the action potential, which switches a third track-hold amplifier into the track state momentarily. The output of this amplifier (the maximum value of the AP) is fed to the other end of the 10-turn potentiometer. This potentiometer is used to select an intermediate value (the percentage repolarization value), which is taken to one input of a comparator. The other input is the filtered action potential signal, so that the comparator produces a positive pulse when the action potential signal voltage reaches the percentage of repolarization selected by the potentiometer. This pulse resets the RS flip-flop, A8a, whose output is a square pulse, the duration

ACTION POTENTIAL DURATION METER (PULSE OUTPUT)





PULSE WIDTH TO VOLTAGE CONVERTER

Figure VI - 10

R5 = 1MO; R6 = 1MO; R7=10K; R8=390K; R9=1KO; R10=15K; R11=1MO; R12=100K;

R18=1KO; R19=1K5; R20=1KO; R22=100K; R24=180K; R25=390K; R26=20K;

R27=3K9; R28=3K9; R29=820K; R30=330K; R33=220K; R34=100K; R35=1MO;

R36=270K; R37=2K4; R38=2K4; RV1 =3KO; RV2=100K; RV3=10K; RV4=47K;

RV5=10K; RV6=47K; RV7=10K; RV8=47K; RV10=200K; RV11 =5KO; RV12=10K 10 turn;

C3 = 47n; C4=33n; C10=100p; C13=15n; C14=2n2; C15 = 2n2; C16=33n; C17=10n;

C18=1μo, (tantalum); C19=1μo (tantalum,) C20=4n7; C22 = 0n; C23=10n; C24=1μo.

(tantalum); C25=10n; C26=1no; C27=1no; D 3,7,=IN4148; D 5,6,=7.5V zener;

A 3,4,6,16,25=741; A5=741s; A8=CD 4013; A 9,10,11,19,20= CD 4047;

A 12= CD4051; A 13,14,15 =15TH-1; A17,18=555; A21= CD4520; A22,23= CD4042;

A24 = AD7530; A26=CD4011.

of which is equal to the duration of the action potential.

Pulse width-to-voltage conversion (Fig.VI-10). The square pulse is fed into the input of the pulse width-to-voltage converter consisting of A19 and A26. The pulse enables the clock oscillator, A26, RV2, R29, R30, and C20, to increment the counter A21. The outputs from this counter are latched into A22 and A23 at the end of the AP pulse when the monostable A19 is triggered. A19 in turn triggers another monostable, A20, which resets the counter to zero. The latched binary number is connected to the inputs of a D to A converter, A24, whose output is buffered by A25. RV11 alters the gain of the converter for calibration purposes. This method of pulse width-to-voltage conversion was chosen because a digital value of the pulse width is generated simultaneously and this can be printed on a line printer or fed directly to a computer for further analysis.

Calibration of recording apparatus. Calibration is effected by switching the input of the counter to a pulse generator whose pulse width is shorter than the period of the oscillator (A26). The counter (A21) is not incremented and hence zero voltage is produced at the output. The counter input may then be switched to a second pulse generator that produces pulses of a given width , (250 ms in this case).

Circuit Performance.

Several artificial signals are fed into the instrument to test its performance. An action potential simulator that can also provide signals of varying an amplitude and duration is used. Instantaneously changing the amplitude only (Fig.VI - 11A) and rapidly changing the offset voltage only (Fig. VI - 11b) produces a negligible change in output (± 2%). The output circuitry uses digital techniques, and the small steplike excursions in the duration signal are due to the digitization of the fluctuations at the input and statistical variations in the counting circuits. The response to relatively large and small variations in duration is also tested with the simulated action potential signal. The duration is increased from 250 to 450 ms. in 5 steps over a period of 6 min.(Fig.VI-12A)and decreased from 325 to 260 ms. in four steps on a beat-by-beat basis (Fig.VI-12A) These simulated tests are more rigorous than those normally found under experimental conditions, and the instrument, within the limits mentioned above, follows the changes in duration faithfully.

An illustration of the instantaneous nature of the response of the instrument is shown in Fig.VI-12C)A short action potential (240 ms) was inserted in a train of long ones (425 ms). The meter picked this individual beat out and responded accurately. It may therefore be used to monitor ectopic rhythms, ie. irregular heart rhythms that often have occasional action potentials of both longer and shorter duration than mormal, and also for action potentials recorded with a microelectrode in which the impalement may not be stable, and either isolated action potentials or small trains

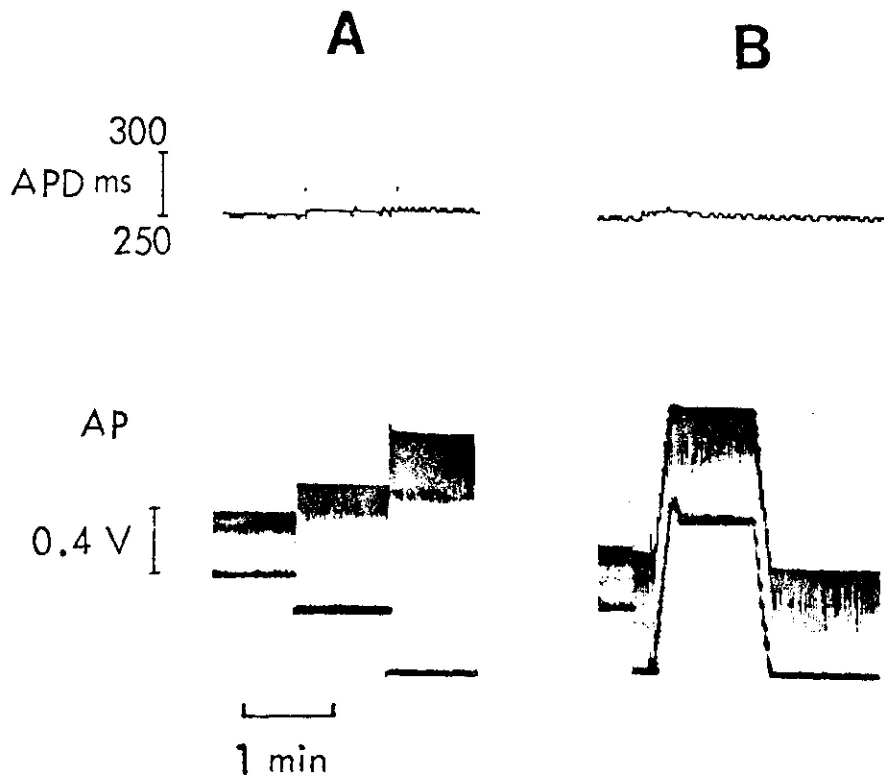


Figure VI-11 Effect of input signal changes on the output of the action potential duration meter.

Part A - the amplitude of the action potential input signal (AP) is abruptly increased in three steps from .2 to about 1 volt. The action potential duration output (APD) remains relatively unaffected.

Part B - A 1 volt offset is rapidly applied to the input signal (lower trace) but the output remains constant (upper trace). The action potential duration was constant at about 250 milliseconds throughout these experiments.

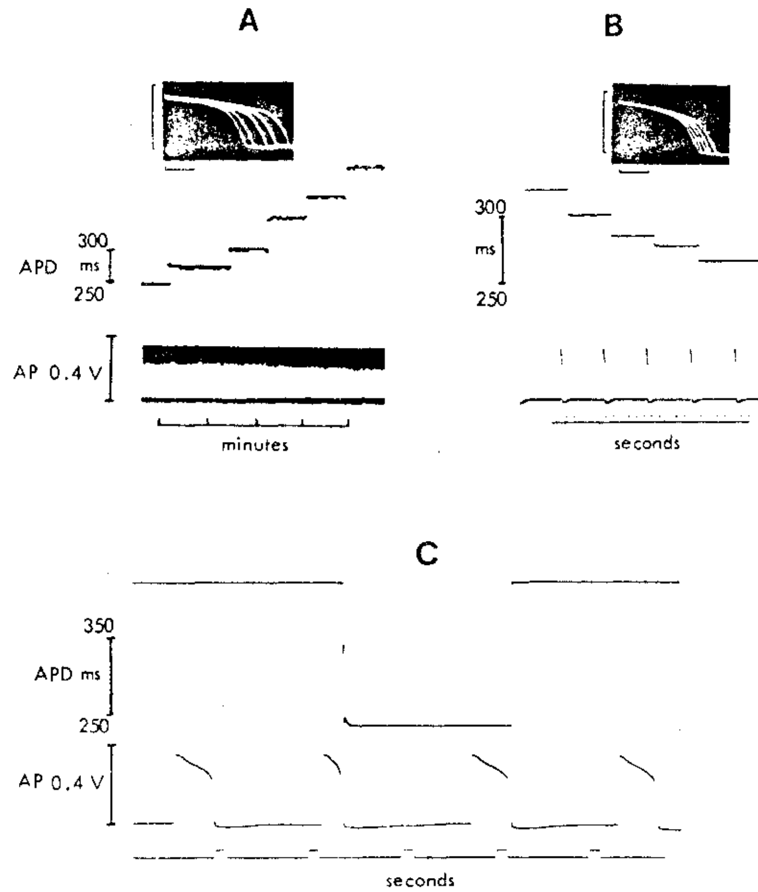


Figure VI-12 Response of the meter to changes in the duration of the action potential.

Part A - the duration was changed in 5 relatively large steps over a period of 6 minutes and the action potential duration output recorded (APD). The action potential input signals (AP) were also recorded and superimposed, on an oscilloscope (inset); vertical calibration bar 0.4V, horizontal, 100 ms.

Part B - the duration was altered in small decrements on five successive beats and the response recorded (upper trace). These changes are not detectable on the input signal (lower trace) but can be seen superimposed on the oscilloscope trace (inset).

Part C - response of the instrument to a single action potential of different duration. A short action potential in a train of longer ones is easily detected.

of action potentials are recorded.

Examples of Use.

The instrument has been used for measuring the duration of action potentials obtained from the intact heart in situ in nonrecovery experiments on pigs in which the front of the chest was removed to expose the heart. The device successfully follows changes in action potential duration recorded with suction electrodes under a variety of circumstances that show similar changes where microelectrodes are used,for example, after changes that affect the action potential of all the cells of the myocardium as when extracellular potassium is increased (Weidmann 1956) and when heart rate is changed (Fig. VI-13A)Changes in action potential duration affecting local areas of the heart are also detected and Fig.VI-13B demonstrates changes in duration induced by local alteration in temperature at the recording site similar to that found with microelectrodes (Toyoshima et al 1965).

The device has been particularly useful for continuous recording of the reduction in duration of action potentials in a segment of ventricular myocardium made ischemic (Lab & Woollard 1949) by a coronary artery tie (Fig.VI-13C)

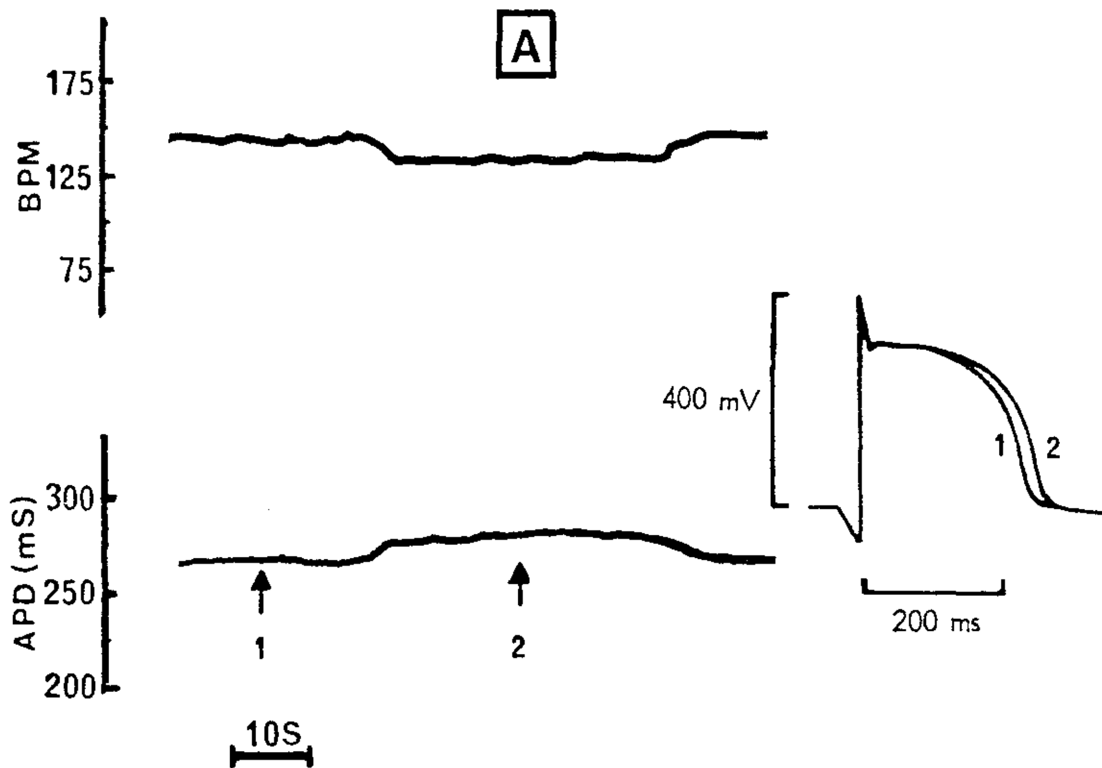


Figure VI-13 Changes in measured action potential duration (APD) following various interventions. Action potentials (AP) were obtained with suction electrodes from the left ventricle of an intact heart in situ.

Part A - Change in action potential due to a transient reduction in heart rate (BPM). The inset shows superimposed action potentials (arrows 1 and 2). Results of a paced animal experiment in which a decrease in heart rate produced an increase in duration of AP.

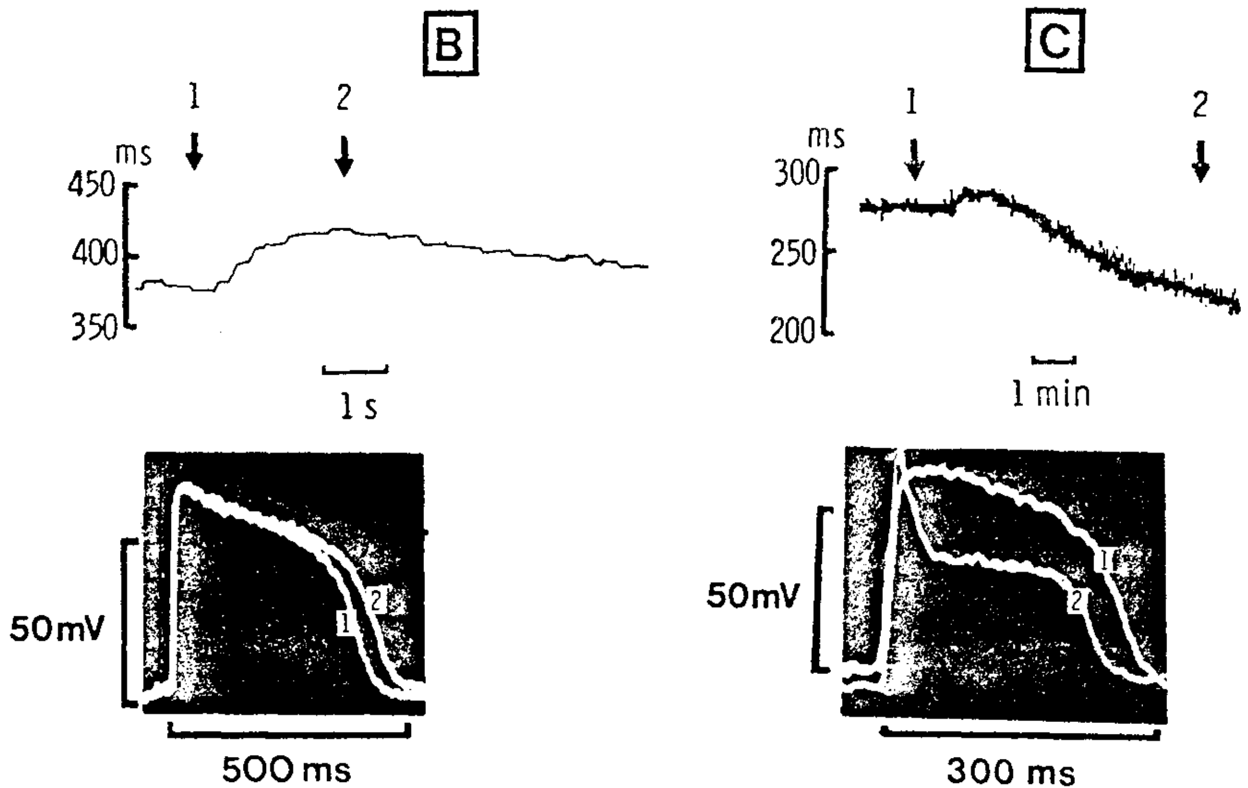


Figure VI·13

Part B - local cooling of the recording area, simply by 5 drops of saline at 10°C, produced a prolongation of action potential. Lower traces are the superimposed action potential recordings at 1 and 2.

Part C - progressive reduction of action potential duration produced by tying a branch of the coronary artery supplying the recording area. Control action potential 1) is from arrow 1 and ischaemic action potential 2) is from arrow 2. Note the transient increase in action potential duration immediately following the tie which is probably due to some cooling of the area because of the reduced blood supply. (See part B).

CHAPTER VII

SOME POSSIBLE ROLES OF CONTRACTION-EXCITATION COUPLING

CONTRIBUTION OF CONTRACTION-EXCITATION FEEDBACK TO THE VENTRICULAR REPOLARIZATION GRADIENT (T-WAVE OF ECG) IN INTACT PIG HEART IN SITU

Introduction.

During isotonic contraction of isolated cat papillary muscle the cellular transmembrane action potential duration is longer than that observed during isometric contraction (Ch.lV;Kaufmann et al 1971, Hennekes et al 1977) ie. there exists some form of excitation-contraction feedback. However there have been objections to the validity of using results obtained from isolated cardiac muscle studies to predict the behaviour of the intact beating heart in situ (Reichel 1976). Although some results from isolated frog ventricle are similar in some respects (Ch.V; Lab 1978)analagous results to those above using intact mammalian ventricles in situ have not yet been reported. One reason for the paucity of microelectrode studies in intact beating ventricles in situ is the difficulty of keeping the electrode in the cell, even if the part studied is immobilised by a ring of perspex sutured to the epicardium and agar allowed to set inside the ring. To verify the previous results such restraints on the ventricle would defeat the purpose of the study since it is necessary to make a comparison between action potentials during an isotonic (auxotonic) contraction,

where the ventricular wall is freely moving, and action potentials of an isovolumic beat. The object of this investigation is therefore to confirm the cellular electrophysiological muscle studies and, in addition, to see if they have their equivalent in the epicardial ECG. For the electrical records the study uses the monophasic action potential, obtained with suction electrodes which can reliably follow the repolarization phase of the action potential (Hoffmann et al 1959) and the epicardial electrocardiogram.
Since whole ventricle mechanics cannot give a true indication of the behaviour of the epicardial muscle from where the electrical recordings are obtained, the mechanical behaviour of the epicardium is also monitored.

Results and Discussion

An experiment is shown (Fig VII - 1A) in which the aorta is occluded for 100s while the action potential duration is automatically monitored. On occlusion the intraventricular pressure rises markedly in systole and a little in diastole, indicating an increase in end-diastolic volume. (Homeometric autoregulation was occasionally observed in these preparations but has not yet been systematically studied). Within the first one or two beats there is a reduction in the duration of the action potential. In experiments on 2 pigs the intraventricular pressure is increased about 1.75 times (76.7% SD 13.7%) using the aortic clamp. The reduction in action potential duration is 20.4 ms SD 9.4 superimposing the electrical and mechanical traces on a faster time base (Fig.VII-1B) shows the increased intraventricular pressure and a reduction in shortening of the epicardial segment during the ejection period with the isovolumic beat.

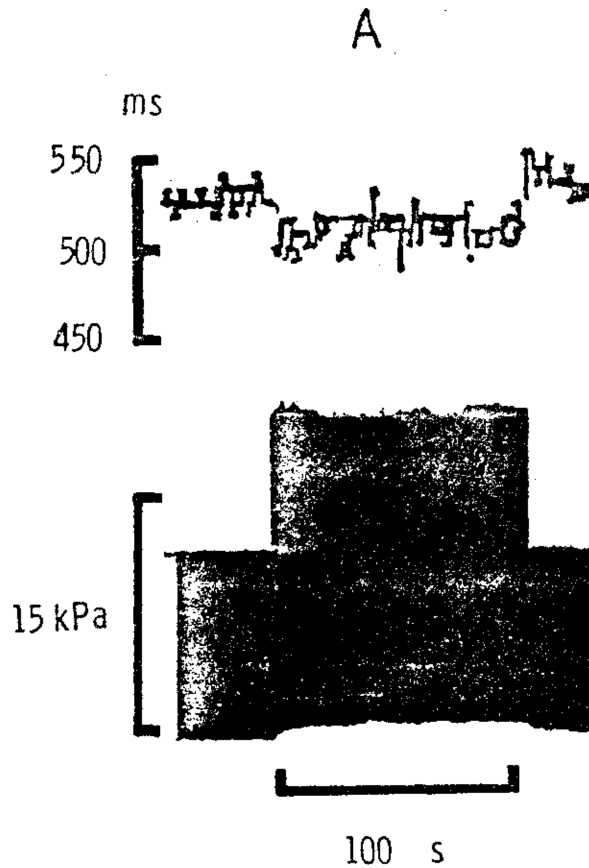


Figure VII-1 Changes in action potential duration, epicardial E.C.G, epicardial motion and intraventricular pressure, on constricting the aorta.

Part A. Constricting the aorta, on commencement of horizontal calibration bar, raises the systolic intraventricular pressure. (lower trace) with a small rise in end-diastolic pressure. The analogue output of the action potential duration (top trace) shows an immediate reduction in duration measured at 70% to repolarisation.

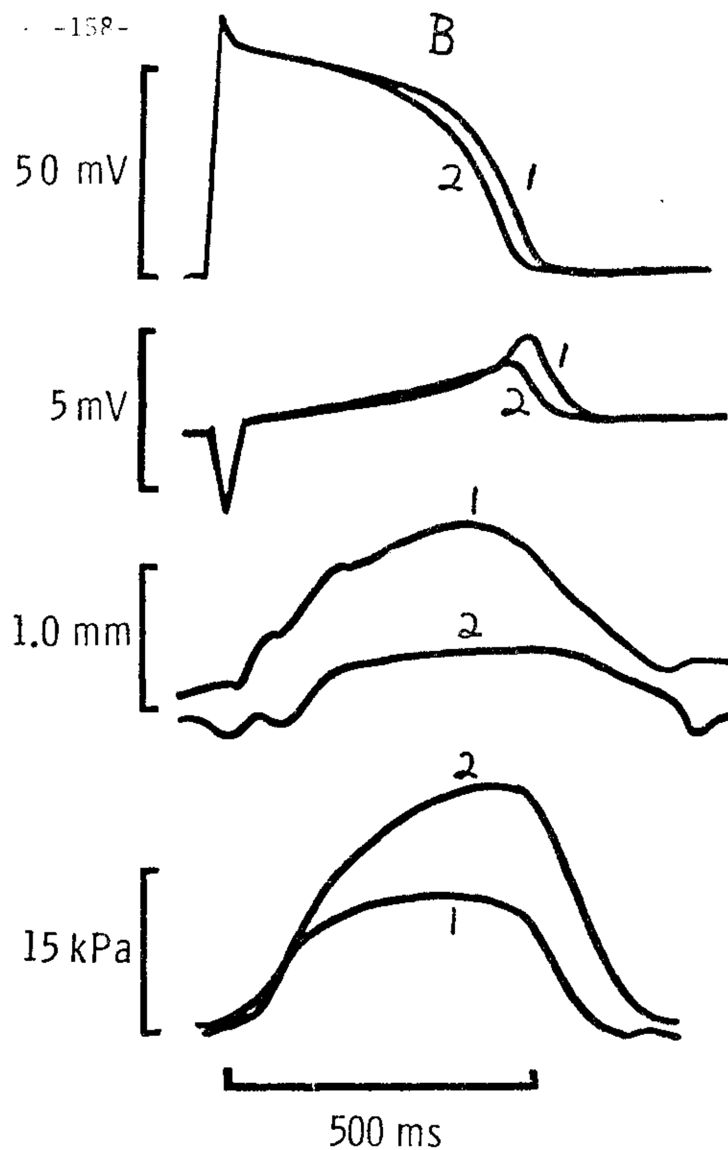


Figure VII. 1

Part B. Superimposed electrical and mechanical records from same experiment as in Part A immediately before (1) and after (2) aortic constriction. (Traces from above down; action potentials, epicardial ECG, epicardial segment motion (shortening is upward), intraventricular pressure). Accompanying the rise in intraventricular pressure, both epicardial segment motion and the action potential duration are reduced. The epicardial ECG shows a reduction in Q-T interval and a smaller T-wave. (28 Kg pig, heart rate 75/min. All records, except pressure, from antero-lateral surface of left ventricle).

In other experiments 'paradoxical' wall motion has been observed, with the epicardium lengthening during the rise in pressure. The action potential duration is clearly seen to be reduced with the isovolumic beat, with a reduction in duration of the Q-T interval of the epicardial electrocardiogram of the same order of magnitude. The QRS amplitudes superimposed in this experiment but the T-wave is smaller with the isovolumic beat than with the auxotonic beat. There is no change in heart rate in these experiments. In some experiments the QRS is also reduced as has been previously observed (Lekven 1979)

The abbreviation of the Q-T interval of the epicardial ECG confirms that the repolarization processes of a large number of cells are accelerated during isovolumic contraction. Further, the observation that the T-wave is smaller during this period indicates that there has been a change in the ventricular repolarization gradient, with a smaller T vector. This change may not simply be explained by an alteration in the geometrical relationship between the electrode and the ventricle during repolarization from base to apex in the thinner walled ventricle because, first, the QRS complex does not change consistently. Secondly, the T wave change is always in the same direction with this mechanical manoeuvre, and, finally, there is also an epi-endocardial repolarization gradient which, with the larger diameter ventricle, should produce a larger repolarization wave front and thus T-vector. It has been alternatively suggested (Ch.V ; Lab 1971) that since an increase in the diameter of the ventricle reduces the difference in the degree of muscle fibre shortening between the epicardium and the endocardium (Kushmer 1970)

the difference in the influence ofcontraction on the excitation process between the two areas is smaller. Cellular repolarization through the ventricle would thus be similar and this would reduce endo-epicardial current flow; and make the T-wave smaller. If the ventricle is allowed to empty, differential shortening is greater between the inside and outside of the ventricle with greater difference in contraction-excitation-feedback influences in the epicardium and endocardium; and thus a larger T-wave.

These results are clearly in keeping with previous ones (Ch.lV,V; Kaufmann et al 1971; Hennekes et al 1977) and the isovolumic contraction reduces epicardial shortening and there is an accompanying reduction in action potential duration from the area studied. There were no transient changes in tension or length observed with changes in mode of contraction in the present experiments which could be analogous to some "mechanical" results of the previous authors (see also Ch.IV). These may have been missed for two reasons. First a detailed mechanical mapping of epicardial segment behaviour has not been undertaken and mechanical transients may have occured in parts of the ventricle that were not monitored. Secondly ventricular wall motion is heterogenous (Fischer et al 1966; Dieudonne & Jean, 1969) so that transient decreases in tension in one part of the heart may have been masked by changes in the geometry of contraction throughout the ventricle.

The mechanism behind these mechanically dependent changes in action potential have been partly discussed in the previous chapters and will be dealt with more thoroughly in the final chapter.

CONTRIBUTION OF CONTRACTION-EXCITATION COUPLING TO VENTRICULAR ARRHYTHMIAS

Introduction

There is no entirely satisfactory explanation for the premature excitation that initiates ventricular fibrillation in the very early stages of myocardial ischaemia (Chapter III). The causes usually given relate to primary electrophysiological disturbances resulting directly from the ischaemia. The disutrbances include differential changes in conduction velocity, reentry, and enhanced automaticity. However, despite the fact that severe mechanical dysfunction occurs during regional ischaemia, and extrasystoles can accompany physical stresses and strains in myocardium, a critical consideration of a mechanical cause for extrasystoles during ischaemia has been neglected. This section briefly discusses the mechanical generation of threshold excitations and considers the possibility that mechanical changes may also induce ectopic impulses that precede ventricular fibrillation during regional ischaemia.

Method

The studies presented here also use intact pig ventricle in situ. Landrace pigs were anaesthetised with 1:1 mixture of N_2O and oxygen, with 1% Halothane, and the chest opened to expose the heart. The tripodal device (Chapter VI) was attached to an area for monitoring mechanical and electrical behaviour of a segment of epicardium which can be deprived of its blood supply using a snare around the coronary vessel supplying it. The experimental protocol necessitates the use of a beating heart that is mechanically unrestrained and which can also be subjected to unphysiological mechanical stress and strain. Microelectrode impalements under these conditions are unstable (Czarnecka et al 1973; Downer et al, 1977) and can produce records which are difficult to interpret. Therefore the

electrical measurement chosen was the monophasic action potential recorded with a suction electrode. The time course of repolarization of the action potential in this type of recording is an accurate predictor of the action potential configuration (Hoffmann et al 1959). The centre of interest in the present experiments is the shape of the repolarization phase only and the records may thus be confidently interpreted.

Results

Figure VII - 2 shows monophasic action potentials and epicardial segment motion before and soon after the small coronary tie, the segment contracts (shortens) as the intraventricular pressure rises, and the ventricular cardiac action potential has the standard configuration. Segment lengthening occurs when membrane repolarization is virtually complete and the ventricle relaxes. After the tie the area becomes dyskinetic and demonstrates paradoxical wall motion. It lengthens during ventricular contraction and actually shortens when the ventricle relaxes. This cycle contains a vulnerable electrical period beginning at about the last third of the repolarization phase and ending at the next action potential, for, associated with the stretch and sudden shortening of the segment, a transient depolarization is seen which can reach threshold for a new propagated action potential. This vulnerable period is roughly, mechanically, defined by the relaxation phase of contraction. The degree of stretch, and shortening, correlated significantly with the amplitude of the depolarization when the measurements were taken during this period ($r = 0.94$ $P < 0.001$, $N=20$, - for both stretch and shortening). An association between transient depolarization and segment shortening during this period of relaxation has been observed in every animal explored and occurred during the first hour of the coronary occlusion. More significantly, if a large coronary artery was occluded, e.g. left anterior descending branch, premature beats were seen to accompany the depolarization, and in

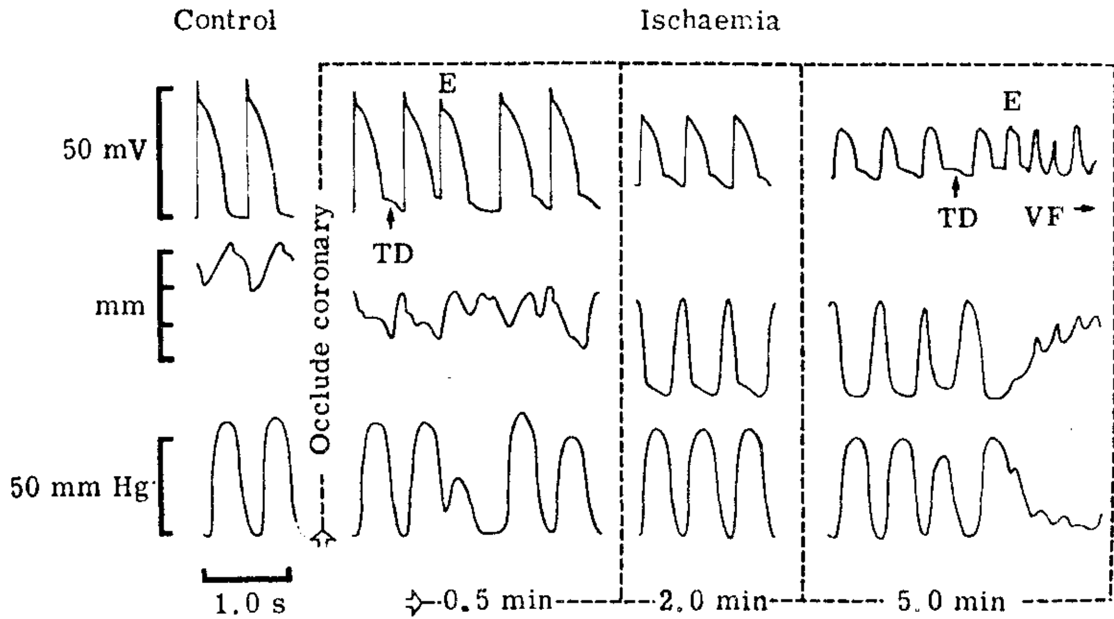


Figure VII-2

Effect of ischaemia on monophasic action potential (upper trace), ventricular segment motion (middle trace), and intraventricular pressure (lower trace). In the control panel segment shortening (upward movement of trace) occurs during the action potential, i.e. mainly during systole. (The configuration of the control action potential was stable over several minutes). After the coronary occlusion the shortening occurs predominantly when repolarization is over, i.e. during diastole, and a transient depolarization appears (TD) which sometimes appears to reach threshold for an extrasystole (E). The transient depolarization of the ectopic action potential is not pronounced, but the length changes are also small. Continued ischaemia progressively reduces the action potential amplitude and duration while the segment shows holosystolic bulging. The final panel demonstrates an extrasystole precipitating ventricular fibrillation (VF). See text for further discussion. (reported in Lab 1978b - figure unpublished). Monophasic action potentials and segment length records as in Lab & Woollard, 1978.

two thirds of the animals ventricular fibrillation followed. The remainder of the animals displayed a short period of multiple extrasystoles disappearing within an hour after the onset of ischaemia. If a small branch of the left anterior descending artery was tied, premature beats were an inconsistent finding and the hearts did not fibrillate. No transient depolarizations were observed in non-ischaemic areas in any of the experiments.

Clearly either of the above correlations does not imply a single casual relation. The complex inhomogeneous intramural stresses and strains precludes the definition of a precise relation between altered mechanics and depolarization in the intact breating ventricle in situ. Further, the re-entry mechanism could explain the depolarization as the result of electrotonic spread of a delayed action potential in an adjacent area of the heart: the retardation a consequence of the conduction defect because of the ischaemia. In fact similar transient depolarizations to those reported here have been observed using microelectrodes and the re-entry mechanism was invoked (Czarnecka et al, 1973; Downer et al, 1977). The nature of suction electrode recording i.e. from a large number of cells, further increases the significance of this difficulty in interpretation. One could strengthen the credibility of a stress-strain cause of arrhythmia by demonstrating mechanically-induced transient depolarizations and accompany premature beats in intact hearts with little possibility of re-entry circuits. In fact this demonstration should be a prerequisite of "contraction-excitation feedback" as a mechanism causing extrasystoles during ischaemia. The specific requirement still would be segment stretch followed by shortening during relaxation. This situation appears possible during ventricular outflow constriction in frog ventricle (Lab, 1978a) and analogous experiments were performed in the intact porcine heart in which the aorta was occluded. Similar electro-mechanical relationships to that found in regional ischaemia appear on aortic clamping. (Fig VII - 3A)

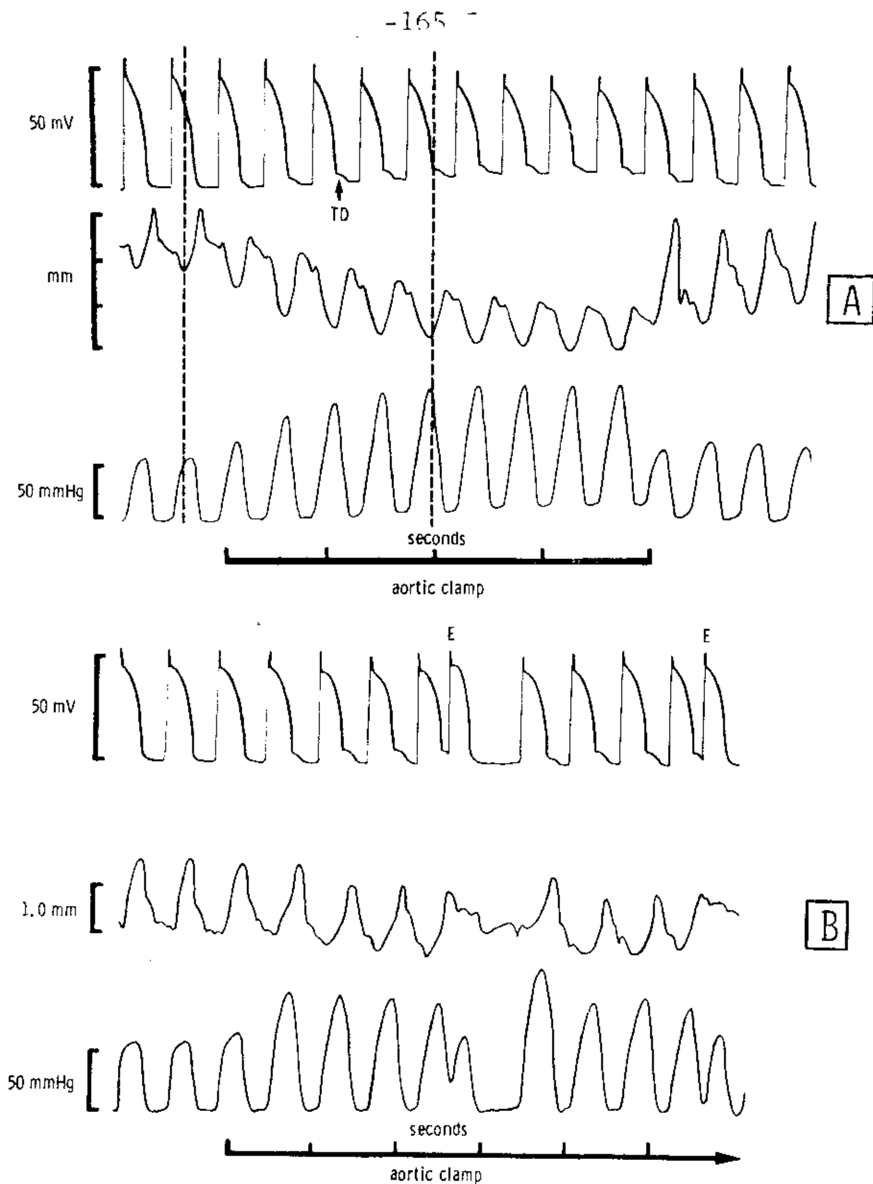


Figure VII-3

A. Effect of aortic occlusion on electromechanical records (Traces as in Fig. 3). Within 1-2 beats after aortic occlusion the end-diastolic segment length increases, lengthening cyclically during ventricular contraction per se and shortening during relaxation. Coincident with the disturbed contraction pattern, transient depolarizations appear (TD). On release of aortic clamp both electrical and mechanical changes fully reverse. B. Example of extrasystoles (E) which occasionally accompany the transient depolarizations. As during ischaemia (Fig. 1) the TD's of the ectopic action potential are reduced (negligible in this case) as are the length changes.

Pardoxical wall motion develops within 1-2 beats, with systolic lengthening followed by diastolic shortening of the segment. These mechanical strains were once more accompanied by transient depolarizations, which appear capable of initiating extrasystoles (Fig. VII - 3B). Again this apparent contraction-excitation feedback was a consistent finding, observed in all but one of the animals explored, (N = 12) and figure VII - 3B is an example of a variation in size of length change and corresponding depolarization, during the vulnerable period, with occasional extrasystoles. Similar to the situation during ischaemia there was a correlation between both, length increase and shortening, with depolarization (R = 0.77, 0.64 - respectively; $P < 0.001$, N = 70, - for both). The action potential duration also shortened with isovolumic contraction and corroborates the findings with isometric contracting cat papillary muscle (Kaufman et al, 1871 and Chapter IV). As in the experiments in Figure VII-2 the mechano-electric relations in figure VII-3 are also not precise because of the inhomogeneous intramural contraction patterns. Nonetheless the results not only support the findings in the ischaemic ventricle but also largely confirm the micro-electrode studies in isolated cat papillary muscle perfused preparations described in Section I: viz "paradoxical" myocardial mechanics may be accompanied by transient depolarizations which can reach threshold.

Discussion

Proposals for the molecular mechanism for the mechanically induced potentials have been presented (Kaufmann et al, 1971; Lab 1978a; 1980) and discussed in a recent review (Lab 1982, & Chapter VIII). It is of interest to see how these mechanisms relate to the changes found in ischaemia and to other hypotheses explaining early ectopics. First, passive mechanical alterations could distort the internal and external membranes and produce permeability changes so that, for example, the membrane potential would move closer to the relevant equilibrium potentials. A non-specific increase in permeability could depolarize

the membrane despite the probability that the final potential reached would be more negative than zero (Takeuchi & Takeuchi, 1960). This increase in permeability could augment the ischaemically induced potassium leak. The changes in tension could also distort intra-cardiac spaces. Changes in potassium movement or accumulation could follow rapidly (Kline & Morad, 1976) and thus infleunce potassium conductance to alter membrane repolarization (Weidman, 1956). However, these hypotheses do not easily explain some of the experimental observations in chapters IV & V. For example a release can induce a transient depolarization but a stretch at the equivalent time does not produce the expected, opposite, change in potential: viz a repolarization. Another possibility is that related to intracellular calcium. Mechanically induced changes in intracellular calcium (Allen & Kurihara 1981) show analogous alterations to the mechanically induced changes in action potential and Figure VII 4 demonstrates this similarity. These calcium variations have been invoked to explain the mehcnaically induced changes in potential (Lab in press). Further, lowered pH can increase sarcoplasmic [Ca^{2+}] in cat papillary muscle (Allan & Orchard, 1981), and this is expected in ischaemia. Myoplasmic calcium can affect: outward currents (Isenberg 1975; Bassingthwaighte, Fry & McGuigan 1976), and an Electrogenic Na/Ca Exchange (Mullins 1979). The transient depolarization observed with strophanthidin (Kass et al 1979) and low potassium (Eisner & Lederer, 1979), may also have common explanations with the foregoing.

It appears that during the critical first hour or so of ischaemia there are coincident wall motion disturbances, after potentials, changes in c-AMP as well as in intracellular [Ca^{2+}]. It is tempting to speculate that all these findings are causally related to the extrasystoles observed in this period. Pollack (1977) has suggested that stretch accelerates diastolic depolarization via a c-AMP mediated mechanism, and there are interactions between Ca^{2+} and c-AMP pertinent

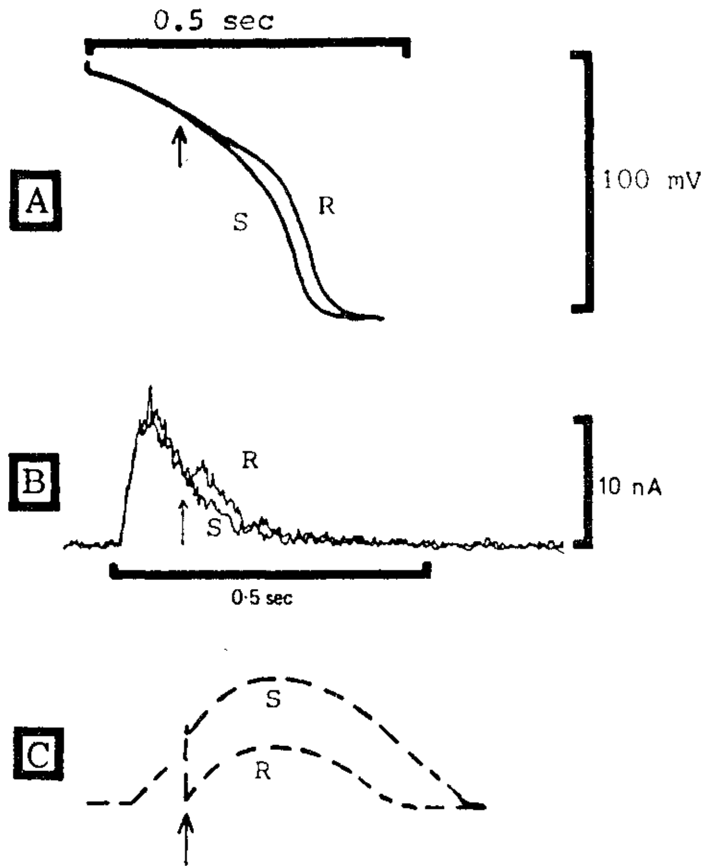


Figure VII-4

Analogous effects on action potential [A] and free sarcoplasmic calcium [B] of mechanical perturbations diagrammatically presented in [C] as dashed lines. Both records were from cat papillary muscle, but were obtained in different laboratories. Sarcoplasmic calcium, $[Ca^{2+}]_s$, is indicated by the light output (nA) of intracellularly injected aequorin. A stretch (s) halfway up developed tension affects neither the action potential nor $[Ca^{2+}]_s$. However a release (R) at the equivalent time delays both the repolarization and the fall in $[Ca^{2+}]_s$. (Panel B after - Allen et al, 1981).

to transmembrane ionic currents and to contraction (Harary et al, 1976; Schneider & Sperelakis, 1975; Reuter & Scholz, 1977; Tsien, 1977; Chapman 1979; Katz 1979; Barany & Barany, 1981). Notwithstanding the undecided mechanism, stress-strain related ectopics via some sort of contraction excitation feedback may be a regular occurrence in very early myocardial ischaemia. This is a subject that needs further investigation as a triggering cause of the premature beat that, in the appropriate ionic, metabolic and electrophysiological milieu, precedes potentially lethal arrhythmia.

REVIEW OF CONTRACTION EXCITATION FEEDBACK IN MYOCARDIUM: ITS EXISTENCE, MECHANISM, PHYSIOLOGICAL ROLE AND CLINICAL RELEVANCE

Excitation-contraction coupling in ventricular myocardium has received exhaustive investigation. Feedback processes in the reverse direction between contraction and excitation are less conspicuous and, not surprisingly, have attracted little notice despite their potential physiological and clinical importance. For the present, contraction-excitation feedback is inferred when changes in mechanical stress or strain cause or precede changes in membrane potentia For example, reduced force development or increased shortening induces greater depolarization. A depolarization, according to the type and timing of the mechanical change producing it, can appear either as a prolongation of the action potential, or as a transient depolarization. This review will describe the circumstances in which contraction-excitation feedback occurs, discuss possible explanations for the phenomenon and speculate on its potential importance in selected clinical situations.

The durations of the action potential and of contraction are of the same order of magnitude. This relationship permits mechanical activation to feed back on the initiating, concurrent action potential. However, the term "feedback" may be a misnomer as the process may comprise initiating factors that cause both electrical and mechanical changes. These factors may also be common to excitation-contraction coupling. Accordingly, a precis of the relevant aspects of excitation-contraction coupling is given in Figure 1. There are comprehensive reviews elsewhere (Huxley, 1974; Fozzard, 1977; Noble, 1975; Tsien, 1977; Coraboeuf, 1978; Carmeliet, 1978; Nayler & Williams, 1978; Vassalle, 1979; Reuter, 1979; Chapman, 1979; Fabiato & Fabiato, 1979; Katz, 1979).

The action potential normally causes a rise in sarcoplasmic calcium concentration, $[Ca^{2+}]_s$ via the mechanisms outlined in Figure 1. This instigates and mainly controls actin and myosin interaction. Relaxation is induced by processes which lower the calcium concentration. Active tension is also modulated by cyclic AMP and changes in binding constants of Ca^{2+} for

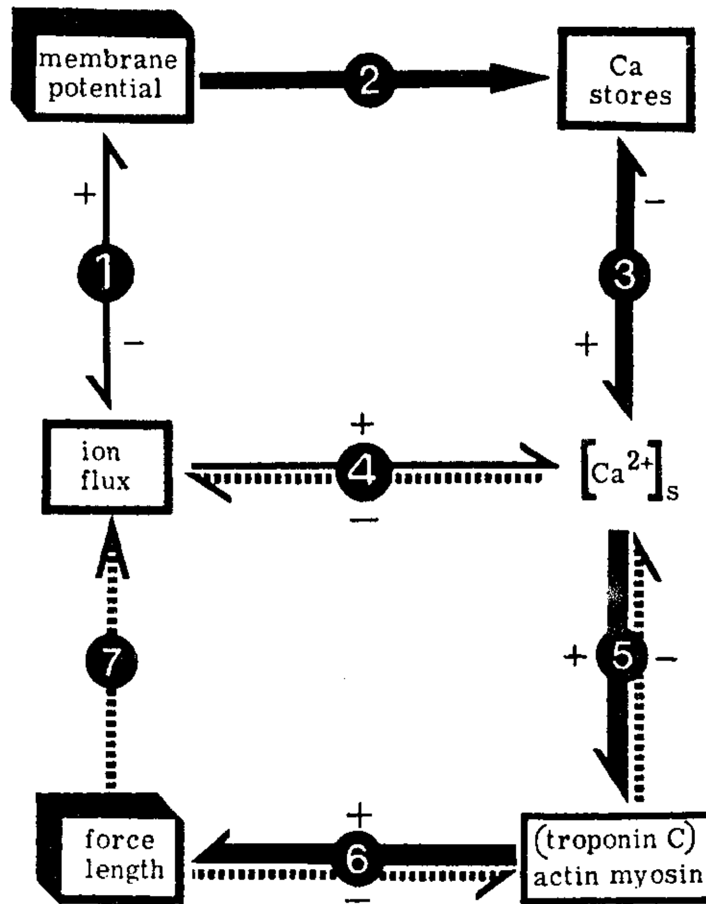


Figure 1

Schematic diagram of some interactions between membrane potential and contraction in heart. The sequence in excitation-contraction coupling may be followed via the heavy black arrows (mainly clockwise). Ion fluxes [1+] determine the membrane potential, which can also provide a driving force for ion movements [1-]. The changes in membrane potential are a function of the ionic equilibrium potentials and conductances (g). At rest g_{Na} is low and g_K is high. The latter mainly contributes to the negative resting potential which is maintained in the long term by an ATP-ase dependent Na/K pump. This keeps the internal Na^+ concentration, $[Na^+]_i$, low and $[K^+]_i$ high. Extracellular Ca^{2+}, $[Ca^{2+}]_o$, is relatively high while sarcoplasmic Ca^{2+} concentration, $[Ca^{2+}]_s$, is very low. With the upstroke of the action potential g_{Na} is rapidly increased and the fast inward sodium current, i_{Na}, reverses the transmembrane potential.

Figure 1 (cont'd)

Despite the consequential increase in the outward driving forces for potassium, g_K decreases and the outward repolarizing current is less than expected. The cardiac action potential is thus prolonged. A slow inward current, carried mainly by Ca^{2+}, i_{Ca}, also prolongs the action potential. The channels for i_{Ca} are influenced by c-AMP dependent protein kinase. (i_{Ca} probably generates action potentials in the sinus node and partially depolarized muscle - Cranefield, 1975) The depolarization [2] results in a rise in sarcoplasmic calcium from the stores [3+] directly, and probably by calcium induced calcium release. In mammalian muscle i_{Ca} does not normally immediately raise $[Ca^{2+}]_s$ to any significant degree unless the action potential is long [4+]. The calcium combines with Troponin-C [5+] which causes Troponin-I to allow actin and myosin interaction. The process, which needs ATP, results in force development [6+]. As or probably before the membrane repolarizes, during relaxation [6-], the sarcoplasmic reticulum sequesters Ca^{2+} [5-; 3-]. Ca^{2+} can also leave the sarcoplasm by a metabolically dependent calcium pump or by Na/Ca exchange [4-]. The exchange system is important for contraction in the frog. Greater binding to Troponin can also lower $[Ca^{2+}]_S$, but this is associated with increased force rather than faster relaxation. Length dependent activation (Jewell, 1977) is incorporated in [6±]. Force and length changes could link with membrane events (contraction-excitation feedback) by processes depicted by the dotted lines. For example mechanical changes could act to change ionic flux by affecting permeability or diffusion gradients directly [7]. Indirectly [6-; 5-], force and length changes could influence the membrane by altering $[Ca^{2+}]_S$. This may influence ionic flux [4-], and hence membrane potential [1+], by modulation of first, the electrochemical gradient for Ca^{2+}, second, outward potassium currents, third, "leak" currents, and finally, the electrogenic Na/Ca exchange.

troponin. The configuration of the action potential can be modified by three basic mechanisms: the degree of inactivation or recovery of ionic currents; ionic accumulation such as internal Ca^{2+} and external K^+; and ionic exchange such as Na/K and Na/Ca.

I. PREVALENCE OF CONTRACTION-EXCITATION FEEDBACK

Electrocardiographic studies by Stauch (1960) provided the first evidence for a feedback between contraction and excitation. He demonstrated shortening of the Q-T interval of the ECG in an isovolumic contracting frog ventricle as compared with the auxotonic beating heart, allowed to empty. Later corroborations used monophasic action potentials in a similar experimental preparation (Stauch, 1966; Lab, 1968). The plateau phase of the action potential was steeper during isovolumic than during auxotonic contraction. Stretch can also induce resting membrane potential changes (Penefsky & Hoffmann, 1963; Kaufmann & Theophile, 1967). Because of this, as well as the length-tension relation and length-dependent changes in activation (Jewell, 1977), this review will briefly include the effects of length changes on membrane potentials.

Before proceeding, we should note that the experimental protocols used in the studies produce results which need cautious interpretation because the mechanical manoeuvres can disturb the electrical relationships between the biological signal generator and the recording electrode. In addition isolated preparations can show internal inhomogeneity in contraction as well as relaxation (Krueger & Strobeck, 1978). This inhomogeneity is exacerbated by damaged and compliant ends associated with anchoring the preparation. Mechanically induced changes in potential may thus vary within and between preparations because of non-representative sampling. However, reasonably consistent results have been found using a variety of preparations and recording techniques, some of which sample many if not all the cells. Further, several predictions based on the existence of this "feedback" have been fulfilled.

(i) MECHANICAL CHANGES IN RESTING MUSCLE

(a) Effect on resting potentials. In several different ventricular preparations a stretch during diastole, to about L_{max} (the length at which maximum tension is produced) can result in an immediate reversible depolarization (Penefsky & Hoffman, 1963; Lab, 1978a; Boland & Troquet, 1980). These depolarizations may produce action potentials (Lab, 1978b) or spontaneous activity (Kaufmann & Theophile, 1967). Boland and Troquet (1980) thought their results in the intact rat ventricle were due to ischaemia: however no blood flow measurements were made and their results were in keeping with those from the superfused isolated preparations cited above. In all these cases the change appears fast enough to induce activity on a beat-to-beat basis. Two studies conflict with the foregoing results by showing no change in resting potential with length change (Spear & Moore, 1972; Allen, 1975). Dudel and Trautwein (1954) found depolarization with large stretches in cat papillary muscle but interpreted this as muscle damage.

(b) Effect on action potentials. When stretched muscle is stimulated the action potential shows a reduced *spike amplitude* (Dudel & Trautwein, 1954; Penefsky & Hoffman, 1963; Lab, 1978a). This is expected with a partially depolarized membrane (see previous section). Spear and Moore (1972) also found reductions in action potential amplitude, and velocity of conduction, recorded from stretched rat ventricle. The latter changes however were slowly reversible and were unlikely to operate on a beat-to-beat basis. This preparation also showed graded and asynchronous electromechanical behaviour, which was absent in guinea-pig, cat and frog.

Several reports show that lengthening also alters the *duration* of action potential, although there are also some inconsistencies. A shortening of action potential duration closely follows stretch in several studies (Dudel & Trautwein, 1954; Allen, 1975; Lepeschkin, 1976b in his Fig. 3; Lab, 1978a; Lab, 1980). Allen showed a small but significant initial shortening during

the first few beats, succeeded by a prolongation as steady state was reached many beats later. Dudel and Trautwein (1954) again considered their particular changes as being irreversible. Inconsistent with the foregoing, two investigations display no effect on the action potential duration (Gennser & Nilsson, 1968; Hennekes et al, 1977), while finally and also inconsistent with both the previous findings, Nomura (1963) using non-vertebrate cardiac muscle found a prolongation of duration with stretch.

In context but on a slightly different tack, stretching then releasing turtle ventricle perfused with 40 mM Ca^{2+} solution could result in small mechanical and electrical oscillations (Bozler & Delahayes, 1971). These authors considered the transmembrane potential change to follow the mechanical change. Stretch of normally perfused resting muscle (normal $[Ca^{2+}]$) produced only small changes in membrane potential if the extension was large (see also Lab, 1978a where large stretches were needed to produce an electrical effect). Bozler and Delahayes also saw oscillations following a twitch in 20 mM Ca^{2+}. The mechanical oscillations occurred with only moderate reliability. The oscillations were absent in some frog ventricles and in all frog atria. There is also another report of an intact frog preparation in which no mechanical induction of a membrane change could be demonstrated (Lab, 1978a). Possible explanations for these discrepancies have already been mentioned (beginning Section I) and are outlined in context below.

In general, moderately large passive length changes in ventricular muscle can induce changes in membrane potential. In a few preparations the response is inconsistent. The discrepancies may be species related and/or be due to varying experimental conditions. Changes in sarcomere length with mechanics are inhomogeneous in any one preparation (Krueger & Pollack, 1975), and difficult to control between preparations. This inhomogeneity is enhanced by the variable damage produced in anchoring the muscle. The differing electrical effects of stretch can thus be the result of partially damaged and

compliant ends. These ends could take up most of a relatively small length change in which case electrical recording from a distant area would show no mechanically induced effect, whereas closer recording sites could demonstrate a variable electrical effect; or even oscillation due to electrotonic spread. A change in internal milieu can also account for some of the discrepant observations. Isolated and electrolyte superfused cardiac preparations undergo changes with time (Reichel, 1976), and most of the above reports are imprecise about the timing of experiments. In keeping with this possibility, Bozler and Delahayes (1971) observed a reduction in amplitude of oscillation after several hours' study. Storage prior to experimentation (how or for how long was not stated) also reduced the oscillations.

(ii) MECHANICAL CHANGES IN ACTIVE MUSCLE

(a) Comparison of action potentials in isometric and isotonic contraction. In contrast to the somewhat confused situation when using length changes in resting muscle, alteration of the mode of contraction of cat papillary muscle affects the action potential clearly and reproducibly. Kaufmann et al (1971) found that the action potential duration associated with isometric contraction was shorter than that associated with an isotonic contraction. Similar but not identical results were found when the action potentials of an isovolumically contracting frog ventricle were compared with those of an isotonic (auxotonic) contraction (Stauch, 1966; Lab, 1978a). These results are not identical because isovolumic contraction of the whole ventricle does not imply isometric contraction of the wall. Changes in epicardial segment length can manifest as paradoxical motion in some areas (Lab, 1978a). The action potential changes from these sites are probably related to the length changes described in the previous sections.

(b) Effect of imposed perturbations during action potentials. Controlled length changes provided further insight into the phenomenon. A quick stretch of cat papillary muscle at any time does not affect the action potential (Hennekes et al, 1978; Hennekes et al, in press). A typical example is seen

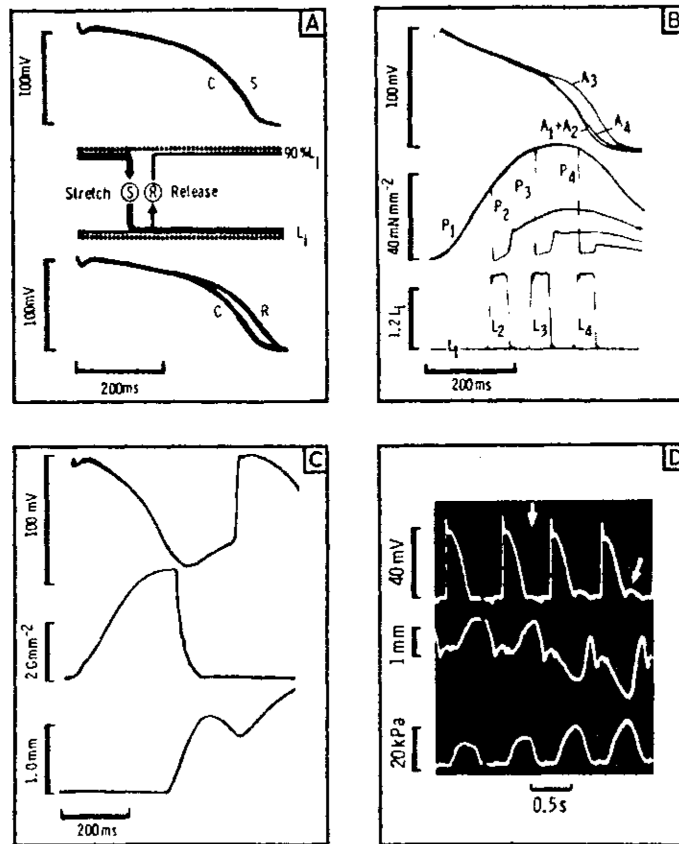


Figure 2

Action potential, length and force changes following imposed mechanical changes in four muscle preparations. A-C cat papillary muscle, D, intact ventricle. (A) The muscle contracts at two initial muscle lengths (dashed lines L_i and 90% L_i). Each produce upper and lower control action potentials (C) with similar time courses. A stretch, (s), from 90% L_i to L_i does not affect the action potential and S and C superimpose in the upper traces. This obtains irrespective of the timing of the stretch. However when the muscle shortens (release - R) the action potential prolongs with a depolarizing potential (lower trace R). (B) The undisturbed isometric muscle (P_1/L_1) has the shortest action potential duration (A_1). Transient

Figure 2 (cont'd)

quick releases (L_2 - L_4) inactives force development (P_2 - P_4). With forces P_3 and P_4 the action potential is prolonged by depolarizing potentials (A3 - A4). Intermediate timing of the intervention (L_3/P_3) produces the greatest action potential change (A_3) whereas the early release (L2/P2) has no effect (modified with permission; Lab 1980). (C) A release near the end of repolarization produces a discrete depolarization which is prominent and reaches threshold for a propagated action potential. (modified with permission, Kaufmann et al, 1971). (D) Recordings from pig left ventricle in situ. Monophasic action potentials from the epicardium (top trace) show a transient depolarization (inclined arrow) on constricting the aorta (vertical arrow). This is apparently related to the rapid stretch then shortening of the epicardial segment (middle trace). Shortening is an upward movement. Lowest trace is intraventricular pressure. (Unpublished record from Lab, 1978b.) Unit conversions 0.1g $\approx$ 1mN; 1kPa $\approx$ 7.5 mmHg

in Figure 2 A - (s). Release during the early rising phase of contraction (first perturbation in Figure 2 B) also leaves the action potential unaffected. By contrast, as seen in Figures 2 A and B, a quick release near the peak of developed force prolongs the action potential (Kaufmann et al, 1971; Hennekes et al, 1978). Concordant changes are found using microelectrodes, insulation gap, or electrograms (Lab, 1980). Once into the time course of delayed repolarization, immediate restretch of the muscle cannot restore control electrical conditions (Figure 2 B). The prolongation of action potential following a late release (Figure 2 C) can be similar in configuration to an early afterdepolarization, as characterised by Cranefield (1977). This transient depolarization is important because it sometimes appeared to reach threshold to produce a propagated action potential (Figure 2 C). This may be relevant to pathological arrhythmias (see Section III(iii)). In intact pig ventricle *in situ*, a disturbance of segmental wall motion analogous to the foregoing mechanical perturbations, may be produced by occluding the aorta. This may also cause both a transient depolarization (Figure 2 D), and accompanying extrasystoles (Lab, 1978b)

The precise relationship between the mechanical intervention and the depolarization is unclear. There is no simple correlation between the change in potential and mechanical alteration such as tension, velocity of shortening, and length (Lab, 1980; Hennekes et al, in press). Further, for any given length change, the change in potential is crucially dependent on time. Both early and late release in a preparation can produce a small depolarization while intermediate timing yields a large one (Figure 2 B). Mechanically induced "uncoupling of the active state" is also a time dependent process (Brady, 1965; Kaufmann et al, 1972). In this process a release of the muscle to a short length during contraction is incapable of producing an active tension appropriate for the new length: a tension deactivation (Julian & Moss, 1976). One suggestion, therefore, is that the release induced depolarization is related to the release induced deactivation (Lab, 1980). This interpretation may be further supported with

some experiments with caffeine which is known to prolong contraction while leaving the action potential relatively unaffected. Under these circumstances release did not produce a voltage change until significant deactivation occurred, and this was after repolarization of the action potential (Hennekes et al, in press): i.e. a very late release produced both a significant deactivation and membrane effect. Extrasystoles followed more frequently under these conditions than during normal perfusion.

Notwithstanding some inconsistencies and difficulties in interpretation, there is evidence that changes in the mechanical nature of contraction affect the action potential. In general a large tension development, e.g. an isometric contraction, is associated with a short action potential. By comparison a lightly loaded muscle that shortens has a long duration action potential. There is also a unidirectional component in contraction-excitation feedback in that muscle lengthening during activity produces no electrical change whereas muscle shortening does.

II. EXPLANATORY MECHANISMS

When we consider explanations for contraction-excitation feedback, three important properties need to be borne in mind: the phenomenon operates mainly when the muscle is activated; it occurs rapidly, with a time lag of only 10 - 20 ms (Kaufmann et al, 1971); and it can be unidirectional. The possible mechanisms are discussed below.

(i) PASSIVE, PHYSICAL MECHANISM (Figure 1 [7])

First an architectural change could passively affect the relationship between the electrical signal and monitoring electrodes. Some studies show changes in the cable properties and electrical constants of muscle with mechanical change (Potapova & Chailakian, 1965; Deck, 1964; Dulhunty & Franzini-Armstrong, 1977; Dominguez & Fozzard, 1979). Second a permeability change dependent on membrane stress or strain could move the membrane potential closer to the relevant equilibrium potentials. Finally, mechanical changes

could distort intercellular spaces. This may alter K^+ movement or accumulation, which can occur rapidly (Kline & Morad, 1976; Weidman, 1956) to influence membrane potential.

It is difficult to invoke these passive changes to explain the observations entirely. A release and a stretch at a given time should produce the appropriate distortions to depolarize and repolarize respectively the membrane of cat papillary muscle; whereas stretch in fact has no effect on the action potential. Further, a release-restretch manoeuvre returns the muscle to the passive mechanical state preceding the intervention but the electrical change is not aborted. These arguments therefore weaken the credibility of a purely passive mechanism.

(ii) ACTIVE INDIRECT MECHANISM

An additional possibility to the preceding is some change in the factors promoting active tension development. Mechanically induced deactivation of tension (Brady, 1965; Kaufmann et al, 1972; Julian & Moss, 1976) has properties akin to those outlined in II above: it occurs during muscle activity, it is rapid, and directional. Further, Gordon and Ridgeway (1976) found a length-dependent change in membrane potential in skeletal muscle which was Ca^{2+} mediated. Also, the mechano-electric effects in cardiac muscle are most prominent when the internal calcium $[Ca^{2+}]_S$ is in decline, i.e. just before peak developed tension (Allen & Blinks, 1978). These authors measured $[Ca^{2+}]_S$ by intracellular micro-injection of aequorin, which is a photoprotein that emits light in the presence of Ca^{2+}. Thus it seems reasonable to implicate the calcium ion in contraction-excitation feedback; for this ion bears upon transmembrane currents and on developed tension. The crucial questions are whether a mechanically dependant $[Ca^{2+}]_S$ change occurs in mammalian cardiac muscle under these circumstances, and whether it does so in a directional manner. Here the observations of Allen and Kurihara (1981) were germane. They clearly showed an increase in light output of aequorin, $[Ca^{2+}]_S$, with release but no change with stretch. How release yields an enhanced $[Ca^{2+}]_S$ in the face of an abridged tension is speculative. Allen

and Kurihara suggested a tension mediated change in the binding constant of troponin for Ca^{2+} (Figure 1 [5±]), attendant on the number of attached crossbridges (Bremel & Weber, 1972). However, because stretch has no effect on $[Ca^{2+}]_s$ and action potential, this suggestion is probably not the whole explanation and crossbridge interaction during tension *deactivation* in particular may be important. Although they may prove related, the load-dependent changes in relaxation observed by Brutsaert et al (1978a and b) remain enigmatic.

These observations suggest that any explanatory hypothesis for contraction-excitation feedback has to account for a rise in $[Ca^{2+}]_s$ together with a reduction in tension and prolongation of action potential in addition to the three properties listed above. Modulation of $[Ca^{2+}]_s$ can affect transmembrane movement of ions in several ways (Figure 1 [4-]). First it can determine i_{Ca} (Reuter, 1979). However there is no release induced change in a calcium mediated action potential (Hennekes et al, in press). Second, it can influence outward currents (Isenberg, 1975; Bassingthwaighte et al, 1976; Di Francesco & McNaughten, 1979). On first inspection neither of these hypotheses explain the above properties because the hypothesis requires a rise in $[Ca^{2+}]_s$ to shorten the action potential and reduce force. More evidence is needed to be certain of their exclusion, for Ca^{2+} compartmentalisation is not fully understood. Third the $[Ca^{2+}]_s$ variation could modulate an electrogenic Na/Ca exchange in the appropriate direction (Mullins, 1979). Kass et al (1978) considered this mechanism for their transient inward current. A mechanically induced alteration in Na/K exchange may also need eventual exclusion because most ion exchanges are electrogenic (Thomas, 1972; Isenberg & Trautwein, 1974; Schwarz et al, 1975). Fourth, calcium could act like an internal "transmitter substance" and modify a non-specific leak current (Kass et al, 1978; Eisner & Lederer, 1979).

Finally $[Ca^{2+}]_s$ may affect ionic currents by Ca-dependent phosphorylation at sarcolemmal sites (Kakiuchi & Yamazaki, 1970; Harary et al, 1976). In the context of phosphorylation processes c-AMP can also modulate i_{si} (Schneider & Sperelakis, 1975; Reuter & Scholz, 1977) and increases in Ca^{2+} can reduce c-AMP levels (see also Tsien, 1977; Chapman, 1979; Katz, 1979 for reviews). Further, there has been some evidence that concentrations or activities of cyclic nucleotides can vary in the heart, on a beat-to-beat basis (Brooker, 1973; Wollenberger et al, 1973) and with length (Flitney & Singh, 1981). This introduces the intriguing, but distant, possibility that mechanically dependent changes in phosphorylation at sites appropriate to membrane and also to contractile activation could mediate in contraction-excitation feedback. This mechanism bears some resemblance to the one proposed by Pollack (1977) for accelerated diastolic depolarizations produced by stretch, and can also account for the changes described in Section I (i) (a).

III. ROLE AND CONTEXT

There are few investigations that specifically study contraction-excitation feedback. However, any electrophysiological study that entails some primary mechanical change should also show elements of the feedback. Such experiments should provide circumstantial evidence relating to the existence and importance of contraction-excitation feedback. These studies were selected from the literature during the period beginning in 1969 to 1980, and the mechanical analogues to those producing contract-excitation feedback extracted. The characteristics shown in Figure 2 were used to predict the electrophysiological and other consequences of the mechanical changes: viz mechanically induced changes in repolarization phase, transient, and/or threshold depolarizations. Finally the expected observations and interpretations were compared with the ones actually obtained.

(i) POSSIBLE EFFECTS OF FEEDBACK AT A CELLULAR LEVEL

Relation between mechanically induced action potential change and ensuing mechanical change. Electrically induced change in action potential duration (Antoni et al, 1969; Wood et al, 1969) initiate transient changes in tension over several beats - presumably by changing $[Ca^{2+}]_s$. Similar tension transients may also follow a mechanical change (Parmley et al, 1968; Jewell & Rovell, 1973)

and these thereafter alter direction with a slower time course (Parmley & Chuck, 1973; Maisch et al, 1975; Suga & Sagawa, 1978). It is probable that the change in action potential which is mechanically induced contributes to the initial tension transients (Kaufmann et al, 1971). Hennekes et al (1977) later presented evidence to show that there may be additional contributions: an interpretation endorsed by Suga and Sagawa (1978).

At present it is not easy to reconcile the feedback with the force changes in homeometric autoregulation ("Anrep phenomenon") in the intact heart. Anrep (1912) showed that an increase in developed pressure accompanies an increase in afterload in the two minutes following the change. However the feedback requires an increased afterload to be accompanied by immediate reductions in force transient (and action potential duration): ostensibly the wrong direction for the Anrep phenomenon. Regional variation in myocardial blood flow is suggested to explain the latter (Monroe et al, 1972) but it could also be related to the slow action potential changes described by Allen (1975)

(ii) INTACT NORMAL VENTRICLE

Action potential duration and ventricular repolarization (Q-T interval of E.C.G.) and left ventricular shortening

One study in ventricle as a whole is a direct corroboration of the initial experiments in excitation-contraction feedback. Isolated myocardium that shortens substantially and rapidly against a small afterload prolongs the action potential duration (Fig. 2A&B). An analogous situation exists in intact human hearts. Ford & Campbell (1980) used amyl nitrite to produce a reduction in afterload, and this speeded wall shortening, reduced systolic time intervals, and prolonged the Q-T interval of the e.c.g. The second heart sound (S_2) occurred earlier and the T-wave later: i.e. S_2 & T times moved in opposite directions and the S_2-T interval lengthened. Other interventions that changed heart rate only moved S_2 and T times in the same directions.

Other studies relate to differential intramural shortening during contraction in intact ventricle (Rushmer, 1970; Fischer et al, 1966; Dieudonne & Jean, 1968; Streeter, 1979), and relaxation (Krueger & Strobeck, 1978). These mechanical inhomogeneities should provide a background for intramural feedback interactions. Further, the ECG is generated through electrical inhomogeneities (Schlant & Hurst, 1976). Therefore the mechano-electric interactions should show predictable alterations in the ECG. Three examples will considered in relation to: the T-wave (repolarization changes - Figure 2A & B); the U-wave (Transient depolarization - Figure 2C and D); and the extrasystoles (Figure 2C).

Action potential duration and ventricular repolarization gradient (T-wave of the ECG). During normal ventricular contraction the epicardial circumference shortens proportionately less than the endocradial circumference (Rushmer, 1970), and the tension distributions are also different (Law of Laplace). According

to contraction-excitation feedback, this length/tension distribution implies an epicardial repolarization (short action potential) ahead of endocardial recovery (long action potential). This repolarization gradient, curiously opposite in direction to depolarization, is in fact normally found. The vector contributes to the "upright" T-wave of the ECG. Further, during increases of ventricular volume the inner and outer circumferences approach each other. Under these circumstances, if mechanical conditions of the endo- and epicardial musculature approximate, so should the electrical conditions. This mechanical manoeuvre produces the expected, similar, endocardial and epicardial action potentials, and consequent flattened T-wave (Lab, 1971). Figure 3 A illustrates a remarkable T-wave change with increase in ventricular volume. Analogous mechano-electric changes can be found in the intact pig ventricle *in situ* (Figure 3 B) and similar manoeuvres can alter regional electrical differences in isolated rabbit hearts (Khatib & Lab, 1982). Finally, the flattened T-wave commonly observed in pathologically dilated hearts is in keeping with the proposal just illustrated. The relevance of the foregoing to steady state conditions or changes in rate (Lab & Yardley, 1979) remains to be seen. However the possibility that the mechanics of intramural contraction modulates the repolarization gradient needs further investigation, especially as the generation of this gradient is not clear (Lepeschkin, 1976a; Burgess, 1979) and Noble and Cohen (1978) have demonstrated electrophysiological differences in isolated ventricular preparations, taken from the endocardium and epicardium.

Transient depolarization and U-wave of ECG. If some mechanical inhomogeneities in the intact ventricle occur after repolarization is complete, discrete depolarizations could occur (Figure 2 B and C) to generate current flow after the T-wave, and thus to form or influence the U-wave. It has already been proposed that the U-wave may be associated with late afterdepolarizations (Lepeschkin, 1941; Lepeschkin & Surawicz, 1964). Further, Lepeschkin (1976b) suggested that its amplitude is some function of contraction. In this context variable diastolic intervals and/or extracellular potassium affect contraction (Hennekes et al, in

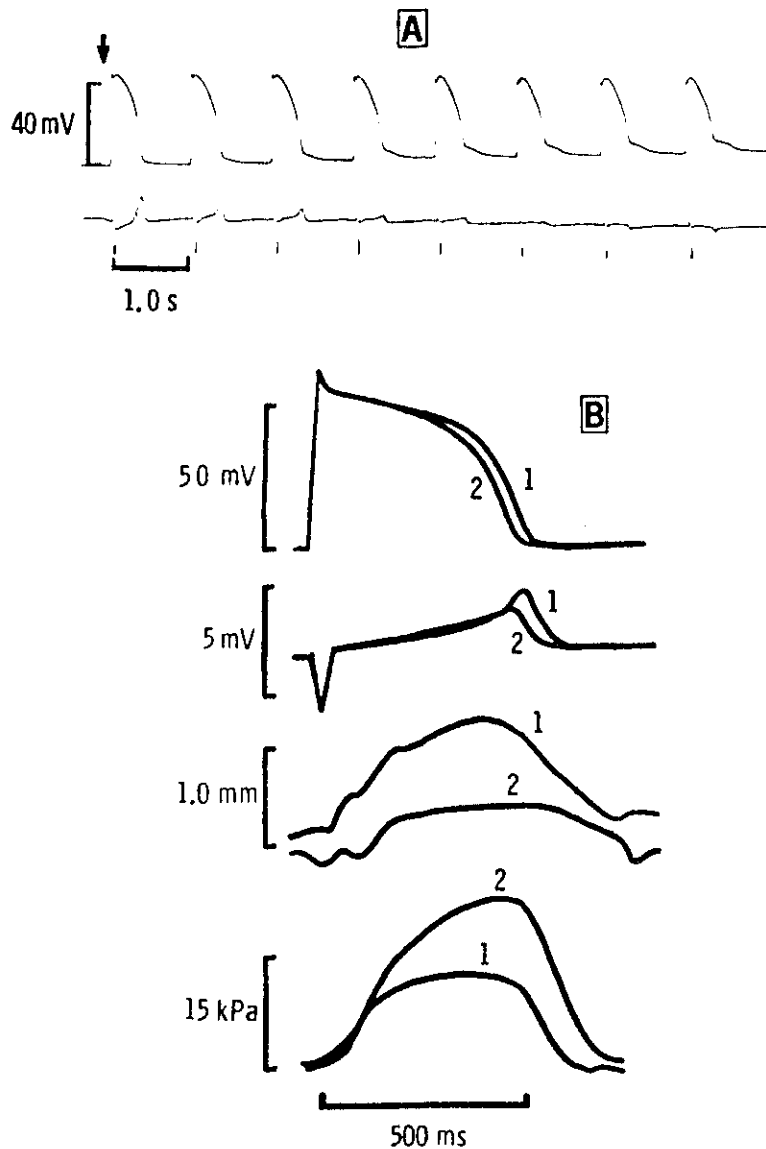


Figure 3

Monophasic action potentials and ECG from ventricular surface in intact ventricles *in situ*, on aortic occlusion. [A] Action potentials (top trace) and electrocardiogram (bottom trace) from frog. After aortic occlusion (arrow) the T-wave inverts within 6 - 7 beats while QRS complex changes are small by comparison. The action potential duration (at T_{70}) shortens and a depolarizing potential appears on the terminal repolarization phase. (Unpublished record. Action potentials similar to those reported in Lab, 1971, 1978a). [B] Electrical and mechanical records taken immediately before (1) and after (2) aortic constriction in the pig. Superimposed traces from above down: action potentials, epicardial ECG, epicardial segment motion (shortening

Figure 3 (cont'd)

is upward), intraventricular pressure. The rise in intraventricular pressure reduces both epicardial segment motion and the action potential duration. The epicardial ECG shows a reduction in Q-T interval and a smaller T-wave. The QRS amplitudes superimpose. (In other records the QRS with the isovolumic beat is smaller than with the auxotonic beat.)
28 Kg pig, heart rate 75/min and unchanged. All records except pressure from antero-lateral surface of left ventricle. (Unpublished record. Experimental preparation and recording techniques described in Lab & Woollard, 1978.)

press), afterdepolarizations (Cranefield, 1977), and U-waves (Lepeschkin, 1976b) in accordance with this hypothesis. The electrical vector responsible for the U-wave may originate from the potential difference between muscle regions with different transient depolarizations. The latter arise from different degrees of contraction-excitation feedback due to inhomogeneous wall contraction.

Threshold depolarization and ventricular ectopics. Mechanical perturbations near or in diastole can induce threshold depolarization (Figure 2 C; Kaufmann & Theophile, 1967; Kaufmann et al, 1971; Lab, 1978a and b). Analogous situations shou exist if extraneous mechanical perturbations were to be imposed on intact hearts. Mechanically induced depolarizations were found in isolated rabbit hearts which also demonstrated appropriate refractory periods to the mechanical intervention (Brooks et al, 1964; Kluge & Vicenzi, 1971). Acetylstrophanthidin reduced this "mechanical" refractory period and could precipitate fibrillation. Strophanthidin alone can generate afterpotentials or transient inward currents (Kass et al, 1978) and perhaps the mechanically induced depolarization facilitates these. Clinical analogues to the extraneous mechanical perturbations also exist. A blow to the chest (Hurst & Logue, 1966) and cardiac catheterization (McIntosh, 1968) can precipitate extrasystoles. Further, Zoll et al (1976) were able to stimulate the heart non-invasively with a "mechanical thumper". The device appeared to be experimentally and clinically successful.

(iii) INTACT ABNORMAL VENTRICLE

During ischaemia contractile and electrical performance at the cellular and gross levels deteriorate (Samson & Scher, 1960; Coraboeuf et al, 1976; Kubler & Katz, 1977; Elharrar & Zipes, 1977; Lazzara et al, 1978). This will cause abnormal wall motion (Tennant & Wiggers, 1935; Tyberg et al, 1974; Forrester et al, 1976; Theroux et al, 1976). Thus, as described above in normal hearts with reference to Figure 2, appropriate timing of ventricular mechanical changes during the action potential could affect corresponding deflections in the ECG of abnormal heart. Several studies indicate that changes in mechanics could initiate electrophysiological variation in a pathological milieu, and examples of three types will briefly be considered.

Reduced spike height and QRS complex during ventricular dilatation. Stretch reduces the spike amplitude of cardiac action potentials (Penefsky & Hoffman, 1963; Lab, 1969; 1978a; Boland & Troquet, 1980). Left ventricular distension should therefore reduce QRS vector by reducing the potential differences across the depolarizing wave front, and, as with the T-wave (Section (ii)) reduce the electromechanical differences between epicardium and endocardium. Reductions in QRS complex with dilatation have been observed, and explained by increases in blood volume (Brody, 1956), and reduced tissue mass under the electrodes (Lekven et al, 1979). However an alternative possibility that needs consideration is a length induced change in action potential spike.

Altered membrane potentials and S-T segment elevation. S-T segment elevation in the ECG results from altered current flow between different membrane potentials and action potential durations in normal and abnormal myocardium. Varying mechanical conditions could modulate the action potentials distinctively in these areas, and thus alter the degree of S-T segment elevation. Lekven et al (1980) found that ventricular distension during regional ishcaemia induced S-T segment changes which depended on whether the distension was produced by volume infusion or aortic constriction. Although their, plausible, explanation was related to differential changes in blood flow, it was of interest that they noted length changes in ischaemic segments which comply with contraction-excitation feedback.

Threshold depolarizations and ventricular arrhythmia. A conduction block probably produces some localised contraction abnormality, and this lends itself to contraction-excitation feedback. Regular coupled extrasystoles, alternating with irregular parasystoles, may relate to mechanically induced depolarizations because, during parasystole, the focus is "protected" from the driving action potential by ar entrance block which may be mechanically transcended for coupling such alternating arrhythmias are difficult to expalin using the re-entry theory (Schamroth, 1980) an Figure 4A from a preliminary report, suggests that mechano-excitation interaction m be an option worth investigating. In this experiment occlusion of the pulmonary

artery in an intect heart in situ produced changes in wall motion, transient depolarizations, and regular coupled extrasystoles often alternating with irregular rhythms (Covell et al, 1981).

Finally, early ischaemic arrhythmia may also be viewed in the light of contraction-excitation feedback. An ischaemic segment is stretched during systole and shortens late in relaxation, i.e. it is dyskinetic (Tyberg et al, 1974; Forrester et al, 1976). In consequence responsive myocardium should give rise to mechanically induced extrasystoles (Figures 2 C and 4 A). Preliminary studies show late depolarizations on the monophasic action potential accompanying segment dyskinesia. The electrical changes are associated with threshold depolarizations (Lab, 1978b) and Figure 4 B is an example in which ventricular fibrillation was precipitated. Within one hour of coronary occlusion ventricular ectopics, and often fibrillation, were regularly found. During this period a delayed transient depolarization was consistently observed together with segment dyskinesia. However one needs to exclude an ubiquitous electrical artifact before accepting contraction-excitation feedback in addition to the other membrane factors as a cause of ventricular ectopics (Arnsdorf, 1977; Hauswirth & Singh, 1978; Cranefield, 1977; Cranefield & Wit, 1979; Hoffman & Rosen, 1981). This cause is worthy of further study as fibrillation could ensue in situations in which there is depressed conduction, re-entry and altered automaticity.

Figure 5 summarises contraction-excitation feedback and its possible ramifications: (1) Contraction and mechanical changes influence myocardial electrophysiology (quadrant C to A). In particular stretch followed by shortening can produce transient depolarizations which prolongs action potentials, or appear as discrete depolarizations. These can precipitate premature beats.
(2) The mechanism is undefined. One or more tenable pathways, common to both mechanical and membrane events, probably lie in changes in calcium kinetics and perhaps in specific phosphorylations at selected intracellular sites, although an altered architecture cannot be excluded. (3) The normally contracting left ventricle provides a suitable mciroenvironment for the expression of contraction-

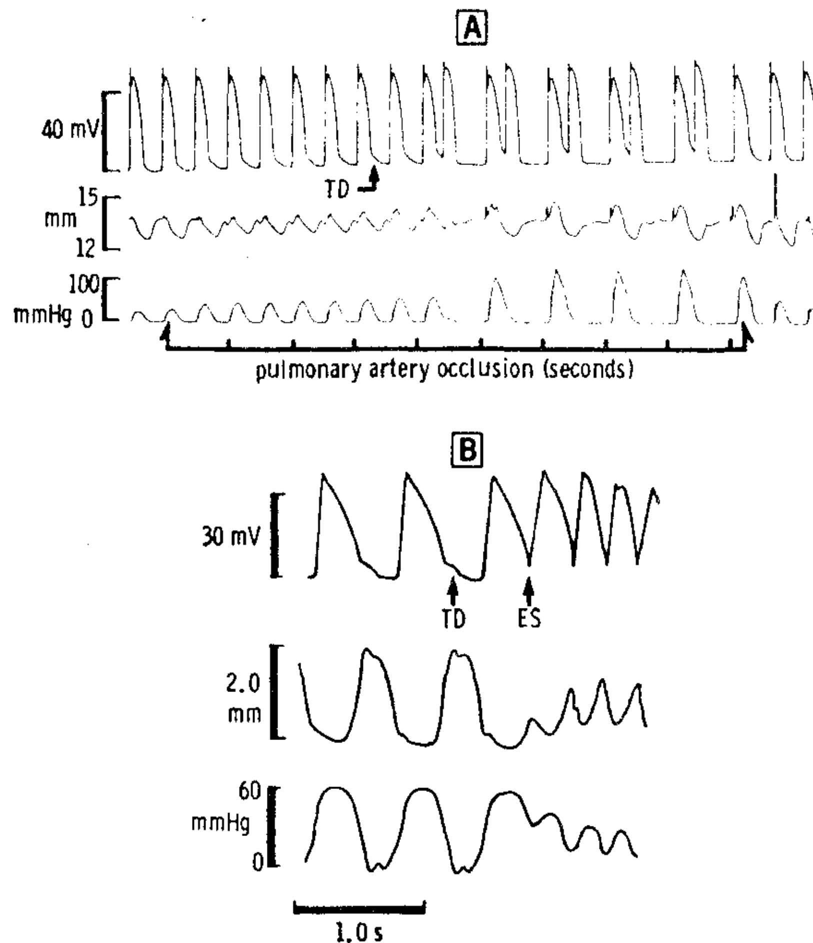


Figure 4

Changes in action potential and segment length from the epicardium of intact ventricles *in situ*. Traces from above down: monophasic action potential, epicardial segment length, intraventricular pressure. [A] Records from right ventricle of an anesthetised dog. Pulmonary artery occlusion increases right ventricular pressure and segment length, causes changes in segment contraction, and produces transient depolarizations (TD) on the action potential. Coupled right ventricular extrasystoles follow. (Modified with permission: Covell et al in press.) [B] Records from left ventricle of pig during regional ischaemia. The action potential shows transient depolarizations (TD) which are related to the stretch and rapid late systolic shortening of the segment (upward is shortening A premature beat arises during the crest of the depolarization (ES) precipitating ventricular tachycardia and eventual fibrillation within 5 minutes of the coronary occlusion (unpublished record, reported in Lab, 1978b).

excitation feedback. Inhomogeneous wall motion can produce electrophysiological inhomogeneity (quadrant D to B). These inhomogeneities can bear on the ECG to influence the T-wave, and conceivably produce U-waves. (4) Regional ischaemia exaggerates mechanical inhomogeneities and expression of the feedback could be conspicuous. Contraction-excitation feedback can generate "extrasystoles" which may contribute to ventricular arrhythmia in early ischaemia.

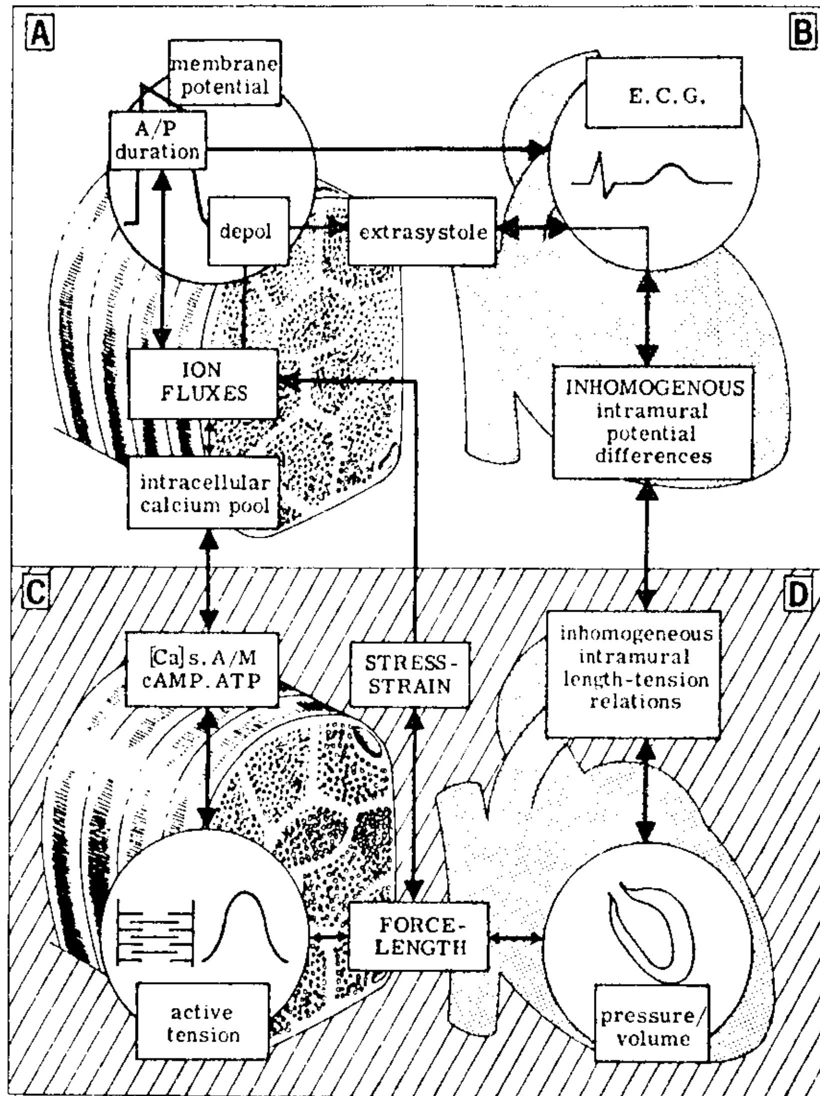


Figure 5

Scheme of some of the interrelationships between excitation-contraction coupling and contraction excitation feedback in ventricular muscle. The diagram has four quadrants with the lower, hatched, half containing the mechanical, and the upper the electrical events. The left hand side of the diagram depicts the events at cellular level, and the right hand side indicates their role in the intact ventricle. (In quadrant C A/M = actin and myosin.) See text for discussion.

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Reprinted from Circulation Research
Vol. 42, No. 4, April 1978

Mechanically Dependent Changes in Action Potentials Recorded from the Intact Frog Ventricle

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SUMMARY **The wall of the ventricle contracts inhomogeneously during an isovolumic beat of an isolated, intact frog ventricle. Some epicardial segments actually lengthen while the pressure is rising. Almost simultaneously, the early repolarization phase of the monophasic action potential recorded from such a segment is accelerated, compared to the same phase for an isotonic beat in which the segment shortens. Segment lengthening during the isovolumic beat also may be seen during the late repolarization phase when, in contrast to the above, it produces an afterdepolarization. These electrical changes disappear when isotonic contraction is restored. Corroborative findings were obtained from microelectrode and insulated gap recordings from isolated frog ventricular strip. Both electrical changes can be seen clearly when the segment is lengthened by intraventricular injections of Ringer's solution. There also is a short transition period toward the end of the action potential plateau when lengthening produces neither depolarization nor repolarization. The accelerated repolarization is manifest as a shortening of the Q-T interval in the ventricular electrogram. In all experimental preparations, the afterdepolarizations reached threshold for a propagated action potential. This mechanism may explain the generation of extrasystoles in myocardial ischemia.**

IT IS WIDELY accepted that during a heart beat there is a progression of events, directionally oriented, which begins with the depolarizing action potential and, via a process of "excitation-contraction coupling," leads to contraction of the muscle.[1,2] From the observations in this report and elsewhere,[3-8] it is suggested that the process is not strictly undirectional. When heart muscle undergoes active or passive mechanical stress or strain, electrical records from the muscle change. Thus the observations on intact frog ventricle presented here, supplemented by studies on isolated ventricular strips, indicate that the mechanics of contraction can influence action potentials and ECG. Furthermore, the mechanism(s) involved can induce opposite polarizing effects, depending on the time of occurrence of an epicardial distortion during the cardiac cycle. These experiments may have some clinical relevance in that the mechanically induced changes may alter the T wave repolarizing vector and be related to the generation of ectopic beats in ventricular myocardium.

Methods

Intact Isolated Ventricle

Frog hearts, obtained from animals that were killed by pithing, during winter and summer, were used in the experiments. The ventricular chamber was cannulated and perfused via the aorta. The cannula was connected to a tap constructed to allow isovolumic or free-loaded contraction, the continuous recording of intraventricular pressure, and rapid externally imposed changes in ventricular volume. Monophasic action potentials from the epicardium and the relative movement of two points on either side of the electrode, monitored by a caliper, were recorded simultaneously (Fig. 1).

Perfusion System

The ventricles of frogs (*Xenopus laevis*) were cannulated via the aorta and perfused with continually oxygenated Ringer's solution (NaCl, 111.3 mM; KCl, 1.8 mM; $CaCl_2$, 1.08 mM; Na HCO_3, 2.4 mM) at room temperature which varied between 20 and 22°C. The cannula was in two sections: a small cannulating section which connected the ventricle to a piston tap and a second vertical section glued to the other end of the tap. A pressure head of 2-3 cm in the cannula provided adequate diastolic filling of the ventricle. The diameter of the ventricle was adjusted by varying the pressure head against which it worked, to be approximately the same as that observed in the intact frog with the chest open. The volume moving up and down the single perfusion cannula with each beat was between 0.1 and 0.3 ml. The contractions were thus free-loaded unless the cannula was closed by the tap, as described below, in which case the contractions were isovolumic. The atria usually were left intact and the heart allowed to beat spontaneously at between 20 and 30 beats/min, but in some cases the atria were tied off and the ventricles electrically stimulated at 24 beats/min.

Piston Tap

Construction. The piston tap consisted of an outer cylinder with a piston inside. The cannula from the ventricle was inserted into a sealing ring in the wall of the outer cylinder. There were holes running through the piston in different directions so that the position of the piston could determine to which of the resevoirs of fluid the ventricle was connected. There were three basic positions of the tap: for free-loaded (auxotonic) contraction, for isovolumic contraction, and for rapid, externally

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Received September 7, 1976; accepted for publication December 2, 1977.

imposed changes in ventricular volume. Ventricular pressure could be monitored at any of the positions.

Operation of the Tap. The relevant position of the piston and thus alignment of the appropriate holes were chosen by setting a mechanical stop onto which the piston was driven by gas under pressure. As shown in Figure 1, the three-way solenoid valve releasing the gas under pressure was opened by the closing of gated relay, 2, controlled by a "Digitimer" (Devices). The piston was spring-loaded and returned, aided by vacuum, when the valve switched back. The "Digitimer" was triggered by a Schmitt Trigger (B) into which the action potential was fed.

Injecting System

Intraventricular injections and withdrawals were accomplished by a motor-driven syringe which was operated when another relay, 1, closed at times preset by the "Digitimer." The relay allowed current to flow first in one then in the other direction to drive the motor forward and then in reverse.

Recording System

Monophasic action potentials from the epicardium were recorded by means of suction electrodes (C) after the method of Hoffman, et al.[9] These authors have drawn attention to some of the pitfalls in the interpretation of the records obtained by this method of recording. However, interest in the present studies is centered on the slower time course changes, for example, the repolarization phase of the monophasic action potential which is not qualitatively different from the repolarization phase found in transmembrane action potentials recorded through a microelectrode.[9] The suction electrode recording was fed into one input of a high input impedance differential amplifier. The other input was a wick electrode on the ventricle. The precise location of the wick did not affect the nature of the recording obtained.

The relative movements of two points on either side of the electrode were monitored by a photoelectric caliper (D) placed either longitudinally or transversely. This caliper was also attached by suction and recorded roughly unidimensional changes in the chord length of the segment studied.[10] The construction of the caliper did not allow for "shearing" between the points of attachment of the caliper to the epicardium. A bipolar electrogram (E, Fig. 1) was recorded from two epicardial wick electrodes, soaked in Ringer's solution, or with one electrode in the intraventricular perfusing fluid. Intraventricular pressure was monitored with a pressure transducer (F), and the volumes injected into the ventricle were monitored by means of a graded density film attached to the plunger of the motor-driven syringe. The film interrupted a light source to a phototransistor (G).[11] The signals from transducers and electrodes were fed into a multichannel pen recorder (H) (Devices) and storage oscilloscope (I) (Tektronix).

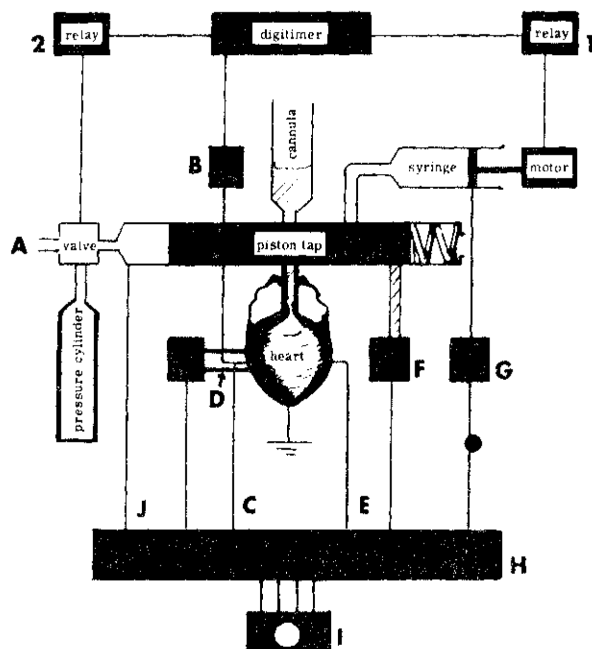

FIGURE 1 *Diagram of experimental arrangement. The frog ventricle was cannulated and attached to the cylinder of the piston. The motor driven syringe was operated by Relay 1 and a "Digitimer." A three-way solenoid valve was connected to a pressure cylinder operated by Relay 2. A, vacuum; B, Schmidt-trigger; C, suction electrode; D, caliper; E, electrocardiogram; F, pressure transducer; G, photoelectric circuit to monitor plunger of syringe; H, multichannel pen recorder; I, storage oscilloscope; J, marker.*

Confirmatory Records from Frog Ventricular Strips

Intracellular Recordings

A ring of muscle was cut from the base of the frog ventricles (*Rana pipiens*), connective and atrial tissues were cut away, and the ring was converted into a strip about 1 mm^2 and 4-8 mm long. The muscle was clamped horizontally in a perfusion chamber and a conventional microelectrode recording system for cardiac muscle was used.[12] The electrodes were floating free from earth and their resistances were not less than 20 MΩ. Records of all the attempted impalements were stored on magnetic tape so that those which were successful could be analyzed later. The number of successful impalements was rather low because frog ventricular cells are small and the muscle length was changing. Tension was measured by an RCA transducer.

Insulated Gap Recordings

The method described by Niedergerke[13] was used for these recordings. The strip of ventricle, prepared as described above, was mounted vertically and clamped near one end in a canal 0.5 mm long and 0.5 mm in diameter. The canal was formed by two sliding plates of acrylicic plastic which created a partition between two chambers. Silicone grease in and around the canal completed the separation between the two chambers. The muscle traversed the gap and lay in the chambers; the first chamber containing Ringer's solution and the second isotonic KCL or 0.5% procaine in 7% sucrose. Action potentials recorded across the insulating gap, between the first and second chambers, were 20-60 mV in amplitude.[14]

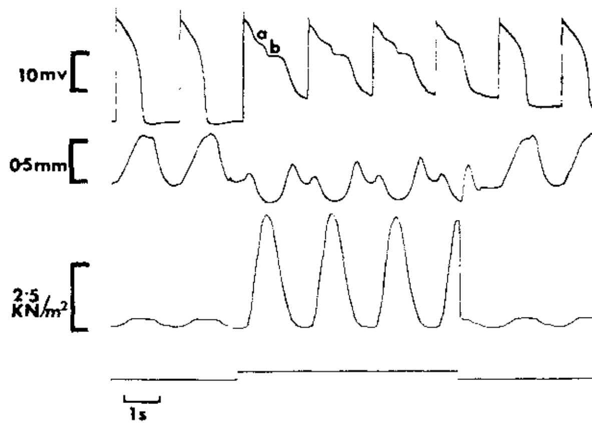

FIGURE 2 *Action potential changes (top trace) and movements of an epicardial segment (middle trace) with cyclic intraventricular pressure changes (bottom trace) due to isovolumic contraction of a frog ventricle indicated by the deflection of the marker (horizontal line). The first two beats show "normal" configuration of action potentials and segment shortening (upward deflection). At point (a) of this record, after the onset of isovolumic contraction, the first part of the repolarization phase of the action potential is accelerated, while the pressure is rising, and the segment lengthens. Note that the segment length during most of the isovolumic contraction is greater than that of the relaxed isotonically contracting ventricle (trough of recording, before isovolumic contraction). During the relaxation phase, when intraventricular pressure declines, the rapid repolarization phase is interrupted (at b) to form a depolarizing afterpotential which does not allow the action potential to return to base line before the next action potential. The ensuing spikes have a reduced amplitude. On resumption of isotonic contraction, the changes are completely reversed.*

Tension and length were recorded by a modified strain gauge lever.[11, 14, 15]

Results

Mechanical and Electrical Changes on Converting Free-Loaded Contraction to Isovolumic Contraction

When the frog ventricle was allowed to contract auxotonically (free-loaded), the action potential displayed the normal configuration except for the reduced amplitude associated with the suction electrode method of recording (first two action potentials on the left side of Fig. 2). The same segment of epicardium showed shortening (upward deflection) and relaxation, as expected. The recorded intraventricular pressure fluctuated little, and this was due to the muscle moving a small pressure head of Ringer's solution in the perfusing cannula. However, when the ventricle was made to contract isovolumically at its end-diastolic volume (marker, horizontal trace), changes were seen in both the action potentials and in the pattern of contraction of segments of the epicardium. On closing the aortic outflow at the appropriate time (diastole), the action potential exhibited several phenomena when compared with the auxotonic contraction. It had a faster initial repolarization followed by a delay in recovery which took on the appearance of hump-like afterpotentials. The potential did not reach the same base line as the pre-isovolumic action potential, and the next action potential was reduced in amplitude.

The changes in epicardial segment length were distinct from those found during a free-loaded, auxotonic beat. In Figure 2, the fibers of the epicardial segment actually lengthened when they were expected to shorten, that is during ventricular contraction per se. This lengthening coincides with the steepening of the plateau phase. Thereafter the segment shortened but, again, at a time when these muscle fibers should "normally" lengthen—when the ventricle relaxes as a whole. The segment length throughout the isovolumic cardiac cycle was greater at any given time than during a free-loaded contraction. Studies of segmental movement on the epicardium of dog ventricle have been carried out previously[16, 17] and, since the time relationship between the mechanical and electrical events of any one segment was the center of interest in this study, the recordings were obtained with the caliper on a convenient surface usually orientated along the longitudinal axis of the surface of the ventricle, anteriorly.

To quantify some of the results, the amplitudes of the action potentials were taken at mid-plateau (at t_1 inset, Fig. 3) and compared before and after aortic closure; the difference was obtained (ΔV_1) and expressed as a percentage of a control amplitude. (This form of expression was desirable because the action potential amplitude obtained by suction electrode varied from preparation to prepara-

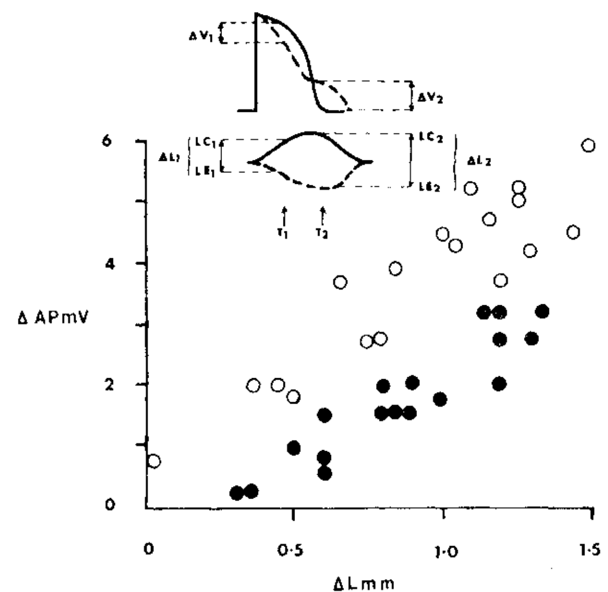

FIGURE 3 *Relationship between changes in segment length and changes in action potential (open circles) and after potential (filled circles). An isovolumically contracting empty ventricle was filled progressively to normal end-diastolic volume with 0.05-ml increments. Changes in plateau and afterpotential voltages were measured and plotted against segment length changes (initial length was 3.5 mm).* ***Inset*** *(see text): Control action potential and segment length (solid lines) superimposed on experimental records (dashed lines). Changes in potential, V, and segment length, L, were taken at times t_1 and t_2; e.g., L_2 is the change in segment length at t_2 obtained from LC_2, the control length, and LE_2 the "experimental" length.*

tion.) The control base-line was established, using action-potentials before and after aortic closure, and the measurements were made from this line drawn through the greatest diastolic potentials. The peak height of the after-potential produced on closing the aorta was measured at t_2 (inset, Fig. 3) and the percent difference obtained (ΔV_2) was again expressed as a percentage of control plateau height. The time t_2 corresponded in most cases with the middle of the fall in intraventricular pressure and, where the peak of the after potential was not clear, t_2 was put at this mid-fall point. Changes in segment length were expressed in strains $\Delta L/L$ at t_1 and t_2 (where, for example, ΔL is the difference in length at t_1 between pre- and postaortic closure (LE-LC), and L is the control segment length at t_1 (LC). Correlation coefficients (r) between potentials and lengths at t_1 and t_2 were 0.52 and 0.235, respectively, with $P < 0.001$, and between 0.05 and 0.01, respectively.

In one experiment, the ventricle was emptied and refilled in 0.05-ml steps to its end-diastolic volume. At each volume the same measurements as above were made, but at each volume the figures obtained were related to the control values for the empty ventricle. The results are plotted in Figure 3. Clearly, the greater the length change the greater the change in potential. For a given length change, the change in the plateau was greater than the change in afterpotential. Not all the changes need be present at the same time. Figure 4 shows an unaltered

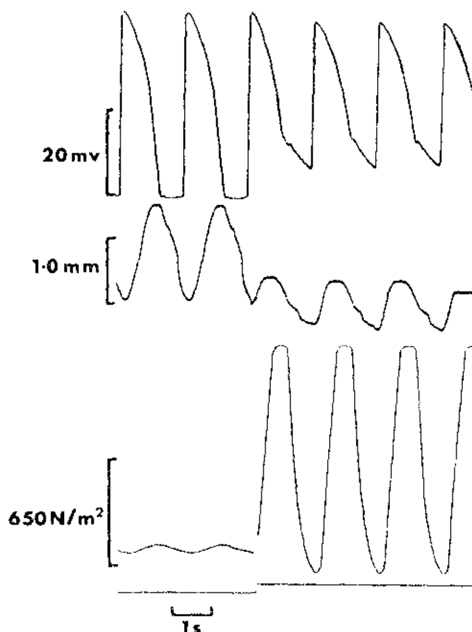

FIGURE 4 *The same type of experiment as in Figure 2 but with no obvious accelerated repolarization of the plateau phase of the action potential (top trace) when the ventricle is made isovolumic (marker). In this case, the predominant lengthening of the epicardial segment (middle trace) takes place during the decline of intraventricular pressure (bottom trace), and the length attained is greater than the isotonic diastolic length—left side of picture. At roughly the same time as the segment lengthens, during the isovolumic contraction, the rapid repolarization phase of the action potential is interrupted by a depolarizing afterpotential.*

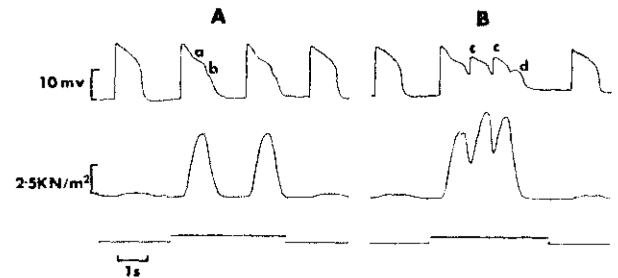

FIGURE 5 *Action potentials (top trace) and intraventricular pressures (middle trace) of frog ventricle during isovolumic contraction (upward deflection of the marker on the bottom trace). In part A, isovolumic contraction resulted in an increased intraventricular pressure, and the accelerated repolarization (a) followed by depolarizing afterpotential (b). In part B of this figure (the same preparation), the afterpotential is accompanied by new propagated action potentials (c).*

plateau phase with the beginnings of a normal (shortening) contraction pattern of the segment of epicardial muscle. Thereafter the segment shows an exaggerated lengthening compared with the same period during isotonic contraction. Associated with the latter, the hump-like after-potential and the rise in base line are observed. Although action potential changes occasionally were seen without segment length changes, only once was a significant length change observed with little accompanying action potential change. These last two observations could be explained if epicardial contraction were inhomogeneous, with the electrode on a segment having a mechanical behavior different from that of the "caliper" segment. Fisher et al.,[16] in fact, found no distinct regular pattern of epicardial length changes in their experiments. It is likely that epicardial segments can lengthen in a direction not monitored by the caliper, no length change being apparent, but with the action potential still changing. The reverse is less likely but possible. An electrical recording which is obtained from a relatively unstrained bit of myocardium could then explain the isolated observation of a segment length change with no change in potential.

Electrical Effects Related to the Recorded Changes in Potential

Threshold Depolarization

If the depolarizing afterpotential observed during isovolumic contraction is nonartifactual, it may be expected to be capable of reaching threshold to initiate a propagated action potential. Figure 5A again shows the steepening of the plateau phase with whole ventricle contraction and the depolarizing afterpotential with whole ventricle relaxation (as designated by the decline in intraventricular pressure). In Figure 5B, this depolarizing potential reaches threshold and produces an action potential capable of initiating a contraction.

Although the afterpotentials or the points at which threshold are reached in Figure 5B are somewhat larger than in Figure 5A, the last potential, d, was the largest of all. Yet no action potential has propagated. The explanation for the last observation, made in several preparations,

is speculative at present. It is possible, first, that ionic fluxes accompanying mechanically induced multiple extrasystoles alter the immediate extracellular environment and thus produce conditions which change the threshold. Second, the cells that actually reached threshold may have been distant from the electrode and many have shown, at the time of recording potential d, a potential of reduced amplitude.

Microelectrode Recordings

The action potentials obtained by suction electrodes are proportional to intracellular potentials but are reliable in following slow changes in potential.[10] However, during the changes in length of the epicardial segments there is inevitably distortion at the tissue-electrode interface. Thus the current flowing into the electrode may vary either by a reduction in the number of cells contributing to the current or by a change in the extracellular shunting resistance. Some confirmatory studies were therefore thought necessary and were done on isolated strips of myocardium using microelectrodes.

It was technically difficult to keep an electrode impaled in a single cell and record reliably while imposing rapid mechanical changes on the muscle. (A success rate of 1 in 60 attempts has been quoted in cat papillary muscle with larger cells[6].) However, the measurement of the duration of action potentials was attempted in these experiments, for even if the full resting potential was not recorded, the duration of the action potential at 80% repolarization was identical with that of an action potential obtained with a good impalement. Action potential durations could thus by compared reliably at two different lengths: at slack lengths and at lengths at which near maximum tension was produced (L_{max}). Using several different impalements, making 16 observations in two preparations, action potential duration at a slack length was 864 ± 106 (mean ± SD) msec and, at near L_{max}, 790 ± 140 msec ($P < 0.001$ by Student's *t*-test).

A good example of a change in action potential duration associated with extension of a muscle from a length at which little active tension is produced to a length less than L_{max} is seen in Figure 6. The two action potentials are consecutive and superimposed, being obtained from the same impalement, and the action potential duration is clearly reduced with stretch.

Although changes in action potential plateau, with alterations in length, were clearly demonstrable, consistent changes in resting potential or the production of afterpotentials following mechanical changes were less obvious. It was extremely difficult to stretch a muscle at rest and keep the electrode impaled so that, when releasing the muscle, the control, full resting potential was reestablished. With resting potentials more negative than about −40 mV, a depolarization was the predominant response with a transient stretch (Fig. 7). This response could be explained by the electrode coming out and reimpaling the cell. However, even with short duration stretches confined to less than L_{max}, a new propagated action potential that produced an active tension could be generated (Fig. 7), which is in keeping with a depolarization being real and reaching threshold to activate contraction. A stretch induced "extrasystole" was seen in every isolated strip in which it was sought ($n > 40$).

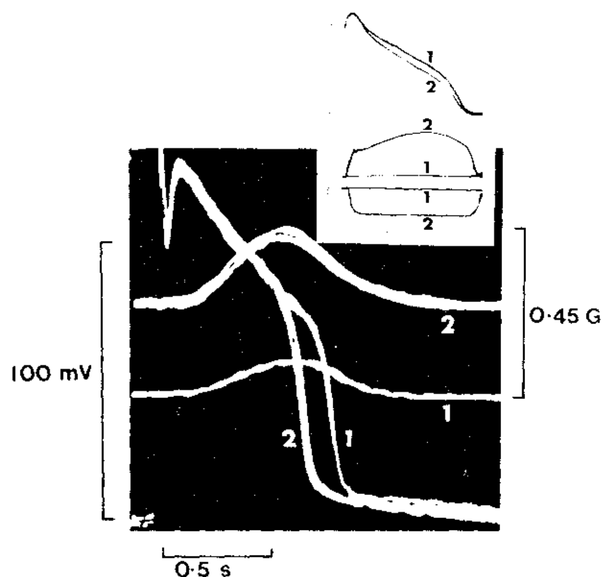

FIGURE 6 *Superimposed traces of action potentials, obtained with microelectrodes, and tensions (two middle traces) during stretch. Same impalement in an isolated strip of frog ventricle. The muscle first contracted (1) at a short length, producing a small tension, and was associated with the action potential with the long duration. On stretching before the second contraction (2), the muscle passive and active tension increased and the action potential duration simultaneously shortened. Stimulation frequency, 30/min; temperature, 18°C; muscle slack length, 2.0 mm. These records were obtained in Dr. B.R. Jewell's laboratory at University College London, with the assistance of Dr. M. Boyett.* ***Inset:*** *Monophasic action potentials, obtained with an insulated gap technique (upper traces, calibration bar, 20 mV), tension (middle traces, calibration bar, 4 mN/mm²), and length (lower traces, calibration bar, downward deflection, L_o to L_{max}) recorded from an isolated strip of frog ventricle. In the first contraction (1) at the short length the muscle produced almost no active tension and was associated with the longer action potential. During the second contraction (2), stretching the muscle to L_{max} resulted in a larger tension and the shorter action potential. Stimulation frequency 30/min; temperature, 20°C; muscle slack length, 5 mm.*

Insulated Gap Recordings

In view of the limitations in interpreting the records with suction electrode and some of the microelectrode recordings, particularly the depolarization on stretch, a third method of recording was used.[13] In this method, one end of the muscle was firmly held in an insulation gap while the mechanical changes were obtained via the other end. The electrical recordings were transgap potentials. Changes in action potential duration, recorded across the insulated gap (inset, Fig. 6) were again confirmed with length changes of the same magnitude as used with the microelectrode studies. With this technique of recording, several transient stretches could be given at different times during consecutive action potentials. Brief repolarizations were seen during the action potential, and transient depolarizations were again produced with stretch which also

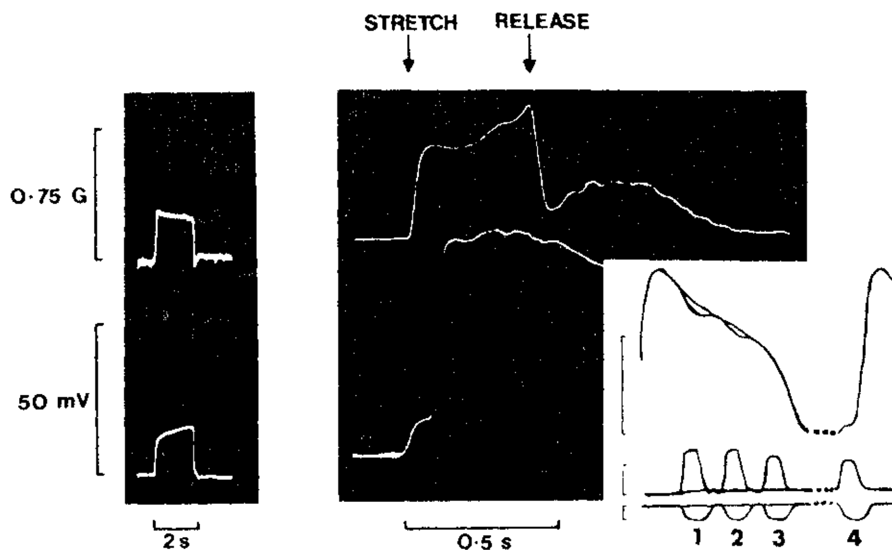

FIGURE 7 *Effects on tension, and potential recorded with microelectrodes, of stretching frog ventricular strip at rest. Left side: A small transient stretch, to about 80% L_{max}, produced an increase in passive tension (upper trace) and an apparent depolarization (lower trace) both reversible and of the same duration as the stretch. Right side: The apparent depolarization (lower trace) was accompanied by a stretch-induced action potential which activated the muscle (upper trace). The stretch, to L_{max}, invariably disturbed the impalement even if the muscle was not released during the induced contraction. Stimulation frequency, 30/min; temperature, 19°C; muscle slack length, 2.5 mm; weight, 3.2 mg. (These records were obtained in Dr. B.R. Jewell's laboratory at University College London, with the assistance of Dr. M. Boyett)* **Inset:** *Effect of transient stretches at different times on recorded potentials. Monophasic action potentials, obtained with an insulated gap technique (upper traces calibration bar, 20 mV), tension (middle traces calibration bar, 10 mN/mm²), and length (lower traces calibration bar, L_0 to L_{max}) recorded from an isolated strip of frog ventricle. The recordings are taken over five consecutive beats. Stretches 1 and 2 during the early and middle of the plateau phase of the action potential produced a transient repolarization tendency, whereas stretch 3 did not disturb the action potential at all. Stretch 4, however, during the resting phase, produced an apparent depolarization that was accompanied by a new action potential.*

could be accompanied by new action potentials as if the depolarizations reached threshold (inset, Fig. 7). There was a period during activity in which a stretch produced no electrical effect.

Changes in the Electrogram of the Intact Ventricle

The Q-T interval and T wave of the electrocardiogram represent the extracellular voltage manifestations of the repolarization wave through the ventricle as a whole, and, as has been seen, mechanical changes are associated with alterations in cellular repolarization.

If, therefore, different segments of ventricular wall have different mechanical behavior during isovolumic compared with isotonic contraction, then contraction under the two conditions should produce different repolarizing rates and vectors. That is, the time course of the repolarization wave through the intact heart (Q-T interval) may be altered. The localized changes in action potential should therefore be reflected in the electrogram, and Figure 8 shows an example of this. The superimposed tracings of the action potentials show both the steepening of plateau (accelerated initial repolarization) and depolarizing afterpotential previously described with isovolumic contraction. The associated electrocardiogram has a shorter Q-T interval and reduction in T wave amplitude compared with auxotonic contraction.

Simulated Mechanical and Electrical Changes in the Intact Ventricle (Intraventricular Injections)

The changes in segment length seen in isovolumic contraction do appear to be associated with alterations in the shape of the action potential. Further, imposed changes in length of an isolated strip of myocardium affect the recorded potentials. It is possible, therefore, that similar electrical effects might be generated if some of the mechanical changes in the epicardium were produced artificially. An experiment was therefore performed in the intact ventricle analogous to that in the isolated strip (see inset, Fig. 7). This was done by injecting small volumes of Ringer's solution into a ventricle contracting isovolumically at a small diastolic volume. The ventricle was not distended beyond its normal diastolic volume and the injections, followed by withdrawals, were given at different times during the cardiac cycle. The results are seen in Figure 9. In all the sections of this figure, the first contraction took place at a small, unchanged intraventricular volume during which time the epicardial segment contracted, the intraventricular pressure rose, and the action potential had a normal configuration. When an injection and withdrawal were made early in the cycle, V_2 in Figure 9, stretching the segment to L_2 and increasing the intraventricular pressure to P_2, there was a transient decrease in the amplitude of the plateau of the action

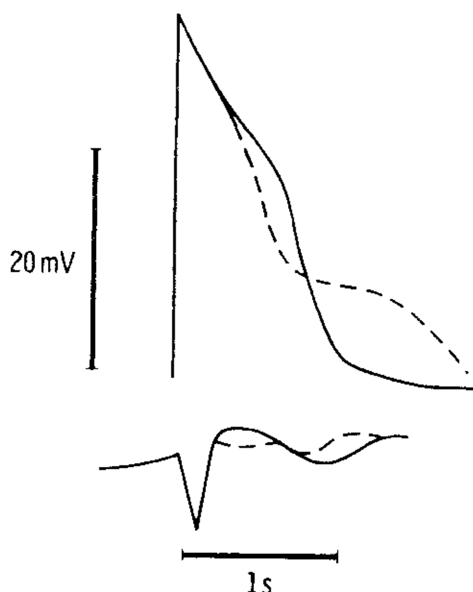

FIGURE 8 *Superimposed traces of electrocardiogram (bottom trace) and action potential (top trace) during isotonic contraction (solid lines) and isovolumic contraction (dotted lines). The action potential shows the previously observed initial acceleration of the plateau phase followed by the depolarizing potential. The ECG of the isovolumically contracting ventricle has a shorter Q-T interval and a lower amplitude T wave than the auxotonically contracting ventricle.*

potential (A_2). If the injection was made near completion of repolarization, the potential change had the opposite polarity producing a transient depolarization (Fig. 9). It is clear that a period should exist during the action potential when an injection has no effect. Such a period would be a transition between the early repolarizing and later depolarizing effects of the injections. Figure 9 demonstrates a period during which even a larger injection than in parts A and B has no effect on the plateau of the action potential. The depolarizing potential seen in Figure 9 also may be associated with the generation of a new action potential, mechanically induced, as was also observed with the isolated strip (Fig. 7). This is demonstrated in Figure 10. The earliest of the intraventricular injections (part A) is associated with a relatively small depolarizing potential as compared with parts B and C where the injections were given later in the cycle. However, the mechanically induced depolarization in part D is accompanied by a new action potential. The epicardial and isolated strip studies thus largely complement each other.

The investigations center on the paradoxical movements of epicardial segments during contraction. Spontaneous lengthenings during isovolumic contraction are observed, mimicked by externally imposed increases in volume, and are accompanied by changes in action potential. The changes during the plateau phase, per se, are directional, and a longer or lengthening segment has a repolarizing tendency compared with a segment that shortens, which has a longer duration action potential (polarizing tendency). Kaufmann et al.[6] observed in cat papillary muscle a prolonged action potential when the muscle is allowed to shorten and, when the muscle contracted isometrically, an acceleration of repolarization. To make a more direct comparison between the former results and the present investigation, a ventricle was made to contract isovolumically at normal end-diastolic volume (Fig. 11). During the next action potential, the ventricle was emptied and immediately refilled on completion of repolarization. The duration of the action potential associated with the empty ventricle is prolonged as compared with the action poten-

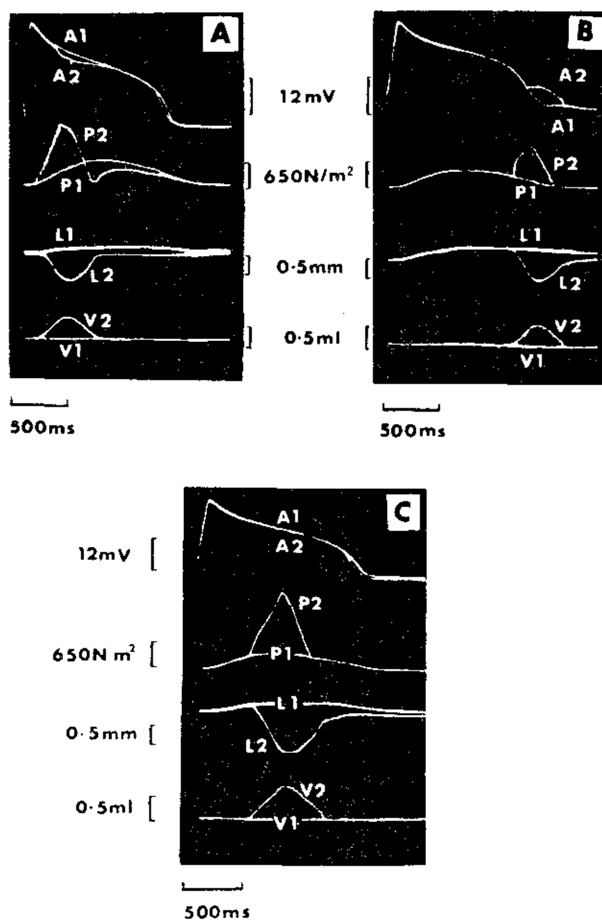

FIGURE 9 *Parts A, B, and C. Effect of transient increases of intraventricular volume at various times in the cardiac cycle during normal isovolumic contraction. In each part of the figure, V_1 is a small intraventricular volume (end systolic); L_1 the change in length of the epicardial segment (upward deflection is shortening); P_1 intraventricular pressure; A_1 action potential with normal time course. Again in each part, a transient rapid increase in intraventricular volume, V_2, produces the mechanical effect of lengthening the epicardial segment; L_2, and increasing the intraventricular pressure, P_2. Although the mechanical changes in each part of the figure have the same direction, the effects on the action potential differ: transient repolarization when the injection is given early in plateau phase (A_2 in part A); and the opposite, namely transient partial depolarization, when the injection is given near completion of repolarization (A_2 in part B). Between these times an even larger injection has no effect (A_2 in part C).*

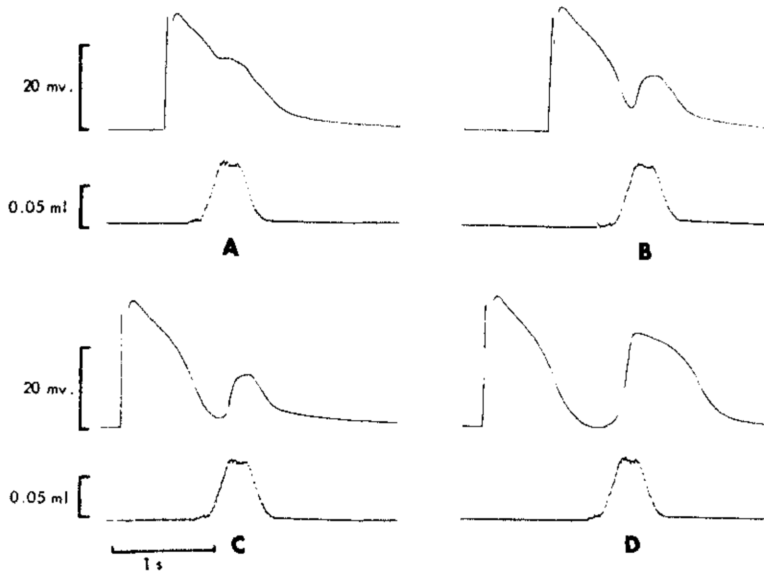

FIGURE 10 *Effect of a transient increase in intraventricular volume (lower traces in A–D) at four different times during the action potential (top traces). The depolarizing potential becomes progressively more distinct as the increase in volume takes place later during the repolarization phase (parts A, B, and C). In part D, however, the depolarization is immediately followed by a new action potential.*

tial of the ventricle with the greater volume, when repolarization is accelerated.

Discussion

It is suggested (for reasons outlined below) that the observed changes in recorded potential, following the mechanical changes, are the result of changes in ionic currents. The changes in duration of the action potential are probably a true reflection of cellular events, for first, suction electrodes are a reliable method of following the time course of the repolarization phase of the cardiac action potential, and second, both the insulated gap and intracellular recordings also show changes in duration. In addition, the electrocardiogram shows changes in Q-T interval and T wave, both manifestations of altered ventricular repolarization as a whole (see also Stauch 1960—cited in Ref. 3). These ECG changes cannot be explained simply by a shift in direction of the repolarization vector

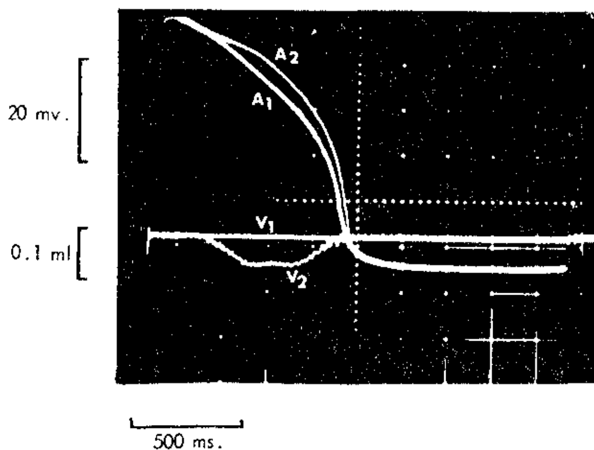

FIGURE 11 *Effect on action potential of reducing ventricular volume. The ventricle was made to contract isovolumically at its normal end-diastolic volume (V_1). The action potential at this volume was A_1. During the next contraction, the ventricle was rapidly emptied to V_2 and then rapidly refilled. The action potential duration increases to A_2 during the change in volume.*

in relation to the recording leads, for example by the rotation of the heart during contraction, for the T wave and Q-T interval changes are always in the same direction, i.e., smaller on isovolumic contraction at normal end-diastolic volume. Furthermore, the Q-T interval per se is a chronological measurement and is unlikely to be influenced significantly by a change in amplitude or direction of the repolarization vector. The changes are therefore more likely to be due to a true alteration in the time course of the ventricular repolarization gradient.

The depolarization is more difficult to interpret, for although it was seen by means of three different recording techniques, each depolarization could be explained by recording artifact. The depolarization seen with the suction electrode could be a manifestation of a distortion at the tissue-electrode interface and altered current flow between the recording and indifferent electrodes. The changes in resting potential observed with microelectrodes could be a result of the electrode leaving and reimpaling the cell. In fact stable resting potentials have been observed despite changes in length (e.g., Fig. 6). [To explain the last observation one would have to postulate that a significantly larger extension of the muscle is needed to alter the resting potential than to change the action potential duration. This possibility is qualitatively in keeping with the graph in Figure 3 and the relatively large extension required to depolarize the muscle to threshold (inset, Fig. 7)]. Finally, the mechanical disturbances, using the insulating gap technique, may produce changes in current flow in and around the gap and thus simulate depolarization.

However, the implications of stretch-induced depolarizations in ventricular muscle are important. Therefore, in view of the above pitfalls in interpretation, additional points in favor of the depolarization not being artifact are worth summarizing even though these points should be treated with caution. To begin with, the depolarizations observed with stretch are in keeping with other observations on Purkinje fibers[18, 19] and papillary muscle,[19] using microelectrodes. Stretch also produced depolarization ca-

pable of reaching threshold in ventricular muscle.[19] (The last two publications were not concerned with action potential durations per se.)

Furthermore, predictions on the expected behavior of the myocardium, based on the results being a true reflection of changes in resting membrane potential, are verified by two observations in these present experiments. First, it is significant that the depolarizing effect, observed by using all three recording techniques when the muscle elongates during relaxation, can apparently reach threshold and be followed by an "extrasystole." Second, this new action potential often has a reduced amplitude, as is expected when the membrane is partially depolarized. One cannot exclude the possibility however that "stretch-sensitive" cells of the pacemaker type may conceivably be found in frog ventricle. If one allows that the length of the segment of muscle fibers was adjusted to be near the physiological optimum (L_{max}), then the final length reached when the segment was stretched, for threshold depolarization, agrees with that found in the isolated ventricular strip.

The above observations and arguments taken together with results of previous experiments strongly suggest that the recorded electrical changes between isovolumic and free-loaded contractions are a true reflection of altered current flow across membranes and, considering the electrocardiographic changes, between groups of cells, in the intact frog ventricle. Furthermore, the epicardial movements under these two conditions show that the mechanical behavior between groups of cells also differs. This is simply because epicardial fiber lengthening in one section of the myocardium, when taken together with a rise in intraventricular pressure, necessitates shortening and tension development in other sections of the myocardium. These experiments are not as far removed from the physiological situation as they first appear. In fact, comprehensive studies of epicardial strains have been carried out by Fischer et al.[16] and Dieudonne and Jean,[17] who also found positive strains (lengthening) during the normal isovolumic phase of contraction of intact dog ventricle. The segments thereafter showed some shortening during isovolumic relaxation.

Having demonstrated that both mechanical and electrical behavior in a particular segment alters at the same time, the question arises as to whether they are causally related and, if so, what mechanisms are involved. It is clear from the effects of stretches on isolated strips (Figs. 6 and 7), as well as the intraventricular injections in Figures 9 and 10 and other experimental situations,[6] that the mechanical change precedes and thus is somehow related to the electrical change. This observed time relationship, together with the evidence described above, is compatible with the existence of a "mechano-electric coupling" in ventricular cardiac muscle. Some "contraction-excitation-contraction" coupling has been described by Kaufmann et al.[6] Keeping cat papillary muscle at a longer (isometric) length results in an abbreviated action potential duration as compared with a muscle that is allowed to shorten (isotonic contraction). The direction of electrical change is identical to that seen in the present studies where isovolumic contraction is accompanied by a shorter action potential than an auxotonic contraction. The switch from isometric to isotonic contraction in the studies on papillary muscle resulted in a small positive mechanical staircase. This mechanical transient may be attributable in part to the same mechanism that causes the transients when the action potential is electrically prolonged.[20] Small pressure or segment length staircases were occasionally observed in the intact frog when altering the mechanical conditions of contraction. To determine whether these small mechanical changes are related to ionic fluxes across the membrane or to the viscoelastic properties of cardiac muscle needs further investigation. In this context, it is worth noting that a prolongation of the action potential in frogs affects only the immediately accompanying contraction and not the subsequent beats;[20] i.e., no tension transients follow. It therefore may be difficult to ascribe the transients that were occasionally seen in these experiments to changes in the action potential per se.

It is appropriate at this stage to begin speculation as to what architectural alteration, cellular or molecular, could take place during the imposed mechanical disturbance to influence the membrane potential. It is significant in this context that both the active state (tension development) and membrane conductance are altered by changes in membrane potential. The calcium ion, implicated in both active state and membrane phenomena,[1,2] could play a role and the time course of repolarization be influenced by a change in membrane properties or internal calcium ion kinetics as a result of the mechanical changes. This change in active state and internal calcium would in turn affect ionic flux across the membrane either directly or indirectly. There is, in fact, considerable evidence that calcium release in muscle may be mechanically dependent or length dependent[21-27] and that changes in internal calcium can alter the outward potassium current.[28] One could speculate further that the calcium ion could be related to the depolarization observed when stretching the muscle at rest again, either by direct or indirect action. Gordon and Ridgeway[27] have found in the single barnacle muscle fiber a length-dependent electromechanical coupling in which a depolarization produced by stretch is calcium mediated. It is thus conceivable that mechanical stress or strain in the membranes of the ventricular muscle could alter the ionic permeability of the membranes thus changing membrane potentials.

Hypotheses alternative to the preceding one are more speculative and will be only briefly recounted, as they are discussed in more detail elsewhere.[14] First, bearing in mind the possible involvement of the active state mentioned above, a link between a mechanically induced change in the muscle and membrane phenomena could be "energetically" mediated, either by cyclic AMP, or ATP.[29-31] Second, the immediate extracellular spaces and/or sarcoplasmic reticulum may be distorted such that an altered diffusion or accumulation of some ion therein could influence the membrane potential to produce potentials analagous to those described under certain conditions with skeletal muscle.[32-34]

Further discussion concerning the mechanism of "mechano-electric coupling" must await more direct experi-

mental evidence. However, if there is sufficient evidence at this stage to support the hypothesis that some forms of "mechano-electric coupling" or mechanically dependent potential changes are to be found in cardiac ventricular muscle, then it is necessary for the hypothesis to fit or explain existing observations. It is thus important to outline the possible role and context of this phenomenon in cardiac ventricular muscle. First, it has suggested that there is a localized control system, operating on a beat-to-beat basis, in which the length-tension relations of cardiac ventricular muscle may influence the duration of the immediate and ensuing action potentials and active state.[6] This mechanism probably is closely related and in fact works in the appropriate direction to be in keeping with the mechanical changes described by Jewell and Rovell.[35] Second, the observation that different degrees of shortening or tension development of ventricular muscle are associated with varying rates of repolarization, as indicated by the duration of action potentials, may be one of the determinants of the ventricular repolarization gradient. This repolarization vector (T wave) is normally in the same direction as the QRS vector and thus deflects in the same directions in the electrocardiogram. The T wave direction indicates, in part, that the epicardial fibers repolarize faster than the endocardial fibers, and it has been suggested[36] that this observation is related to the fact that the epicardial fibers shorten less than the endocardial fibers.[37] This point cannot be more than a suggestion at this stage, for no systematic study has been done on the time course of these action potential changes following mechanical changes, over a prolonged period of time.

Finally, mechano-electric coupling may have important clinical relevance. It is now well established that there can be exaggerated inhomogeneity of ventricular wall contraction after a myocardial infarct.[38, 39] It is feasible that the ventricular dysynergy which can follow such an episode results in an appropriately timed deforming stretch of the surrounding viable myocardial tissue to produce ectopic beats or extrasystoles and thus facilitate ventricular arrhythmias.[40]

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INSTRUMENTS AND TECHNIQUES

MONOPHASIC ACTION POTENTIALS, ELECTROCARDIOGRAMS AND MECHANICAL PERFORMANCE IN NORMAL AND ISCHAEMIC EPICARDIAL SEGMENTS OF THE PIG VENTRICAL *IN SITU*

BY

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Reprinted from Cardiovascular Research
Volume XII, No. 9, *pages* 555–565, *September* 1978

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LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, LONDON WC1

Cardiovascular Research, 1978, **12**, 555–565

Instruments and Techniques

Monophasic action potentials, electrocardiograms and mechanical performance in normal and ischaemic epicardial segments of the pig ventricle *in situ*

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SUMMARY Few studies report simultaneous electrical and mechanical recordings from the epicardium of intact beating hearts *in situ* during ischaemia. We use suction to apply transducers and electrodes to areas of the epicardium. This interferes little with its behaviour and allows: i) free mobility over the surface; ii) simultaneous tridirectional length changes to be recorded and summed for an overall impression of mechanical behaviour; iii) detection of changes in direction of movement; iv) simultaneous recordings of monophasic action potential and epicardial ECG. During ischaemia we can detect impaired contraction with dyskinesis and a change in direction of epicardial forces. The action potential duration shortens and we have noted impaired conduction, inexitability and recordings consistent with re-entry leading to ventricular fibrillation.

There are serious difficulties in trying to measure mechanical performance in the intact heart and in making comparisons between isolated and intact preparations (Sonnenblick, 1974; Reichel, 1976). Some attempt has been made to bridge the gap between the above types of studies, and mechanical measurements have been obtained from epicardial segments by the use of strain gauges sutured to the heart (Forrester *et al.*, 1974; Tyberg *et al.*, 1974), or ultrasonic crystals implanted in the heart (Bugge-Aspheim *et al.*, 1969; Theroux *et al.*, 1974). These methods have two disadvantages. Firstly they are permanently placed, and secondly they usually provide an indication of strain in one direction only. Levers, from potentiometers fixed above the heart, which are not permanently sutured to, but stab the heart, have been used to record segment lengths (Linden and Mitchell, 1960). However these also show unidirectional length changes at any one time. For measurements in three directions on the heart's surface a tripodal device has been previously developed (Dieudonne *et al.*, 1972) but the feet of this instrument have to be stabbed into the heart and may reach a variable depth. Great care also has to be taken to ensure that the instrument does not fall off during vigorous contraction of the heart. With the appropriate calculations an indication of the strains in the tissue to which it is attached can be obtained. The electrical changes in epicardial segments can be obtained extracellularly by the use of wick electrodes on the ventricular surface. Ideally, for measuring electrical events in tissues, microelectrode recordings are desirable. However the technical difficulties of reliably and consistently recording transmembrane potentials in the intact beating ventricle *in situ* are almost prohibitive (Toyoshima *et al.*, 1965; Downer *et al.*, 1977b). Further, this technique frequently necessitates the partial immobilisation of the area to be studied which may affect mechano-electric interrelationships (Kaufmann *et al.*, 1971; Hennekes *et al.*, 1977; Lab, 1978). A new instrument partly described previously (Lab and Price, 1977) has been constructed for electromechanical correlation in epicardial segments, that overcomes some of the above difficulties. The object of this study is therefore, first, to develop a relatively simple but reliable method of monitoring action potentials and epicardial ECG's with mechanical performance of small areas of epicardium and, secondly, use this system to record electrophysiological and mechanical changes in ischaemic myocardium.

Method

Pigs are anaesthetised and the heart exposed (*cf* Downer, 1977). Intraventricular and aortic pressures are measured and a catgut suture placed around a small coronary artery to produce a small ischaemic segment of myocardium.

A tripodal device modified from Lab and Price (1977) is attached by vacuum through its legs to the area of the epicardium to be made ischaemic. Each leg of the tripod (Fig. 1) consists of a thin strip of phosphobronze shim (5 mm × 18 mm) with the lower end bent horizontally forming a 5 mm^2 foot. A strain gauge (micro-measurements -09-03 IDE-120) is glued to the upright part of the leg and the collar of a 5 mm length of 'perspex' tube I.D. 1.5 mm, O.D. 3 mm is inserted through a hole in the horizontal part. A short piece of flexible vinyl tubing (I.D. 0.75 mm, O.D. 1.5 mm) is then placed over this collar. Each of these three pieces of flexible tubing are glued to a vacuum manifold which consists of a 10 mm hollow perspex cylinder (I.D. 2 mm, O.D. 4 mm) closed at both ends. The cylinder is connected to vacuum (approx 400 mmHg = 50 to 60 kPa), *via* a long piece of flexible tubing. One end of this cylinder is glued to the underside of a hexagonal perspex plate (diameter 11 mm, thickness 3 mm). The three legs of the tripod are screwed, at the top ends, to alternate edges of this plate, overlapping it by 3 mm. This leaves 10 mm of the vertical section of the leg to bend in response to movement of its base and to thereby deform the strain gauge. The remaining three faces of the hexagonal plate have solder tags glued to them for attachment of the wires from the gauges and amplifiers. The arrangement above leaves the feet of the tripod on a 16 mm pitch circle. The instrument(s) weigh 2.0 to 2.5 g.

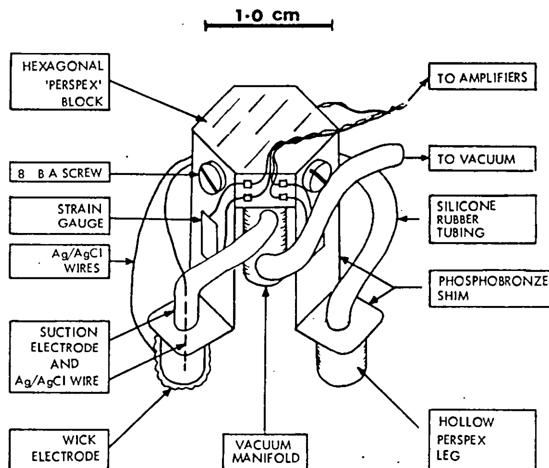

Fig. 1 *Diagram of tripodal device used in mechano-electrical studies. The three legs (third one obscured by vacuum manifold in the diagram) are attached to the surface of the heart by vacuum. The movement of the legs deform strain gauges giving three different outputs. One of the legs is both a suction electrode for monophasic action potentials, and a wick electrode for epicardial ECG's.*

Movement of a foot, relative to the baseplate, produces an output from the strain gauge which can be calibrated in units of length as shown below. The horizontal distance from the centre of the baseplate to each of the feet is 7 mm and the unstrained output of the strain gauge is allocated this value. The calibration of the instrument is then carried out by moving each leg in turn along its axis of movement (from the foot to the central point of the baseplate) in 0.5 mm steps over the range of values encountered experimentally (6.5 to 8.5 mm). During this procedure, the central point of the baseplate is fixed and the feet are attached in turn, to a calibrated precision screw. The device output is linear over this range to within $\pm 5\%$.

The compliance of each leg is tested by applying forces along the axis capable of producing the length changes described above, and is between 0.1 to 0.2 mm·g^{-1}. The compliance perpendicular to the axis is unobtainable with the forces used and is less than 0.0004 mm·g^{-1}. With any given force, therefore, the output of a gauge should vary with the cosine of the angle between the direction of the force and the axis in which the leg moves (Fig. 2A). To test this the tripod is placed with the feet in the same vertical plane and a weight hung from one of them while the device is rotated (Fig. 2A). The actual output (Fig. 2B) varies as predicted.

Detailed geometric analysis of the signals from the tripod in use could indicate the true magnitude and direction (vectors) of the length changes (Dutetre *et al.*, 1972) however our interest is not in these absolute values, but rather in detecting changes in overall contractile behaviour. We have arithmetically summed the three leg outputs continuously, *via* summing amplifiers, to indicate this overall behaviour and accepted an inherent error. We could reduce this error by merely rotating the tripod on the heart until we obtained a maximum displacement from one of the legs, which would ensure that the axis of maximum shortening was parallel to that leg. But in practice it is better to place the tripod to match the shape of the infarct, and to avoid any underlying vessels.

In order to display the length changes in relation to the phases of the cardiac cycle, we have constructed pressure-length loops (Tyberg *et al.*, 1974)

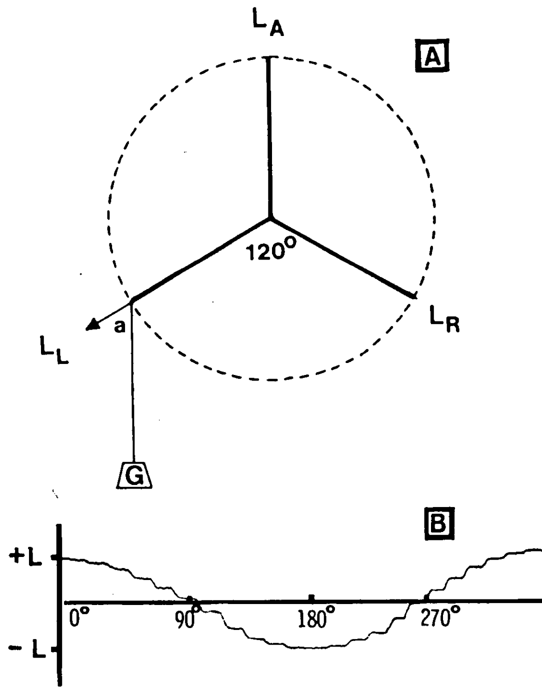

Fig. 2 *Method used for determining some of the performance characteristics of the device.*
A *The device is rotated while a force (G) is applied to one of the legs (L_L). Since the angle between the legs is fixed at 120°, angle a varies between 0° and 360°, and the resultant force vector along the axis L_L varies as the cosine of this angle.*
B *The actual voltage output (force vector) during the procedure in Part A. The output is a cosine wave with maximum output when the force is along the axis of the leg movement and zero output when perpendicular to it. Shortening is upward.*

which are in some respects analgous to the pressure-volume loops used to describe ventricular function.

Using a moving coil driven by a signal generator and amplifier the frequency response of the tripodal device is found to be flat up to 50 Hz. Occasionally oscillations approaching this frequency, are obtained from the tripod in use. They are likely to be artefacts produced by wobbling of the device during rapid movements of the whole heart.

The suction electrode consists of one of the legs of the tripod, or a separate similar hollow perspex tube. A thin Ag/Ag Cl wire passes into a leg through the wall of the flexible tubing and makes contact with the epicardium directly (Churney and Oshima 1964) or by KCl injected into the leg before suction is applied (Szekeres and Szurgent 1974). This wire forms one input to a differential amplifier while the other input is derived from a second, external, Ag/Ag Cl wire electrode in contact with cotton-wool glued around the outside wall of the suction electrode. The two inputs are thus never more than 1.0 mm apart. This cotton-wool also provides the epicardial ECG electrode which forms a stable contact with the epicardium. The petechiae from the suction are not a source of ectopic impulse formation and nor do they affect the mechanical performance of the segments. Using the suction and wick electrode we are able to monitor monophasic action potentials and epicardial electrocardiograms from virtually the same areas as the mechanical records are obtained. In practice, these electrical records have the configuration expected of them and the injury produced by the suction has little effect on the electrocardiogram.

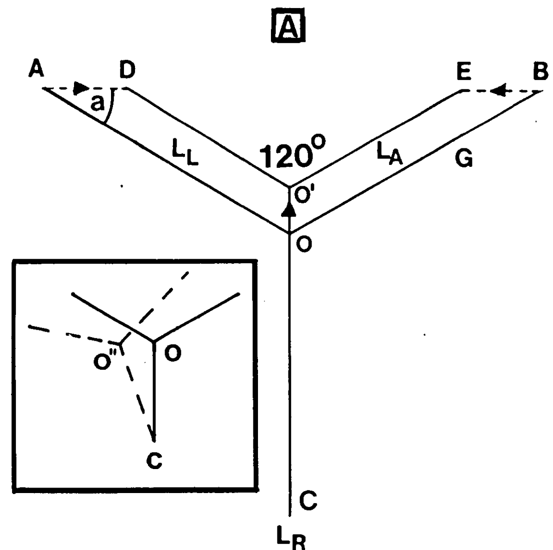

Fig. 3 A *Analysis of the behaviour of the tripod when epicardial shortening (horizontal arrows) occurs at 30° (angle a) to two of the axes and perpendicular to the third axis. Because the angles between the legs are fixed, a change in the direction AB, of (AD+BE (2Y in the text)) results in the position of the tripod moving from ABC to DEC with the centre 0 moving vertically to 0*[1]*. Simple calculations shows that L_A and L_L produce an output less than if the length change were along the axes. However L_R will show an artefactual lengthening. Summing the outputs will produce an over-all small underestimate. (The result is the same if the forces are all in the same direction:* viz. *perpendicular to OC in the direction from A to B. In this case the centre 0 is moved lateral to the right of OC.)*
Inset. The centre primarily moves, perpendicular to OC, from 0 on the solid Y to 0″ on the dashed Y. This would occur when gravity or momentum tilts the tripod during diastole (when the myocardium is most distensible). A small lengthening artefact may thus be recorded (OC to 0″C). During systole, however, epicardial distortion would be less and the movement of 0 would be restricted.

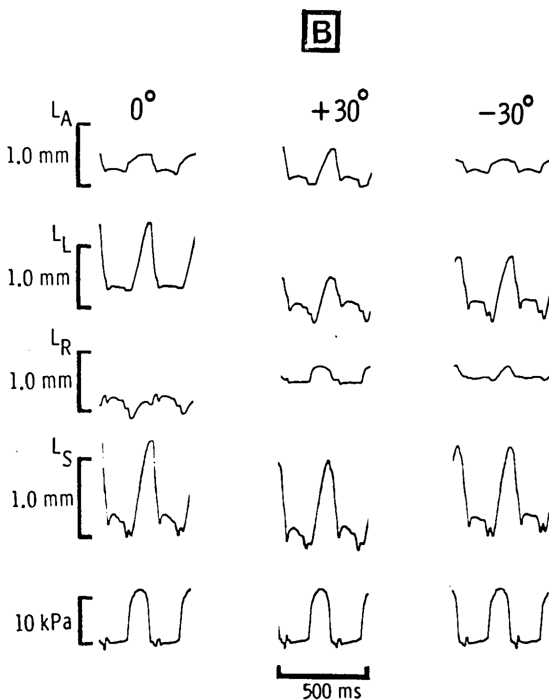

Fig. 3 B *Mechanical outputs of the tripod at 0°, + 30° and −30° over an area of epicardium with different contraction patterns along different directions. Shortening (upward) of the individual segment lengths, and their sum, at the three positions. Despite the differences in the outputs along the three axes, L_A, L_L and L_R, summed lengths L_S produce length changes with the small errors that are within the limits expected of the device. Lowest trace is the intraventricular pressure.*

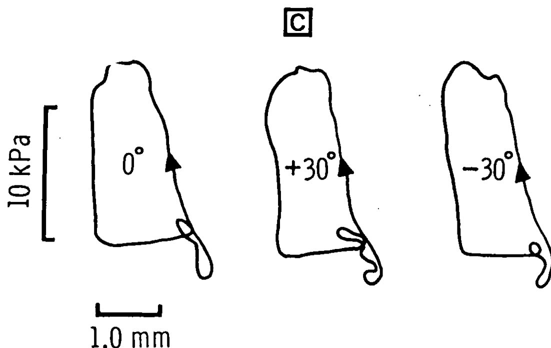

Fig. 3 C *Pressure-length loops using the summed lengths, showing, first, very similar phase relations at the three positions, and secondly, very little change in loop area which is an index of segment 'work'.*

Results

The legs of the tripod are orientated at 120° to each other and for practical purposes, can only move along these axes. Whatever the change in shape of the underlying myocardium, the legs move along their axes to adopt that shape. However, in doing so, the central point of the tripod may be required to shift in relation to the epicardium, thereby partly invalidating the output of the individual gauges as a true measure of the epicardial segment length in the direction of the leg movement. This shift of the central point will not occur if there is symmetrical expansion and contraction of the epicardium, but will occur if the underlying length change is predominantly in one direction and is not parallel to any of the tripod axes. Such a situation is illustrated in Fig. 3A where the length change is a shortening entirely at 30° to two of the axes (perpendicular to the third axis). The tripod moves from its resting position ABC to its new position DEC – with the angles maintained at 120°. If the true length change in the direction AB is 2Y then L_A and L_L will both show a reduction in length of Y/cos 30° and L_R will show an increase in length of Y tan 30°. The combined outputs of the three legs will therefore show a change of (2Y/cos 30° – Y tan 30°) which is approximately 1.75 Y *ie* an underestimation by $12\frac{1}{2}\%$ of the true figure. From these theoretical considerations we would expect to observe a variation in the summed length change of not more than 10 to 15% when rotating the tripod through 30° on an area of epicardium which did vary in length largely in one direction. This experiment is demonstrated in Fig. 3B and C. In Fig. 3B the individual outputs are displayed in the conventional manner showing marked variations in the three 'directions', at the three positions, but the summed outputs are remarkably similar. In Fig. 3C we have displayed pressure-length loops (intraventricular pressure plotted against summed outputs) derived from such a situation to show the phase relationships. Clearly the loops at the three positions are similar. Artefactual recordings due to inertial forces moving the centre of the tripod (inset Fig. 3A) would account for the 'wobble' during diastole just before the rise in pressure. This artefact does not affect the area within the loop which may be taken as an index of segment 'work' (Tyberg *et al.*, 1974). Thus selecting the ejection period (when myocardial forces are large) to be reliable for measurement of length changes, then the overall ejection shortening varies from 1.0 mm at the zero position to 0.9 mm at +30° and 0.9 mm at -30°.

The question arises as to whether the method of mechanical recording has any advantage over

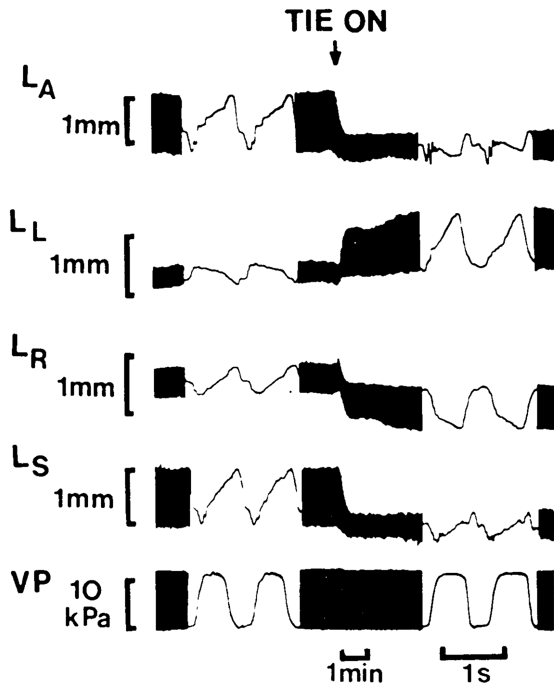

Fig. 4 *Length changes recorded by the device during occlusion of a small coronary artery. The records L_A, L_L and L_R show that there are directional as well as quantitative changes in motion in the epicardial segment. Monitoring in the direction of any of the axes in isolation would not have provided a true record of the overall mechanical behaviour of the underlying myocardium. The summed length records (L_S) however, reveal the major length change during the onset of ischaemia. Despite the loss of directionality and the underestimate in recording the length changes, the summed lengths detect the qualitative change in mechanical behaviour. VP is the intraventricular pressure.*

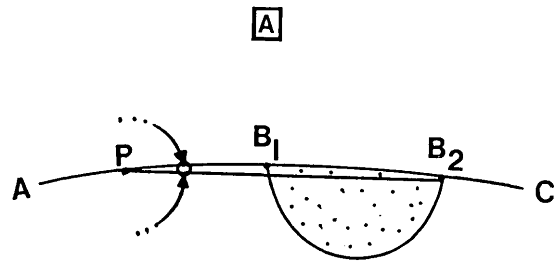

Fig. 5
A *Diagrammatic representation of method used to obtain the solid angle and thus the potential at point P. Curvature A to C represents the epicardium and the stipled area is the injury produced by the suction electrode. Point P is the epicardial ECG surrounded by the partially dotted unit sphere. The solid angle, indicated by the two arrows, is small; being formed by lines from the boundary of interest B_1 and B_2 to point P. (In practice, the distance between B_1 and B_2 is 2-3 mm and P is 1 mm away from B_1. The radius of curvature of the arc AC is about 5 cm. The potential at point P is thus very small.)*

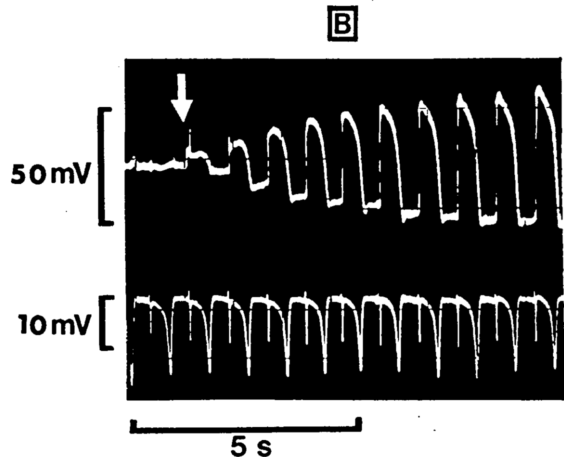

Fig. 5
B *Simultaneous recordings of monophasic action potentials (upper trace) and epicardial electrocardiograms (lower trace) while suction is applied for obtaining the monophasic action potential. The injury produced by the suction causes little distortion of the epicardial electrocardiogram despite the close proximity of the two recording electrodes. (These particular records were obtained from electrodes which were not part of the tripod.)*

previous methods. In Fig. 4 we have displayed length changes recorded from the legs during a 5 min period of ischaemia on the anterior surface of the left ventricle. The ejection period has been visualised by reference to the aortic pressure trace. During the control period, the greatest length changes occur in L_A (ejection shortening of 0.6 mm) and this leg is therefore likely to be closest to the axis of maximum epicardial length change. The 'paradoxical' lengthening of L_L during the ejection period could then be explained as being artefactual by the mechanism described above. On occluding a small branch of the left coronary artery, the major length change (ejection shortening 0.25 mm) now occurs in L_L, while L_A and L_R both show some lengthening during the ejection period. The three outputs, L_A, L_R and L_L are summed in the lower part of Fig. 4 (L_S). Although the directionality is lost this record clearly shows some ejection shortening during ischaemia which would not have been detected if length changes

only in the direction of maximum normal shortening (L_A) were used for the study. The summed leg recording shows ejection period shortening of 0.8 mm during normal perfusion and this falls to about 30% (0.25 mm) of that value 4 min after coronary occlusion. It is, nevertheless, an advantage over previous methods to record movement in three directions since qualitative changes, at least, may still be observed.

The epicardial ECG is recorded from an area immediately surrounding the point of suction which produces a small hemisphere of injury. Using the multiple dipole theory all the electrical vectors radiate outwards from the hemisphere and mostly cancel each other except for those opposite the point of suction. The resultant vector should be small. To facilitate visualising just how small this potential should be, the solid angle theory is used (Holland and Arnsdorf, 1977) (Fig. 5A). The ECG electrode is at a point which is outside the injury produced by the suction. The potential at each of an infinite number of points surrounding the injury may be described by the relationship: $E = K (V_m - V_{in}) \Omega / 4$, where K is interstitial conductivity V_m is the normal membrane potential V_{in} is the transmembrane potential at the site of injury produced by the suction and Ω is the solid angle. This angle is given by an area which is cut off the surface of a unit sphere surrounding each of the points P. The area is determined by lines drawn from the points P to every point on the 'boundary of interest' (not the injured tissue mass) having current flow due to $V_m - V_{in}$. The various values in the above equation remain constant on a beat to beat basis and, as P and the boundary of interest are close and both lie on the surface of a sphere with a relatively large radius of curvature, E is small because Ω must be very small. These predictions may be put to the test by a simple experiment, showing that the electrical records obtained by the epicardial electrode have the configuration expected of them and the injury produced by the suction has little effect on this ECG. While recording the epicardial ECG (Fig. 5B) vacuum is applied and the monophasic action potential obtained

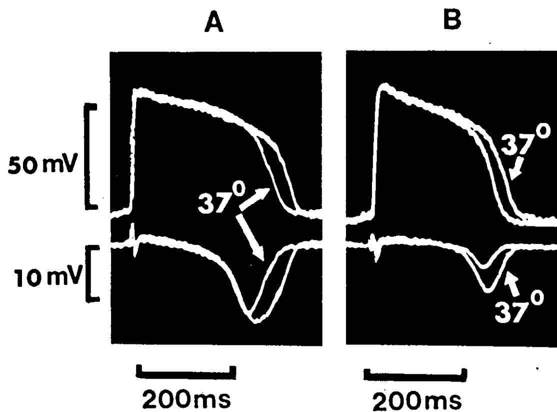

Fig. 6

A *Superimposed on control (37°) action potential (upper trace) and epicardial ECG (lower trace) are an action potential and ECG recorded on lowering the temperature of the epicardium locally with 3 drops of saline at 10°C at the recording site. The action potential is prolonged and the T wave of the ECG deepens.*

B *In this part saline heated to 55°C abbreviated the action potential and reduced the amplitude of the T wave compared to the control records at 37°C.*

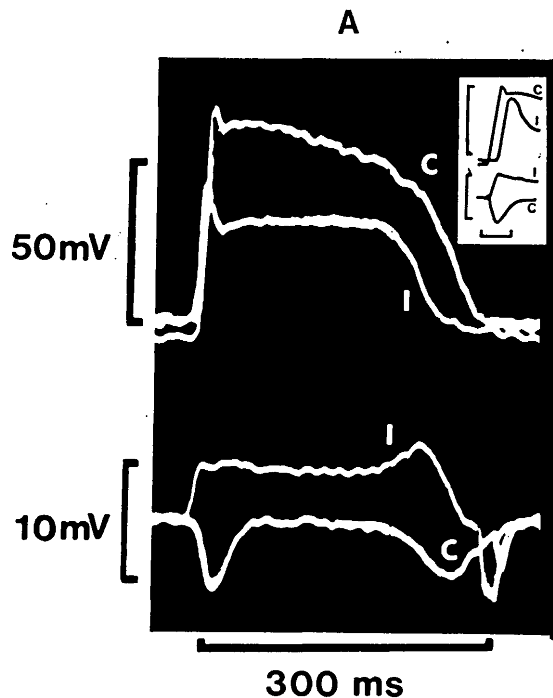

Fig. 7 *Effects of ischaemia on monophasic action potentials (upper traces in each part) and epicardial electrocardiograms (lower traces in each part).*

A *Compared with the control situation (C), mild ischaemia (I) produces a reduction in duration of action potentials and a raised S-T segment. The base-lines of the epicardial ECG are superimposed. There is in fact significant T-Q depression. The sharp downward deflection at the end of the T-waves are conducted atrial pacing spikes. They are not detected in the action potential.*

Inset *Beginning of electrical activity on expanded time base. The 'ischaemic' action potential is delayed compared with the epicardial ECG. Action potential calibration 50 mV, ECG calibration 10 mV, horizontal calibration 50 ms.*

with relatively little distortion of the ECG. There is a small reduction in the R-wave and a small elevation of the S-T segment both of which are predictable and these observations are consistent in all the records obtained using this method.

The action potential has a normal configuration with a reduced amplitude and rise time as compared with transmembrane action potentials. The durations of repolarisation, however, are comparable (Hoffman *et al.*, 1959). Thus, although some qualitative interpretations of changes in amplitude and base line can be made occasionally, change in duration of action potential is the measurement which can be most reliably used. In a simple experiment we tested the validity of this assumption and altered the temperature of the area from which the electrical recordings are made since microelectrode recordings have previously shown changes in duration of action potentials under similar circumstances (Toyoshima *et al.*, 1965). Local cooling of the myocardium resulted in a prolongation of the action potential whereas warming the area abbreviated it (Fig. 6A and B). Simultaneous epicardial ECGs showed that cooling lengthened the Q-T interval and made the T-wave more negative. Warming the area produced the expected and opposite effects on the action potential and ECG. These observations are almost identical to those of the last authors.

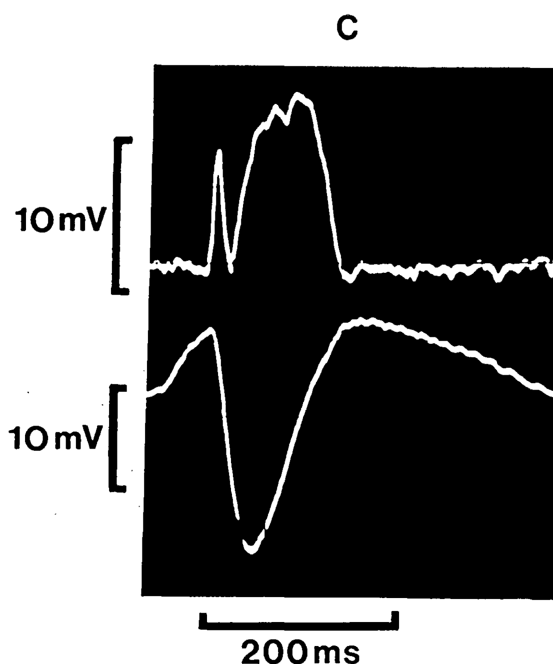

Fig. 7
C *Prominent example of a notched action potential frequently observed when recording from ischaemic areas.*

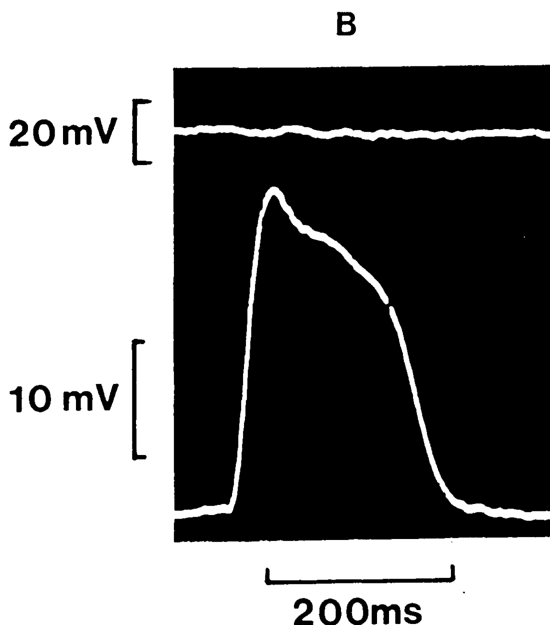

Fig. 7
B *Marked ischaemia causes a monophasic type of response in the epicardial ECG (lower trace) while no electrical activity is recorded by the suction electrode (upper trace).*

The results just described suggest that the electrical recording sytem used here may be a simple and useful alternative to microelectrodes where mechanical changes need to be followed as well. We therefore used this technique to record electrical changes during myocardial ischaemia in our experimental preparation. Following the occlusion of a small coronary artery the action potential duration reduces (Fig. 7A) and the S-T segment elevates. The T wave also changes direction. There is a clear difference in the onset of the deflection of ECG and action potential (inset Fig. 7A). During prolonged ischaemia the electrocardiogram becomes completely monophasic whereas the action potential shows very little or no evidence of electrical activity (Fig. 7B). Action potentials during ischaemia may also be observed that have two components, a spike and a plateau separated by a notch and Fig. 7C is a prominent example.

The results thus far indicate that the mechanical records are similar to those obtained by other techniques (Forrester *et al.*, 1974; Tyberg *et al.*, 1974; Theroux *et al.*, 1974) and in fact provides useful information that might otherwise be missed.

Further, the action potentials recorded with the suction electrode are in keeping with microelectrode recordings previously obtained. (Samson and Sher 1960; Toyoshima *et al.*, 1965; Downer *et al.*, 1977a and b). If these monophasic action potentials are from very localised areas of myocardium and are relatively unaffected by nearby changes in current flow (Fig. 7B) they should prove valuable in the study of the electrical behaviour of ischaemic muscle. In Fig. 8 are continuously displayed simultaneous mechanical and electrical recordings during the onset of a period of ischaemia. The summed mechanical output of the device and the action potential as well as the epicardial ECG show normal configurations before the coronary tie. On tightening the coronary snare the epicardial segment rapidly becomes dyskinetic and the onset of the action potential is delayed (inset) with its duration at 70% repolarisation a little shortened. There is an upward shift in baseline of the monophasic action potential and its amplitude and rise time are reduced. (The latter two observations could be explained by a true depolarisation of the resting membrane.) Approximately 1 min after the tie, occasional action potentials become even more delayed and markedly reduced in amplitude and duration. As the duration of ischaemia progresses these action potentials become smaller until sometimes no action potential is seen between beats and an apparent two to one conduction block manifests. A run of these may be observed in Fig. 8 preceding ventricular tachycardia which in this case progressed to ventricular fibrillation.

Discussion

All methods of measuring epicardial segment-length changes as an indicator of regional myocardial performance have some inherent disadvantages. However, the results we have obtained by measuring length changes from strain gauges attached to the legs of a suction applied tripod, suggest that it may be an improvement on previous methods and the loss of accuracy of mechanical recording obtained with more sophisticated techniques is made up for by the simplicity of its use. The advantage of suction application is self-evident, in that it allows full manoeuvrability of the device around the anterior and lateral surfaces of the exposed heart.

By allowing the legs of the tripod to move only along their axes at 120° to each other, we have simplified the analysis of the outputs from the strain gauges (*cf* Dieudonne *et al.*, 1972) without sig-

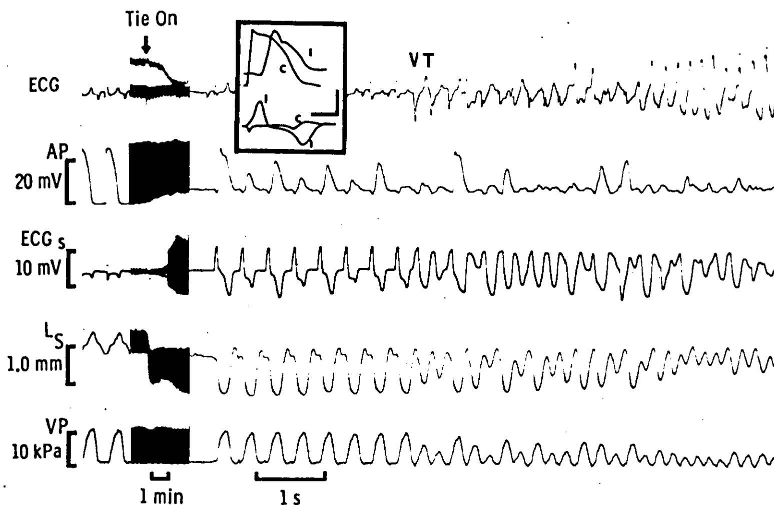

Fig. 8 *Simultaneous and continuous recordings of action potentials (AP), epicardial ECG, (ECGS), segment length (LS) and intraventricular pressure (VP) during the onset of ischaemia. Top trace is conventional ECG. On the left hand side is demonstrated the control recording. On occluding the artery the action potential duration and amplitude reduces while the segment becomes dyskinetic. The changes in the action potentials thereafter indicate conduction delay and partial to complete block in the underlying epicardium. (In the* inset *compare the time relationship between the epicardial ECG (lower trace) and the onset of the action potential (upper trace) in the control and ischaemic periods, Horizontal calibration bar 100 ms; vertrical calibration bars 10 mV for action potential, 5 mV for ECG.) The uninterrupted recording shows the onset of ventricular tachycardia (VT) which progressed to fibrillation (not shown). Variation in mechanical and electrical behaviour is clear.*

nificantly constraining the epicardial movement. In particular, we have not seen the major isovolumic lengthening during normal perfusion which has been suggested to be an artefact (Bugge-Aspheim, 1969), in some recordings from mercury in rubber strain gauges.

The major isovolumic lengthening displayed by ischaemic tissue is unlikely to be artefactual, as it is unchanged by reapplication during the ischaemia, to allow the tripod to revert to its resting length. As we have shown however, the restriction of the movement of the legs of the tripod to these axes produces outputs from the individual legs, which, if examined in isolation, are not necessarily directly related to epicardial length changes in that direction. This is because of movement of the central point of the tripod, either through forces transmitted via the legs or directly by inertial forces, in relation to the underlying epicardium. We could possibly have obtained the 'true' direction and magnitude of the length changes in the underlying epicardium by vector analysis of the three leg signals, taking into account any estimated shift in the central point; or we could have used the variation in the calculated area enclosed by the feet of the tripod as our indicator of local contractile behaviour. However, in theory and apparently in practice, simple arithmetical summing of the three leg outputs produces a value for epicardial length change which has a maximum error of about 12% underestimate. Analysis by the more complicated methods suggested before, (Dutetre *et al.*, 1974) would have severely restricted our use of the tripod and we have accepted the small possible error inherent in the simpler method. This underestimate depends upon the angle between the direction of epicardial length change and the nearest leg axis. The error is therefore not a constant factor for a single position of the tripod if some intervention, such as a coronary occlusion, changes the direction of the major epicardial length change. The area within the loop described when plotting segment length against interventricular pressure has been used as an index of segment work (Tyberg *et al.*, 1974). The artefact produced by inertial movements of the centre of the tripod during diastole does not affect the loop area (Fig. 3C) particularly if 'work' during the ejection period is used.

Our demonstration of a change in the axis of major length change induced by ischaemia illustrates another possible advantage of our method of recording these length changes. Gauges measuring in only one fixed direction, decided upon before coronary occlusion, could fail to detect the epicardial shortening occurring close to the edge of the infarct which presumably is contributing to the ejection of blood from the ventricle. From our study shown in Fig. 3 where the tripod is rotated and reapplied over a small area of epicardium that had its major length change in one direction, it is apparent that the summed leg output would always detect any shortening even if there was a significant direction change. It is clear from the above that it is neither necessary nor particularly desirable to spend time orientating the tripod, to ensure that one of the legs lies parallel to the axis of maximum shortening of the epicardium.

The recording of the summed legs simplifies matters but at the expense of directionality. We therefore continue to measure all three outputs. It is worth noting that the device must reflect three dimensional changes of the underlying myocardium, not just on the surface of the epicardium. The inhomogeneous patterns of normal and ischaemic myocardial wall contraction can be complex, and thus the change in direction of contraction induced in epicardium by ischaemia must be cautiously interpreted. It might be that the fibres in the direction of L_L in Fig. 4 are unable to shorten during normal ejection because of the geometry of fibre orientation. With ischaemia, these fibres, being close to the edge of the ischaemic area, may maintain their tension bearing ability, and therefore shorten against the reduced resistance of the more ischaemic muscle. This mechanism would be similar to that described by Tyberg *et al.*, (1969) in their tandem muscle experiments. A second possibility is that the fibres showing an increase in contraction during ischaemia, may have moved along their length-active tension curves. Finally, if gauge L_L is, in this case, recording transverse to the bulk of the muscle fibres, it could have been measuring their increase in diameter during normal contraction and their reduction in diameter when the fibres are stretched during ischaemia. The last two explanations although they may partially contribute, could not on their own account for the magnitude of the changes in direction of contraction.

The recording of transmembrane potentials from the intact beating ventricle *in situ* is difficult, sometimes necessitating the suturing of a ring onto the area of the ventricle to be studied to limit epicardial movements, and even then, recordings may be subject to difficulties in interpretation (Downer *et al.*, 1977). Since we are interested in simultaneously monitoring electrical and mechanical events during ischaemia, such restraints placed on the myocardium defeat the object of the exercise. There is also the possibility that interfering with mechanical behaviour may directly affect recorded potentials (Kaufmann *et al.*, 1971; Hennekes *et al.*, 1977; Lab, 1978). We have, therefore, accepted the limitations of the monophasic action potential and, according to

our results which are in keeping with many microelectrode recordings obtained from ischaemic myocardium, we feel that much useful information may be obtained from the records, particularly the repolarisation process, at a qualitative level. The close proximity of the epicardial ECG to the suction electrode is also a valuable adjunct in that comparisons between these ECGs and the monophasic action potential may be made. The suction has no significant effect on the recorded epicardial ECG, and in fact stabilises the contact between the wick and epicardium to provide more consistent recordings without the baseline drift which may be found when the degree of contact varies.

Several of our observations using the monophasic action potential and wick electrodes confirm those of Toyoshima *et al.* (1965) and Downer *et al.* (1977a). The latter authors used microelectrodes recordings in almost an identical experimental preparation to ours. As with the microelectrodes in the intact preparation it is difficult to interpret the absolute amplitude of action potential and resting potential using the monophasic action potential. With the latter, however, it may be possible to comment on them in a qualitative manner, which is impossible with a disturbed impalement using microelectrodes in a vigorously beating heart. Finally, a two-component type of action potential similar to ours was also observed in their intact heart studies and has been described in detail in an earlier study (Downer *et al.*, 1977b). Our observations strongly suggest that the monophasic action potential we use provides a highly localised record of the onset and duration of electrophysiological events and which may also reflect some qualitative changes at the membrane level in response to ischaemia. Thus the action potential duration is reduced with mild ischaemia and no action potentials are recorded in prolonged or severe ischaemia: we have observed conduction delays and two to one response of the action potential. The action potential changes in Fig. 8 thus indicate alterations in myocardial conduction and excitability which precede the ventricular fibrillation and are in keeping with the re-entry mechanism of ventricular arrhythmia. Although we cannot measure differences in action potential in cells separated by a mm or so we can often find two to one blocks or a type of electrical alternans by simply exploring the ischaemic area with the suction electrode. This type of exploration may also provide a better method of timing the arrival of the impulse in ischaemic myocardium than epicardial ECG.s

We wish to thank Mr R. Price for constructing the device and helping with its design; and Mrs R. Kingaby for her technical assistance in some of the experiments.

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(*Reprinted from Nature New Biology, Vol.* 230, *No.* 13, *pp.* 150–151, *March* 31, 1971)

Demonstration of an "Excitation–Contraction Recoupling" Mechanism in Mammalian Ventricular Myocardium

A PROCESS called "excitation–contraction coupling" has been generally accepted to take place only in the direction of excitation to contraction. Through this mechanism a propagated action potential initiates an active state in skeletal or cardiac muscle, and the muscle contracts. We propose that, in the mammalian ventricular myocardium at least, the process is not unidirectional and an important reverse coupling between the contractile system and the excitable plasma membrane has been overlooked. Through this feedback interaction the mode of contraction (that is, isotonic or isometric) not only determines the instantaneous electrical state of the plasma membrane, but also influences the mechanical events of the subsequent beats. Thus when Kaufmann *et al.*[1] recorded intracellular action potentials from cat papillary muscle, the time course of the repolarization was altered depending on the mode of contraction. Some kind of contraction–excitation feedback has also been suggested by Stauch[2] and Lab[3,4]. They showed a difference in the shape of the monophasic action potential, as recorded by a suction electrode, when comparing isotonic and isovolumic contraction of the intact ventricle. But their experimental conditions did not allow satisfactory analysis of the phenomenon.

In this series of experiments cat papillary muscles were taken from the right ventricle and laid horizontally in a muscle chamber to make possible conventional microelectrode recording of intracellular action potentials. The muscle was attached at its tendinous end to a modified galvanometer indicator. The load (including sudden changes in it), which was seen by the muscle, is generated by feeding the appropriate current into the galvanometer coil, thus deflecting the modified indicator lever. Displacement of a rear extension of this lever was detected by a photodiode assembly. The other end of the muscle was attached to a force transducer (RCA 57 34).

Fig. 1 shows consecutive traces of the mechanical activity of an isolated muscle stimulated at a rate of 24/min. In contrast to the changes in the electrical phenomena which are immediate and persist as long as the particular mode of

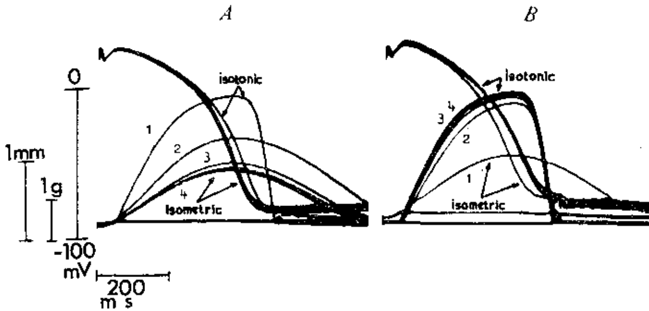

Fig. 1 Cat papillary muscle showing action potentials and mechanical transients after a sudden change in the mode of contraction. In *A* the first beat (trace 1) is isotonic against a small load of 0.3 g/mm^2. The beats that follow are isometric, and show transient peaks of decreasing tension (traces 2 and 3) stabilizing at a new level in the next few beats (trace 4). The procedure is reversed in *B* with the first contraction isometric and the following isotonic beats showing a positive staircase. Simultaneously recorded intracellular action potentials show that the action potential is shortened by passing from isotonic to isometric contraction and is again lengthened by the reverse operation. The mechanical transients are thought to result from the alterations in the duration of the action potential.

contraction does, the changes in mechanical events take place over several beats.

Are the alterations in action potential and the mechanical transients independent or interrelated phenomena? The arguments in favour of the mechanical transients being a result of the electrical changes are as follows. The duration and intensity of the active state in cardiac muscle are dependent on the duration of the action potential[5–7]. Further, by clamping of the membrane potential for different durations Beeler and Reuter[8] showed that the tension developed was greater the longer the clamping period. It has also been recognized from several recent investigations[6,8] that sudden changes in the duration of action potential produced by electrical polarization, voltage clamping or sudden temperature changes also typically result in a staircase-like mechanical response developing over five or six subsequent beats. It is therefore reasonable to conclude that in this series of experiments the changes in the duration of the action potential quantitatively reflect in the subsequent contractile activity of the muscle.

A related or possibly even a controlling parameter in this contraction–excitation feedback system seems to be the force–velocity relation of the muscle under study. It seems as though the action potential duration is abbreviated if the maximum

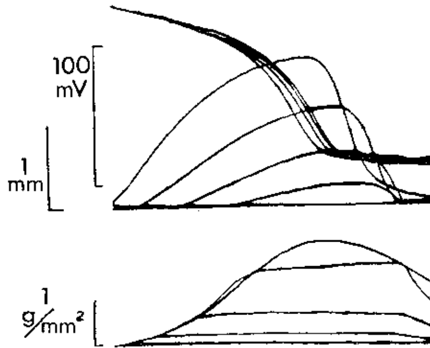

Fig. 2 Action potential duration as a function of the shortening velocity. Top traces show superimposed action potentials, middle traces isotonic displacements and lower traces force development. In this experiment initial muscle length was kept constant and the muscle shortened against increasing afterloads (0.3, 0.6, 1.2, 2.4 g/mm²). The last contraction was purely isometric (action potential is not recorded). It can be seen that the action potential is abbreviated roughly in proportion to the tension development and inversely to the shortening velocity.

velocity of shortening is decreased, with a corresponding increase in the tension developed. In Fig. 2 the afterload seen by the muscle is increased stepwise during subsequent contractions. When the muscle was preloaded starting its contraction from different initial muscle lengths, approximately the same relation between shortening velocity and action potential duration is found.

If the action potential duration is indeed influenced by the instantaneous force–velocity relation, then the duration of the action potential should be increased to a maximum when the muscle contracts at V_{max} against zero load. We have tried to show this in the next series of experiments.

In order to approach V_{max}, quick release experiments were carried out and are shown in Fig. 3. The prolongation of the action potential induced here seems to result from a depolarizing current with its own time course. This is clearly seen when the release is made after the action potential is virtually complete (*C* and *D*). Occasionally, this new depolarization reaches threshold with the initiation of a propagated action potential (*F*). Moreover, the time delay of this feedback between mechanical and electrical events is relatively short. The time taken from the beginning of the release to the point where the change of shape of the action potential can be detected is less than 10 ms.

It thus seems that shortening of the muscle produces a

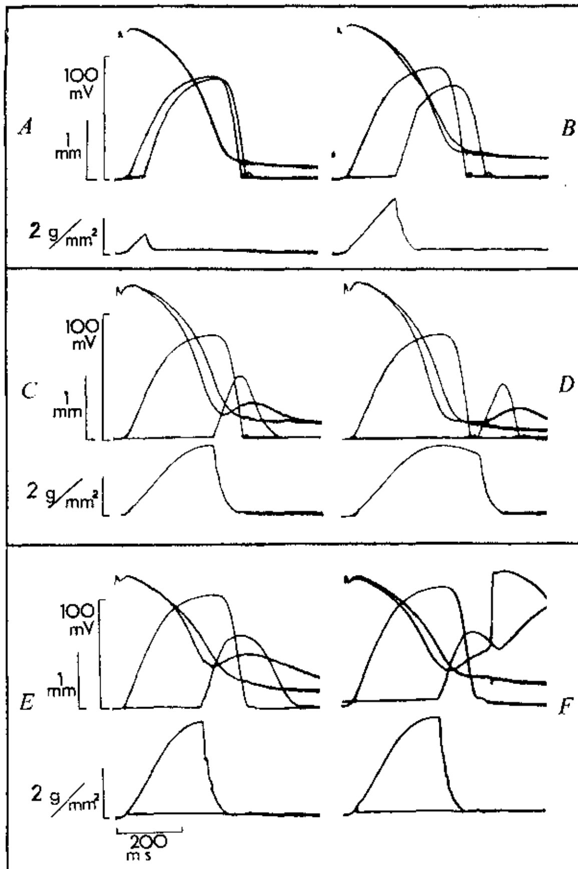

Fig. 3 Quick release experiments, performed on cat papillary muscle preparations. One preparation produced *A* to *D*; another, *E* and *F*. In each set of recordings, that is, *A* to *F*, the muscle action potential, length and tension appear from above downwards at zero time. In all recordings two consecutive beats are shown; the first beat is wholly isotonic, the second beginning isometrically with releases to a standard light load (0.3 g/mm²) at four progressively longer durations of isometric contraction (*A* to *D*). The corresponding action potential recordings exhibit two different effects. During the isometric period the action potential is shortened, but a few ms after the start of the release a depolarizing current interrupts this trend, producing a further prolongation (*B*) or a new wave of depolarization (*C* and *D*). In (*F*) an experiment is shown where this depolarization reached threshold and initiated a new propagated action potential although, in (*E*), it did not do so.

depolarizing current at the plasma membrane, whereas tension development is associated either with a decrease in this current or an increase of a repolarizing current. A current such as the latter has not been directly demonstrated, for in contrast to isometric conditions, both the isotonic beat and the action potential are complete almost at the same time.

Any proposed mechanism for this phenomenon must explain, first, the rapidity of the interaction and, second, how shortening of the muscle can generate an inward current. Changes in length may alter cellular geometry or the molecular arrangement of the membranes and thus change their electrical characteristics. But passive changes imposed on a muscle perfused with agents which uncouple excitation–contraction (for example, Ni or Co) have so far had no effect on the time course of the action potential. A third prerequisite for any hypothesis may therefore be the presence of an active state of the contractile system. An interesting possibility now is that of contractile protein interaction affecting ionic flux across the membrane. It is not difficult to visualize this if we consider calcium as a possible mediator, for it is involved in the acto-myosin complex associated with active contraction, as well as with ion movements across cardiac muscle membranes.

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Received August 10, 1970.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

Pflügers Arch. 324, 100—123 (1971)
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Feedback Interaction of Mechanical and Electrical Events in the Isolated Mammalian Ventricular Myocardium (Cat Papillary Muscle)* **

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Received July 24, 1970

* Preliminary reports of the results were already presented at the 36th and 37th meeting of the Deutsche Physiologische Gesellschaft [Pflügers Arch. **312**, 26 (1969) and Pflügers Arch. **316**, R 11 (1970)] and on the occasion of the Gordon Research Conference on Heart Muscle 1970. August 2—7 at Plymouth, New Hampshire.

** This work was partly supported by grants of the Deutsche Forschungsgemeinschaft (Ka 287/1) and the ministry of Bildung und Wissenschaft (St.Sch. 02 39).

Summary. Measurements of transmembrane potentials were performed under different contractile conditions on isolated cat papillary muscles.

It was found that the duration of the action potential (within limits of about 20%) depends on the mode of contraction. Isotonic shortening tends to prolong, isometric tension development tends to shorten the duration of the action potential.

As a result of the action potential alterations negative or positive inotropic mechanical transients are observed during 5—10 subsequent beats.

The decrease in action potential duration is roughly proportional to the force development, and the increase of action potential duration is related to the shortening velocity.

By applying a controlled stretch the shortening velocity of the contractile element (V_{CE}) was reduced below its value during purely isometric conditions. A further decrease of the action potential duration was observed. Increasing V_{CE} by release experiments increased the action potential duration beyond that observed under lightly loaded isotonic contractions.

A quick release taking place after repolarization is complete produces a new distinct wave of depolarization (10—15 mV) which can sometimes initiate a new action potential.

The quick release experiments fascilitated the estimation of the time delay of the feedback interaction which is less than 10 msecs.

The possibility that passive geometrical changes of the plasma membrane is a causitive factor of the described phenomenon was experimentally excluded.

Alternative explanations are discussed. It seems likely that a controlling parameter of this excitation contraction feedback system is contained in the force velocity relation of the contractile element influencing the internal Ca^{++}-transients by its mode of contraction.

Key-Words: Contraction-Excitation Recoupling — Cardiac Force Velocity Relation — Quick Release — Controlled Release — Active State — Intracellular Action Potentials.

Zusammenfassung. An isolierten Katzenpapillarmuskeln wurden intracelluläre Potentialmessungen bei verschiedenen Kontraktions-Bedingungen durchgeführt.

Es wurde gefunden, daß die Aktionspotentialdauer (in Grenzen von etwa 20%) von der Kontraktionsform abhängig ist. Während isotonischer Verkürzung wird das Aktionspotential verlängert, bei isometrischer Spannungsentwicklung abgekürzt.

Als Folge dieser Aktionspotential-Veränderungen entwickeln sich treppenartige Zu- oder Abnahmen der mechanischen Aktivität während der folgenden 5—10 Kontraktionen.

Durch Anwendung einer kontrollierten Dehnung konnte die Verkürzungsgeschwindigkeit des contractilen Elements (V_{CE}) kleiner als bei isometrischen Bedingungen gemacht werden. Dabei wurde eine weitere Aktionspotentialverkürzung beobachtet. Wurde V_{CE} dagegen durch Entlastungsexperimente (quick release) über die bei leicht belasteten isotonischen Kontraktionen entwickelte Verkürzungsgeschwindigkeit hinaus erhöht, so ergab sich eine weitere Zunahme der Aktionspotentialdauer.

Release-Experimente, die nach der vollständigen Repolarisation durchgeführt wurden, führten zur Auslösung einer neuen Repolarisationswelle von 10—15 mV Amplitude. Zuweilen wurde hierdurch ein neues Aktionspotential ausgelöst.

Die Entlastungsexperimente ermöglichten die Abschätzung der „mechano-elektrischen Latenzzeit“ des beschriebenen Rückkoppelungssystems. Diese betrug weniger als 10 msec.

Die beschriebenen Phänomene lassen sich vermutlich nicht auf Änderungen der membranären Oberflächengeometrie zurückführen.

Andere Erklärungsmöglichkeiten werden als Arbeitshypothesen diskutiert. Es erscheint zumindest sicher, daß der Control-Parameter des beschriebenen Rückkoppelungssystems in der Kraft-Geschwindigkeits-Relation des contractilen Elementes selbst zu suchen ist. Möglicherweise bestimmt dessen Kontraktionsform die Dynamik der kontraktionswirksamen Calciumbewegungen.

Schlüsselwörter: Mechano-elektrische Rückkoppelung — Kraft-Geschwindigkeitsrelation — „quick release“ — kontrollierter Release — „active state“ — intracelluläre Aktionspotentiale.

It is known that in heart muscle more than in skeletal muscle the time course of the active state depends on the mode of contraction (Brady, 1965; Jewell and Wilkie, 1958, 1960). For instance any displacement of the contractile element during activity as produced by active shortening or passive stretching tends to shorten the time course of the active state (an uncoupling effect as defined by Brady, 1965, 1968). Conversely one finds that the production of tension within the contractile element tends to prolong the active state (Hill, 1963; Blinks, 1970).

This is one of the reasons which make the measuring and defining of the active state in cardiac muscle rather difficult (Brady, 1968; Sonnenblick, 1965, 1967). It is not our intention to reconsider the actual concept of active state applied to cardiac muscle or to make a new analytical approach. Instead it is likely that the already complex situation will be made more intricate by this paper. We propose that the mode of contraction not only determines the instantaneous active state

during a given contractile cycle (Brady, 1965, 1968), but also influences the mechanical events of the subsequent beats by a feedback interaction between the contractile system and the excitable plasma membrane.

We first suspected such a type of internal control when Parmley, Brutsaert, and Sonnenblick (1968)—changing the mode of contraction from isotonic to isometric conditions—observed that peak tension of subsequent isometric beats declined in a staircase like manner. A mechanical transient in the opposite direction was obtained by passing from isometric to isotonic conditions. Parmley *et al.* attributed their results to slow changes of the elastic or viscous properties of the heart cell. However, when Kaufmann *et al.* (1969) performed intracellular microelectrode recordings under similar experimental conditions a surprising explanation emerged. The time course of the action potential alters depending on whether the mode of contraction is isotonic or isometric. The observed mechanical transients could be conveniently explained both qualitatively and quantitatively as a consequence of the altered action potential (Antoni, Jacob, and Kaufmann, 1969).

The suggestion that some kind of contraction-excitation feedback does exist in cardiac muscle is not new. In intact amphibian and rat hearts, changes in the shape of the extracellular action potential as recorded by means of a suction electrode could be detected when the mode of contraction was suddenly changed from isotonic to isovolumic (Stauch, 1966; Lab, 1968/69). But the experimental conditions given in these investigations did not allow qualitative or quantitative analysis of the phenomenon. The possibility that the altered shape of the excitation process is in turn reflected on the subsequent contractile activity was not recognized. This feedback interaction between mechanical and electrical events in the mammalian myocardium forming a control loop between the contractile system and the plasma membrane is the subject of the following investigation.

Methods

The need for simultaneous recording of both mechanical and electrical events in small cardiac muscle preparations excluded the use of experimental setups usually designed for the analysis of muscular mechanics. Since the microelectrodes must be freely suspended in order to follow displacements of the contracting muscle, the preparation has to be horizontal. Sources of additional mechanical vibration have to be carefully avoided as for example, switching on and off of mechanical stops, loading the isotonic lever by airstream and manipulation in close proximity to the muscle chamber. These requirements led to the construction of a special recording system.

Muscle Chamber

This is an open acrylic plastic chamber measuring 35 mm long, 8 mm deep, and 6 mm wide. The chamber was perfused with oxygenated Tyrode solution at

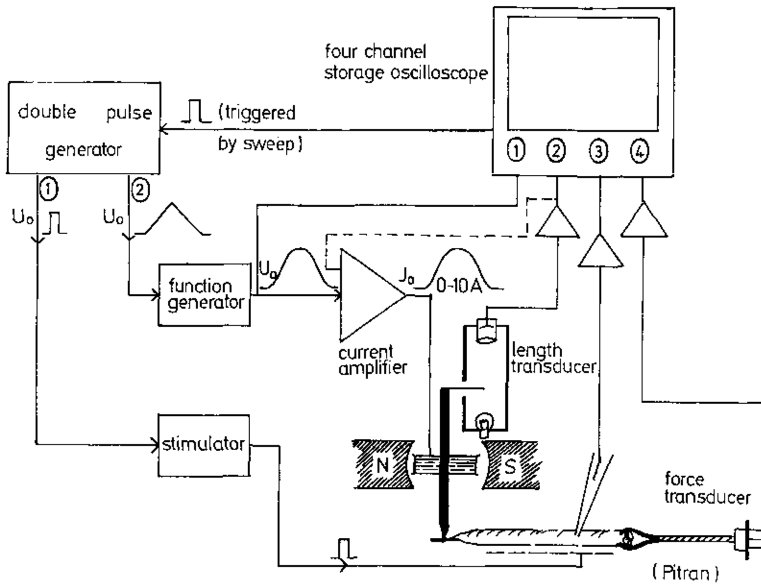

Fig. 1. See text for explanation

31° C. The rate of the perfusion was adjusted to about 15 ml/min. Twice threshold stimuli were applied with Ag/AgCl electrodes which were parallel to the muscle.

Force Transducer

During the first part of the experiments force measurements were made by a RCA 5734 transducer tube. The transducer pin was extended by a 10 mm long stainless steel tube to which a thin piece of glass tubing was adhered in parallel. The proximal part of the glass tube was connected to a suction pump. The lower end of the papillary muscle was fixed to the distal orifice of the glass tube by the negative pressure. The compliance at the tip of the extension was about 0.02 mm/g load.

The transducer tube was mounted so that the tip of the extension reached the middle of the perfused chamber without obstructing the field of view or the microelectrode.

In the latter part of the investigation, when stretch experiments were performed, the displacement of the extended transducer pin contributed too much to the series elasticity of the system. A new kind of force transducer was thus constructed using a pressure sensitive transistor—('Pitran'). For our purposes this transducer has no mechanical displacement, yet has the advantage of delivering an output signal of about 0.5 V/g. This particular use of the 'Pitran' transducer will be published in detail elsewhere.

Isotonic Lever Displacement Transducer and Load Generator

This system was made from a coil-type galvanometer in which the reset spring was detached and the indicator replaced by an aluminium lever about 25 mm long.

The tip of this lever consisted of a flattened forceps-like shaft 8 mm long which was directed downwards at right angles to the moving plane of the lever. This shaft was immersed in the perfusion chamber and clamped the tendinous end of the preparation. A controlled d.c. current flow through the coil of a galvanometer generated a force of up to 10 g at the tip of the lever. This force was seen by the muscle as a load. Adjustable screws allowed fixation of the initial and final muscle lengths. The displacement of a 15 mm rear extension of the lever varied the amount of light falling on a photo-diode. The output of the system was linear over about 3.5 mm movement at the tip of the lever. The equivalent mass of the movable system was reduced during the stepwise improvement of the experimental setup and was finally at about 200 mg.

Load Control Unit

With the device described above changes in load such as quick stretch or quick release, constant or varied afterloading, and controlled stretch could be imposed by means of a load control unit. This unit consisted of a d.c. amplifier with an output of 0—6 amps through the 1 Ohm resistance of the coil. The output of the amplifier may be manually controlled by a potentiometer calibrated in grams. A voltage signal may also be fed into the unit to control the time course of the load seen by the muscle. It was also possible to fix the initial muscle length using negative feedback from the length transducer to the load control unit. The muscle length could be electrically adjusted by the balance potentiometer in the differential preamplifier of the unit. Here the force transducer could be omitted since the force developed by the muscle was represented by the time course of the load generating current and could be taken as the isometric tension curve. This method is a mechanical analogue of the voltage clamp technique used by electrophysiologists.

Controlled Stretch

An open loop was used. It consisted of a function generator delivering an output signal which could be adjusted to an exact analogue of the isometric force developed by the muscle. This signal controlled the current amplifier of the load control unit which fed the muscle puller described above.

Intracellular Potential Recordings

Microelectrodes (tip diameter less than 1 μ) were filled with 3-molar KCl solution and freely suspended on the end of a thin silver wire which was attached via a connector to the lever of a micromanipulator. The resistance of the microelectrodes used was in the range of 10—30 MOhm. It was empirically found that a certain ratio between the weight of the microelectrodes and the free length of the suspending wire gave the best results as far as artefacts produced by mechanical oscillations of the microelectrode-wire system were concerned. Even then the ratio between impalements and successful recordings was about 60:1.

Documentation and Data Evaluation

The output signals of all the recording systems were displayed simultaneously on a four channel storage oscilloscope (Tektronix 564 A). The shortening velocity was measured by electrically differentiating the output signal of the length transducer. A cathode follower was used for impedance transformation of the signals recorded by the microelectrode. Successful recordings were photographed from the stored oscilloscope display.

Material and Preparation

Male and female adult cats weighing 1.5—2.5 kg were sacrificed while under light ether anaesthesia. The hearts were rapidly excised and transferred to a chamber containing oxygenated Tyrode solution. After opening the right ventricle along the anterior border of the ventricular septum, papillary muscles about 5—8 mm long and 0.5—1.0 mm diameter were removed and immediately transferred to the muscle chamber. The first reason for the careful selection according to size was the limited performance of the load generating system (10 g). But even more important was the fact that only muscles functioning at their optimal energetic state exhibited the phenomena which were investigated. In fact the best results were obtained when the muscle had a relatively small and uniform diameter (0.6—0.9 mm) with no side branches.

The muscles were equilibrated in the muscle chamber for at least one hour. During this time the muscles were stimulated at 24/min and allowed to shorten isotonically against a small load (0.3 g/mm^2). Only those preparations which did not show spontaneous activity and reached an isometric peak tension of at least 3 g/mm^2 were used. The muscles were allowed to take up an initial length produced by a load of 0.5 g/mm^2. This length was used during all the experiments if not otherwise indicated.

Solutions

The composition of the Tyrode solution used in these experiments was (in mM/l): NaCl: 136.9; KCl: 2.68; $NaHCO_3$: 11.9; $CaCl_2$: 2.5; NaH_2PO_4: 0.42; Glucose: 5.6.

Results

A. The Action Potential as Dependent on the Mode of Contraction

By way of introduction an experiment similar to that performed by Parmley, Brutsaert, and Sonnenblick (1969) is shown in Fig. 2. The upper part of this figure contains consecutive traces of the mechanical activity of an isolated papillary muscle stimulated at 24/min. In part (A) the muscle first shortened isotonically against a load of 0.5 g/mm^2 and is subsequently made to contract isometrically at the same initial muscle length. During the following isometric contractions the peak tension and the rate of tension development declined in a staircase like manner reaching a new steady state after about 8—10 beats. In part (B) the sequence is reversed, the muscle first contracting isometrically and then allowed to shorten. In this type of experiment the subsequent 8—10 isotonic contractions showed an increase in the amplitude and the velocity of shortening forming a positive staircase.

The lower recordings of Fig. 2 show the same experiment on a different preparation but contain in addition superimposed intracellular action potential recordings. In part (A) the duration of the action potential is immediately reduced when the mode of contraction is changed from isotonic to isometric conditions. This abbreviation is first detectable about $^1/_3$ the way through the plateau and becomes increasingly prominent

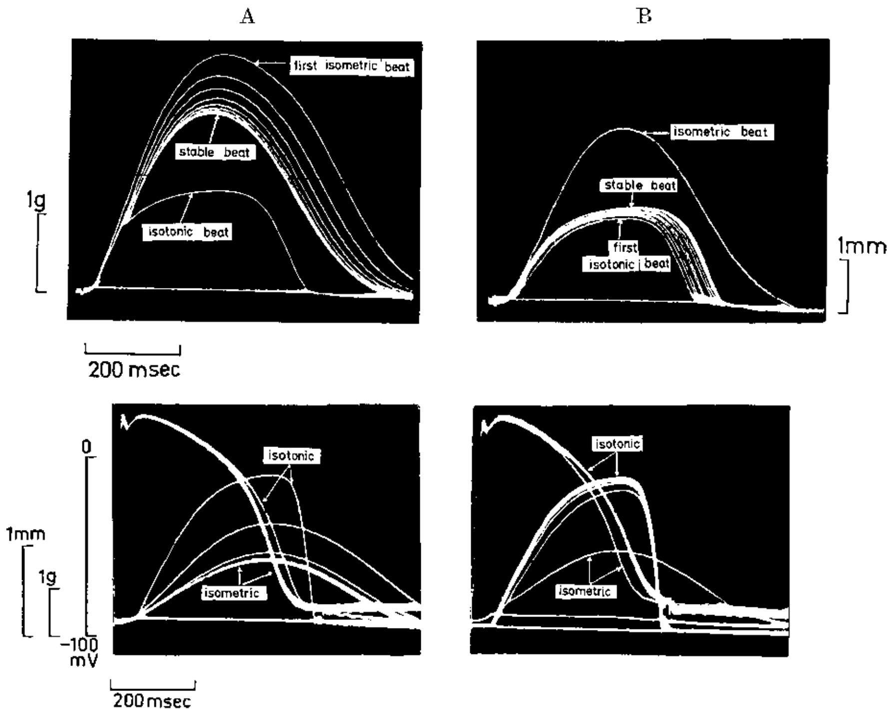

Fig. 2 A and B. Mechanical transients following a sudden change in the mode of contraction. In part I A the first beat is isotonic against a small load of 0.3 g/mm². The second and subsequent isometric beats show peaks of decreasing tension stabilizing at a new level after 6—8 beats. The procedure is reversed in part I B with the first contraction isometric and the following 6—8 isotonic beats showing a positive staircase. The same experiments are shown in part II with simultaneously recorded intracellular action potentials. The action potential is shortened by passing from isotonic to isometric contractions and is again lenghtened by the reverse operation. The mechanical transients are thought to be due to the alterations in the duration of the action potential

during further repolarization. Switching back from isometric to isotonic conditions [part (B)] leads to an immediate broadening of the action potential to the time course observed at the beginning of the experiment.

In contrast to the changes in the electrical phenomena which are immediate and persist as long as the particular mode of contraction does, the changes in contractility show the characteristics of transients taking place over several subsequent beats. Under given experimental conditions the amount of prolongation or shortening of the action potential varies considerably between the different preparations ranging from

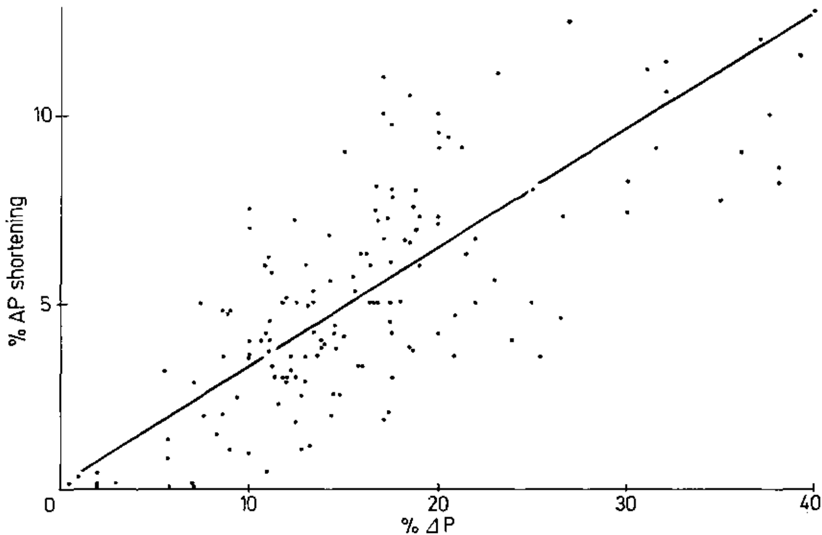

Fig. 3. The percentage change in isometric peak tension (% ΔP) following a change from isotonic to isometric conditions is plotted against the % abbreviation in the duration of the associated action potentials

only a few msecs to 60 msecs. It is felt that this variation depends on the energetic state of the preparations in so far as muscles of more than 1.2 mm diameter, in which the oxygen supply by diffusion is presumably critical, mostly show weak effects.

The question now arises as to whether both the alterations in action potential duration and the mechanical transients are independent or interrelated phenomena. The arguments in favour of the mechanical transients being a result of the electrical changes are as follows. The duration and intensity of the contractile activity in cardiac muscle is strongly dependent on the duration of the action potential (Kaufmann and Flekkenstein, 1965; Antoni, Jacob, and Kaufmann, 1969; Wood, Heppner, and Weidmann, 1969). Further, clamping of the membrane potential for different durations Beeler and Reuter (1970) showed that the inward Ca^{++} current and tension developed was greater the longer the clamping period. It has also been recognized from several recent investigations (Heppner, Weidmann, and Wood, 1968; Antoni, Jacob, and Kaufmann, 1969; Beeler and Reuter, 1970; Braveny and Sumbera, 1967) that sudden changes in the duration of action potential produced by electrical polarization, voltage clamping or sudden temperature changes also

typically result in a staircase like mechanical response developing over 5—6 subsequent beats.

Therefore it is reasonable to conclude that also in this series of experiments the changes in the duration of the action potential quantitatively reflect in the subsequent contractile activity of the muscle. Indeed we can show in Fig. 3 that there is a relationship between the amount of action potential shortening measured arbitrarily at 80% repolarization and the degree of the negative mechanical transient following a sudden change from isotonic to isometric conditions. A similar correlation is found when the intensity of the positive inotropic transient is plotted against the prolongation of the action potential induced by passing from isotonic to isometric contractions. The quantitative relation shown in Fig. 3 agrees with that found in experiments where the duration of the action potential was electrically altered (Antoni, Jacob, and Kaufmann, 1969).

In the following section we will be concerned with the first part of this two-way contraction-excitation—contraction interaction, i.e. the feedback between contractile events and the action potential.

B. The Force-Velocity Relation of the Muscle as a Controlling Parameter of the Action Potential

Previous work on this aspect has already been mentioned (Stauch, 1966; Lab, 1968, 1969). It appears that a controlling parameter in the mechano-electrical feedback system is the force velocity relation of the muscle under study. This can be roughly concluded from the experiments shown in Fig. 4. In Fig. 4A the afterload seen by the muscle is increased stepwise during subsequent contractions. The corresponding action potentials are shortened roughly in proportion to the reduction in shortening velocity. The initial muscle length here was kept constant. However, when the muscle was preloaded starting its isotonic contraction from different initial muscle length, approximately the same relation between shortening velocity and action potential duration was found (Fig. 4B).

Also the graphic evaluation of six similar experiments given in Fig. 5 consistently show that the action potential duration is equally controlled by the velocity of shortening in both the preloaded and the afterloaded contractions. The extent to which this mechanism can vary the action potential under our experimental conditions is limited to about 20% of the total duration of the action potential (measured at 80% repolarization).

It is also possible to demonstrate a similar relationship between action potential duration and shortening velocity of the muscle when release

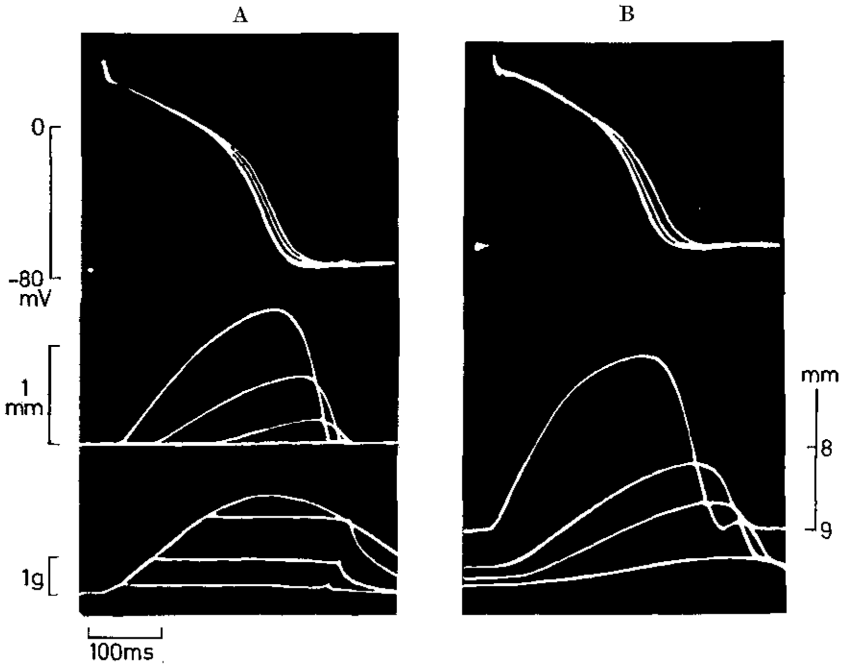

Fig. 4 A and B. Action potential duration as dependent on the shortening velocity of the muscle under different experimental conditions. In each part of the figure the upper recordings represent superimposed action potentials of 4 subsequent beats. During the experiment shown in part (A) initial muscle length was kept constant and the muscle shortened against increasing afterloads (0.3, 1.0, 2.0, 2.5 g/mm²). The last contraction was purely isometric (isotonic displacements: middle traces, tension developed: lower traces). In part (B) increasing preloads were applied to the muscle resulting also in decreasing shortening velocity but at different initial muscle length. It can be seen that in both cases the duration of the action potential is abbreviated roughly in proportion to the decreasing shortening velocity

experiments were performed. In Fig. 6 such an experiment is shown. This figure contains the traces of four subsequent beats each starting its contractile cycle under isometric conditions (lower traces in Fig. 6). But the muscle was not allowed to complete its isometric contraction. About 150 msec after the onset of contraction the muscle was released to 0.3 g/mm². A particular feature of this experiment was that the speed of the release i.e. the decay of tension, was controlled and set to about -300, -150, -60, and -10 g/sec resp. in four subsequent beats. As a result the sortening velocities of the muscle during the releases show different slopes. This can be seen from the displacement recordings

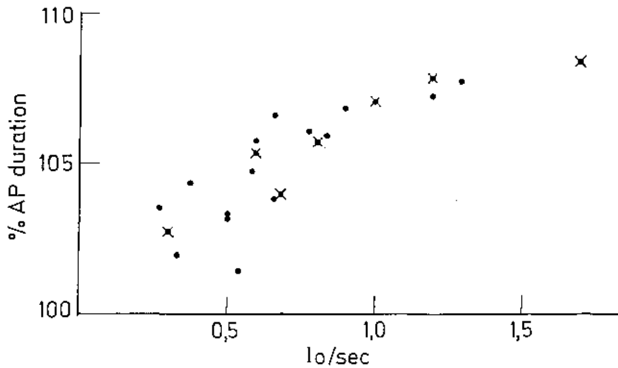

Fig. 5. Graphic evaluation of six experiments as shown in Fig. 4. The amount of changes in action potential duration is plotted against the shortening velocity of the muscle (in terms of l_0/sec). The filled circles represent experiments with increasing preloads (4 experiments performed on 4 preparations) the crosses are measurements under different afterloads at a given muscle length (2 experiments performed on 2 preparations)

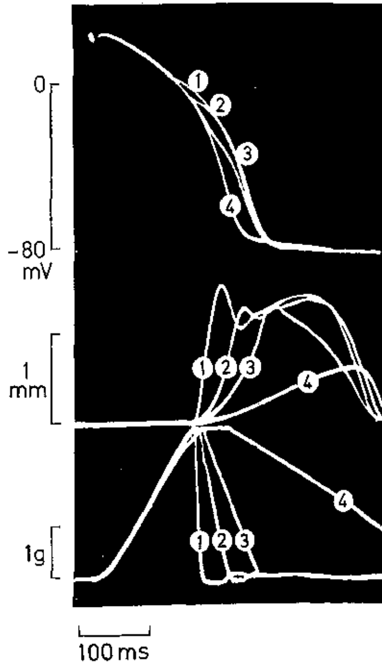

Fig. 6. Controlled release experiments. In four subsequent beats the time course of the isometric contraction (lower traces) was interrupted by a release starting about 150 msecs after the onset of the contractile cycle. The decay of tension during the release was -300 g/sec (trace *1*), -150 g/sec (trace *2*), -60 g/sec (trace *3*), and -10 g/sec (trace *4*). The displacements of the muscle during the releases and thereafter are shown in the middle recordings, the time courses of the action potentials are given in the upper part. (See text for further explanation)

(middle traces in Fig. 6). For instance during the fast release (trace *1*) the shortening velocity is rapid soon after the release and stays nearly constant as long as the release develops. The corresponding action potential is immediately broadened exhibiting a clear deflexion a few msec after the onset of the release (see also chapter C). If the decay of tension is slow (as for instance in trace *3*) the shortening velocity at the beginning of the release is also slow (depending on the instantaneous force-velocity relation) but finally reaches a value which approaches the shortening velocity during the fast release. Consistent with the above the time course of the corresponding action potential is first broadened, rather more smoothly, but also finally reaches the same absolute degree of prolongation as produced by the faster release.

C. The Force-Velocity Relation of the Contractile Element (CE) as a Controlling Parameter of the Action Potential

To elucidate further the role played by the force velocity parameters of the whole muscle as opposed to that of its contractile element we will base our interpretations of the following experiments on a generally accepted mechanical analogue of muscle; a passive elastic element (SE) in series with a contractile element (CE). When the whole muscle is kept isometric there is still some internal shortening of the contractile element due to passive stretching of the series elastic element. The shortening velocity of the contractile element (V_{CE}) is thus not zero. If the force-velocity relation of the contractile element itself contains the determining parameter governing the duration of the action potential, then from our previous results we could expect a further reduction in duration of action potential when V_{CE} tends to zero.

To achieve this aim controlled stretch experiments such as those proposed by Brady (1965) were performed. The rational behind of this type of experiment is to extend the series elastic element during contraction of the muscle in such a way that a constant contractile element length is maintained. This would require a sophisticated setup, but in our experiments the technical procedure was considerably simplified as we did not intend any detailed active state measurements. We were only concerned with reducing V_{CE} below that of normal isometric conditions. Therefore in these particular experiments the stress-strain characteristic of the parallel elastic elements was not determined prior to performing the stretch and there was no device for length control of the sarcomere. The amount of stretch applied to the muscle was only roughly estimated according to the available data of the mechanical properties of the cardiac muscle.

The recordings in Fig. 7 were taken from a papillary muscle where controlled stretches of different amplitude were applied. In the left

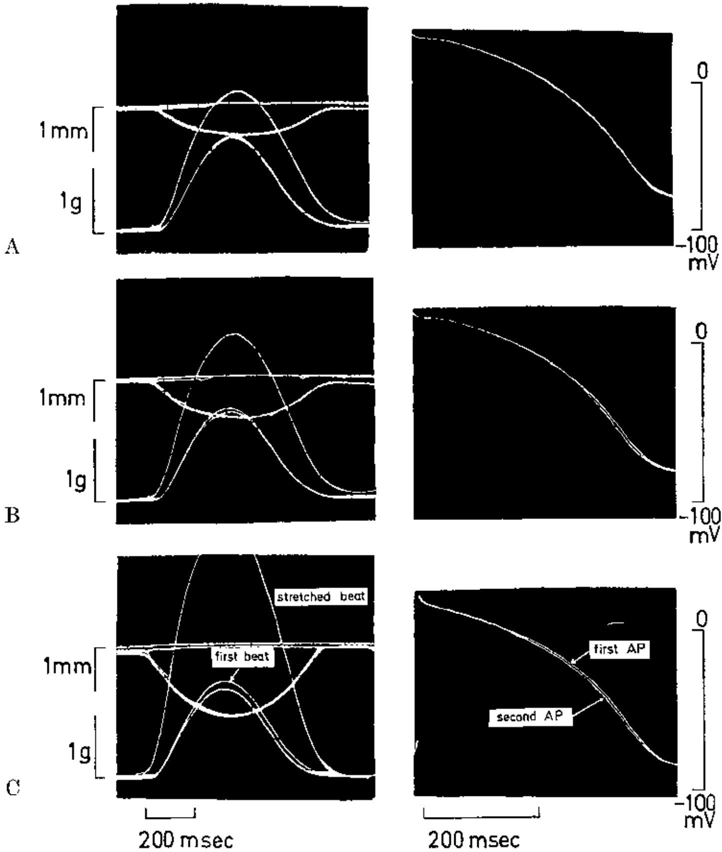

Fig. 7 A—C. Controlled stretch experiments performed on a papillary muscle of 7 mm in length and 0.9 mm in diameter. Each recording in the left column contains three superimposed traces of mechanical activity. The first beat is isometric, the second is performed under a controlled stretch (increasing in magnitude trough A, B, and C). The lengthening of the muscle produced by the stretch is indicated by the downward deflection of the top traces. The third beat was again purely isometric. The right column shows action potentials recorded with a faster oscilloscope sweep (see text) during the first and the "stretched beat". A small but reproducible reduction in the duration of the action potential can be seen associated with the "stretched beat". This reduction can explain the small negative transient during the third isometric beat (part B and C)

column of Fig. 7 each picture contains the traces of three successive contractions. The first is a normal isometric beat at a muscle length which falls on the lower ascending limb of the Frank-Starling curve (preload 0.8 g/mm^2). During the second beat the muscle was stretched

according to the procedure described above. The third contraction was again performed under the initial isometric conditions. In part (A), where only a moderate stretch was imposed, both the contraction before and after the "stretched beat" are of the same shape and amplitude. In part (B) and even more so in (C) the isometric contractions following the stretch show a clearly depressed peak tension reaching its original value after 4—5 beats (not shown here). One may indeed assume that this phenomenon could be the consequence of a small abbreviation of action potential brought about during the application of the stretch. It was difficult to obtain direct evidence for this since, from Fig. 3 the expected action potential shortening should be only 3—4% (8 msec) of the total action potential duration. Such small alterations of the action potential could not be detected on the storage oscilloscope using the sweep speed of the mechanical recordings. Therefore the sweep speed was doubled for the action potential recordings. Consequently we were unable to obtain simultaneous recordings of the mechanical and electrical events but had to perform successive recordings under the same conditions on the same preparation (shown in the right side of Fig. 7). Fig. 7 shows that the suggested action potential shortening actually does take place in part (B) and (C). These results are in favour of the action potential duration indeed being linked to the force velocity relation of the contractile element itself.

It appears thus far that the feedback link between contraction and excitation works as follows: the action potential duration is abbreviated when the shortening velocity of the contractile element decreases and the corresponding tension development increases. If this is true, then the duration of the action potential should be increased to a maximum when the muscle contracts at V_{max} against zero load. However, in cardiac muscle V_{max} as defined by A. V. Hill, cannot be experimentally obtained due to the high resting tension present even in the lower part of the passive length tension course. Despite this difficulty an attempt was undertaken to make the shortening velocity at least faster than in the isotonic contractions at the smallest preload (0.3 g/mm^2).

This was done performing quick release experiments as shown in Fig. 8. In all parts of this figure the muscle was stimulated at 24/min and made to contract isotonically at first against a preload of 0.3 g/mm^2. Thereafter in each part of the figure the subsequent beat was initially isometric. Then, at progressively longer times after the onset of the isometric beat the muscle was released by an exponential decay of tension and the contraction continued isotonically. The exponential decay of tension was used in order to approach a uniform shortening velocity during the release (see for comparison the linear releases of Fig. 6). The shortening velocity in these experiments could be adjusted by choosing

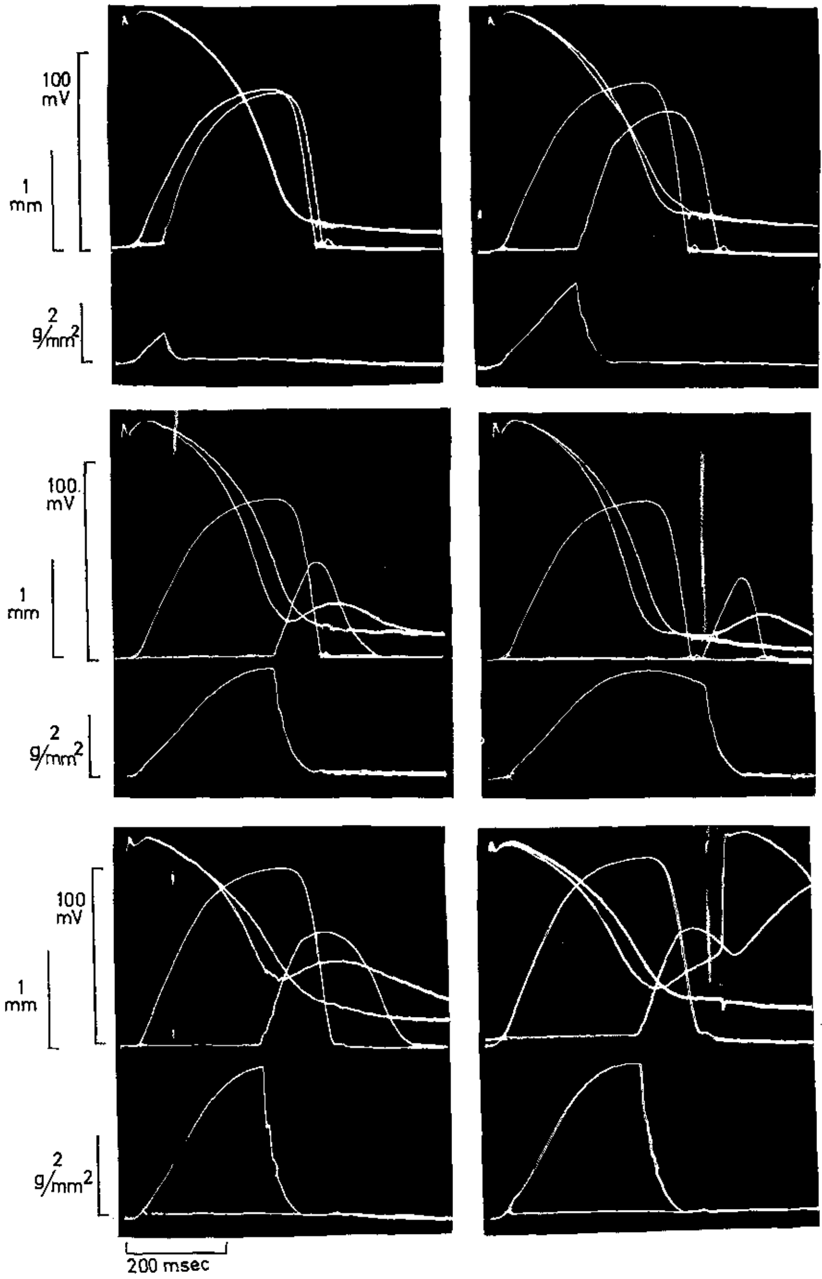

Fig. 8

the appropriate time constant of tension decay. In the experiments shown in Fig. 8 the time constant governing the exponential decay of tension was set to 20 msec resulting in a shortening velocity, which was much higher than that during the preceding isotonic beat at 0.3 g/mm². For instance during the experiment demonstrated in the upper right part of Fig. 8 the shortening velocity during the release was 1.9 l_i/sec as compared with a value of 1.1 l_i/sec developed during the preceding isotonic beat at 0.3 g/mm². Each part of Fig. 8 also contains two superimposed action potentials belonging to the isotonic and to the subsequent released isometric beat. Comparing the time course of each pair of action potentials one can differentiate two effects: the first is an abbreviation of the action potential associated with switching from isotonic to isometric conditions [already described in chapter (A)]. However in these experiments the muscle is not allowed to complete its isometric contraction but is released in such a way that a fast "isovelocic" displacement takes place starting at different points during the isometric cycle. As a consequence of this release the action potential is prolonged as expected from previous results but is now longer than that of the normal isotonic beat. This is seen in the upper recording of Fig. 8 where the release was made 100 and 170 msec after the onset of the isometric contraction. The action potential associated with these releases crossed over the "isotonic" one about half way down the repolarization phase. This shows that by shifting the shortening velocity of the muscle towards V_{max} the duration of the action potential can in fact be further increased.

The release experiments shown in Fig. 8 bear two additional features of this contraction-excitation recoupling system. First the prolongation of the action potential induced by fast displacements appears to be due to a substantial depolarizing current with its own time course. This is clearly seen when the release is made after the action potential is virtually complete (middle, right and left recordings). A new wave of depolari-

Fig. 8. Quick release experiments performed on two different preparations. In each recording the first beat is purely isotonic against a 0.3 g/mm² load. The second beat begins isometrically and is released at different times after its onset to 0.3 g/mm² load, so that the contraction is completed isotonically. The decay of tension in these release experiments is exponential in order to obtain a nearly uniform shortening velocity during the release (for comparison see also Fig. 6). The corresponding action potential recordings exhibit two different effects. During the isometric period the action potential is again shortened (as previously described). But a few msecs after the start of the release a depolarizing current interrupts this trend producing a further prolongation (upper right part), or a new wave of depolarization (middle, left, and right part). In the lower parts an experiment is shown where this depolarization reached threshold and initiated a new propagated action potential

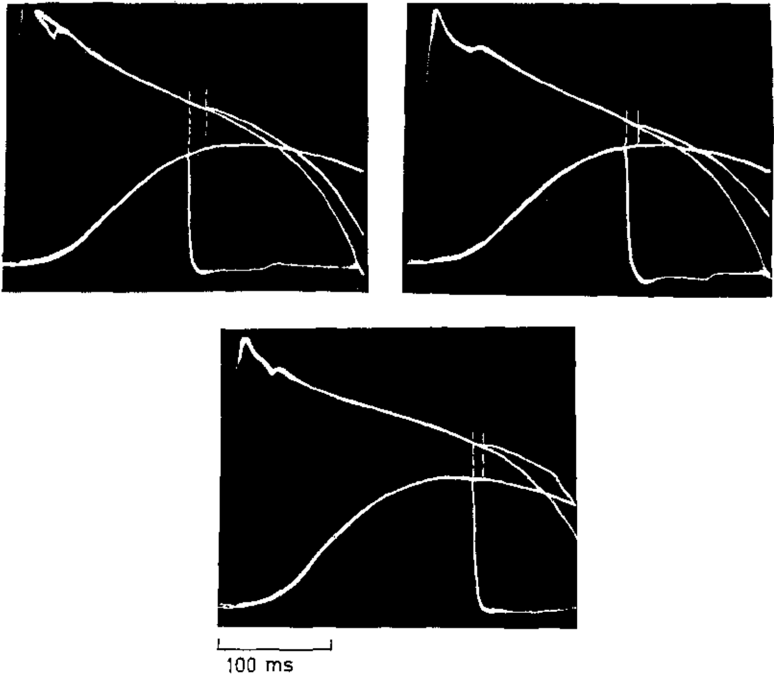

Fig. 9. Quick release experiments. The period of the "mechano-electrical delay" could be estimated by measuring the interval between the onset of the release and the first detectable deviation of the action potential at a high sweep speed. In 14 such experiments this interval averaged 8.9 ± 1.9 msec regardless at which time during an isometric beat the release was performed

zation (ranging from about 12—20 mV in amplitude and 150—200 msec in duration) appears. Occasionally this new depolarization may reach threshold with the initiation of a propagated action potential (lower right recording).

The second additional feature seen here is that the time delay of this feedback between mechanical and electrical events is relatively short. From the beginning of the release to the point where the change of shape of the action potential could be detected an interval of less than 10 msec could be measured (see Fig. 9).

It seems thus far that shortening of the contractile element produces a depolarizing current at the plasma membrane whereas tension development is associated either with a decrease in this current or an increase of a repolarizing current. A current such as the latter has not been demonstrated since in contrast to isometric contractions both the iso-

tonic beat and the action potential are complete almost at the same time.

Discussion

The foregoing experiments have shown that in cat papillary muscle there is a feedback mechanism by which the force velocity relation of the contractile element determines the duration of the action potential to a certain degree. Increasing the shortening velocity (V_{CE}) tends to prolong the action potential whereas increasing tension development produces a shortening of the action potential. As a result of these changes in the time course of the electrical excitation, mechanical transients are produced over the next 5—8 beats until a new steady-state is reached.

Before arriving at the above general concept we have first to exclude several other possibilities as to how contractile events could influence the electrical activity of the plasma membrane. For example one may assume that during changes in muscle length the cellular geometry or the molecular arrangement of the membrane is altered and this may change its electrical characteristics. Several experiments make this unlikely. When sudden changes in length were simulated by passively stretching and releasing the muscle, no change in the membrane potential was detected (as long as all changes took place on the lower part of the passive length tension curve). However if such a muscle was further stretched the membrane became progressively depolarized until a critical length threshold was reached for the production of spontaneous repetitive action potentials (Kaufmann and Theophile, 1967). This phenomenon is probably unrelated to the one we describe here, since a depolarizing current in our series of experiments was only found when the muscle in fact shortened in contrast to Kaufmann's mentioned experiments where the muscle was lengthened. In addition when the muscle was lengthened during activity by controlled or quick sustained stretch the repolarization was facilitated instead of being delayed as one would expect if it were due to passive membrane stretching. One may argue that the passive stretch experiments were carried out on an electrically quiescent muscle and that geometric changes of the membrane will affect its properties only during an action potential. Therefore similar passive changes were imposed on a muscle perfused with an agent which uncoupled excitation-contraction (Ni^{++} or Co^{++}). These preparations show no mechanical activity yet have action potentials which are virtually unaffected (Kaufmann and Fleckenstein, 1965). Sudden passive stretches in length on this type of preparation also had no effect on the time course of the action potential. Therefore we believe that the feedback system we have observed is not directly due to changes in the cellular or molecular geometry of the sarcoplasma.

At the very least one may say that this phenomenon requires the presence of an active state in the contractile machinery. The active state may be classically represented either by the ability of the contractile element to developed tension or to shorten. This led us to an examination of these parameters as being possibly related to the degree of electrical alteration. We found that an increase in the shortening velocity of the contractile element consistently prolonged the duration of the action potential. A similar correlation was found between force development and abbreviation of the action potential. Restated, it appears that the actual force-velocity relation of the contractile element and not of the muscle as a whole determines within certain limits the time course of repolarization. This concept was born out by the results of the controlled stretch and the release experiment. The aim of the former was to approximate V_{CE} to zero. Under these conditions there was a further small reduction of the action potential duration as compared to a normal isometric contraction (Fig. 4). In the quick release experiments the object was to increase V_{CE} beyond that of a lightly loaded isotonic contraction. When such experiments were performed the action potential duration was further prolonged than under pure isotonic conditions. However the interpretation of the latter experiment is complex since a significant proportion of the initial displacement after the release is thought to be due to SE rather than to CE shortening (Ritchie and Wilkie, 1958). If this is so then the prolongation and the new wave of depolarization induced by the release (Fig. 8) may be attributed to SE instead of CE displacement. The question now arises as to whether series elasticity is more or less an integral part of the contractile element (Sonnenblick, 1964; Brady, 1967) so that SE shortening may imply a simultaneous and significant displacement within the sliding filaments. This would be a necessary prerequisite within the framework of our intended hypothesis. In order to obtain some evidence for this as yet unresolved problem an experiment (similar to Brady's, 1967) was carried out for the purpose of this discussion. An isometrically contracting muscle was released for only 5 msec. After this brief period, during which CE shortening is supposed not to have taken place, the muscle was returned to its initial isometric conditions (Fig. 10). The rational behind this experiment is based on the fact that any displacement of the contractile element in heart muscle more so than in skeletal muscle tends to reduce the intencity and the duration of the active state ("uncoupling effect" of Brady, 1965). If indeed during such short releases as in our experiments contractile element displacement does take place then an uncoupling effect should appear. Conversely if contractile element interaction was stable the isometric tension curve should resume its original time course. Fig. 10 shows some of the features relevant to our dis-

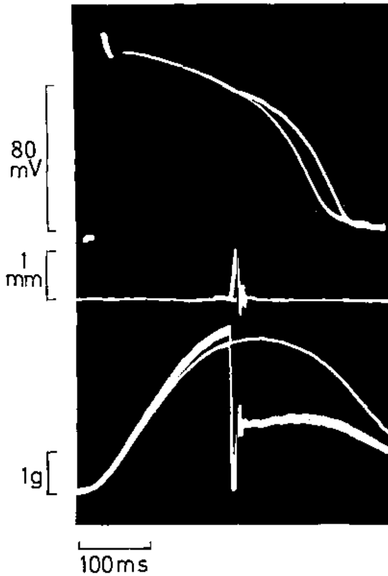

Fig. 10. Tension and action potential recordings of a papillary muscle. The first tension curve is a completed isometric contraction, whereas the second is interrupted (190 msec after its onset) by a 5 msec release period. Isometric contraction at the same muscle length is thus resumed. An "uncoupling effect" (Brady, 1966) is manifest as a reduction in the subsequent isometric tension development. Simultaneously recorded action potentials show that the 5 msec release produces an increase in the duration of action potential

cussion. When the isometric contraction was interrupted by this release 190 msec after the onset of contraction a prominent uncoupling effect is observed. This supports the view that in fact even during such a brief intercalated period of release a significant amount of displacement between actin and myosin may occur. As a corollary, simultaneous action potential recordings exhibit the expected prologation associated with the proposed contractile element shortening.

Although the experimental findings are fairly clear cut the formulation of a unifying working hypothesis at this stage is highly speculative. We feel however that an attempt should be made in order to give some direction to future research in this contraction excitation feedback mechanism. Such an hypothesis must take into account the following:

1. The basic requirement is the presence of an active state

2. The instantaneous force velocity relation of the contractile element contains the controlling parameter

3. Increasing V_{CE} tends to prolong, increasing P_{CE} tends to shorten the action potential (virtually) by generating an appropriate inward or outward membrane current

4. The feedback mechanism takes less than 10 msec to operate.

Having more or less excluded direct effects on the plasma membrane we have to explain the extremely short latency of the feedback system. It is highly improbable that the link involved between contraction and excitation operates via the diffusion of some ion from the contracting internes of the fibre to the plasma membrane. Perhaps a system which could work fast enough to explain this short delay are the transverse tubules. These structures are thought to beradial conductors for the rapid inward spread of excitation in fast working muscle fibres with large diameters (Huxley and Taylor, 1966; Freygang, 1965; Eisenberg and Gage, 1967). If this is true then there is no reason to believe that the same system cannot conduct an electrical potential, generated somewhere in its deeper parts, in the outward direction. For better understanding we will introduce a simplified equivalent circuit of the heart cell including the T-system as proposed by Fozzard (1966). In this analogue model the transverse tubular system is represented by: a resistor R_t which corresponds to the luminar resistance in series with a membrane capacitance C_t. As Falk and Fatt (1964) suggested, this model would require the presence of a transtubular membrane potential of the same size as the trans-surface membrane potential ($E_t = E_m$). If E_m changes, as it does during an action potential, the potential across C_m and C_t will be unequal. Consequently a current will flow through R_t, thus contributing to the time course of the action potential as seen by the microelectrode. Any changes in the electrical properties of the tubular system may therefore be reflected on the action potential. For the sake of simplicity C_t is regarded as being constant and R_{mt} as having a pure ohmic current-voltage characteristic. Then those variable parameters altering the amount of current flow through R_t are E_t (more precisely the instantaneous difference between E_t and E_m) and R_t itself.

Is it possible that R_t or E_t can vary during contractile activity? Consider R_t first. It is conceivable that the lumina of the T-tubules (represented by R_t) are somehow distorted or locally narrowed during contraction thus changing its electrical resistance. For example an active shortening may increase its value. An effect such as this was simulated by feeding a computed action potential (Krause, Antoni and Fleckenstein, 1965) into this analogue model where R_t was made variable. Using appropriate values both a prolongation of the action potential and a new distinct wave of depolarization could be produced (Fig. 8). This is at least qualitatively in accordance with our experimental findings. How-

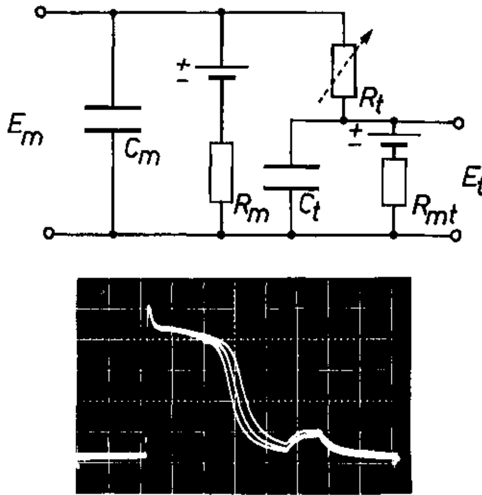

Fig. 11. The upper part shows a simplified equivalent circuit of the ventricular cardiac fibre. C_m and R_m represent the capacitance and the resistance of the plasma membrane. R_t and R_{mt} the capacitance and the resistance of the transverse tubular membranes. The series resistor R_t is thought to be due to the resistance of the tubular lumina. In the resting fibre the transmembrane potential E_m is assumed to be equal to a probable transtubular potential E_t. If during action potential E_m and E_t become unequal a current would flow through R_t contributing to the time course of the action potential. — In the lower part action potentials are shown which were produced on this circuit by analogue computation (Krause, Antoni and Fleckenstein, 1965). The following values were used: $C_m = 1\ \mu F$; $C_t = 6\ \mu F$; $R_t = 200$ to 1000 Ohms; $R_{mt} = 10$ kOhms. The time course of three action potentials are shown corresponding to three different values of R_t (100, 200, and 400 Ohms). With increasing R_t the action potential shortens. A new wave of depolarization could also be produced by a sudden change of R_t from the preceding value to 1000 Ohms after the repolarization was virtually complete

ever it is difficult to precisely imagine how mechanical forces could produce rapid changes of the tubular lumina. Perhaps even more so how the velocity of shortening could affect it. We will therefore deal with the other proposed possibility i.e. changes of E_t (thought to be result of an electro-chemical gradient across the tubular membrane).

Speculation as to which ions or which specific membrane conductivities are involved in producing such a transtubular potential is hazardous at this stage. Theoretically any of the ions which at this point can establish a transmembrane concentration gradient, or may influence the tubular membrane conductivity, can contribute to the formation of E_t. Reconsidering the contraction excitation feedback we may ask which ion has its internal kinetics specifically controlled by the mode of contraction ? It is becoming apparent that such an ion is calcium although

the molecular mechanism of its internal control is as yet unknown (as reviewed by Langer, 1968). In the cardiac plasma membrane, which is amenable to electro-physiological investigations, Ca^{++} can influence E_m either by its own contribution or by affecting the membrane conductivity for other ions. It is reasonable to suppose that Ca^{++} may act in a similar way at the tubular membrane thus influencing E_t. This would provide the link between the force velocity relation of the contractile element, which presumably controls the internal concentration of free Ca^{++}, and the tubular transmembrane potential. This potential could partly depend on the internal Ca^{++}-concentration. From this point changes of E_t could be rapidly reflected on the time course of the action potential as described above.

Whatever mechanism underlies the feedback between contraction and excitation it seems to be an integral part of a system by which the instantaneous control of the contractility of the myocardial cell takes place. This is a mechanism which is distinct from that of Brady's (1965, 1968). A characteristic feature of the present particular system is that it also governs (with diminishing intensity) the active state of the following 4—8 beats.

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TRANSIENT DEPOLARISATION AND ACTION POTENTIAL ALTERATIONS FOLLOWING MECHANICAL CHANGES IN ISOLATED MYOCARDIUM

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Reprinted from Cardiovascular Research
Volume XIV, *No.* 11, pages 624-637, *November* 1980

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LONDON
BRITISH MEDICAL ASSOCIATION
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Cardiovascular Research, 1980, **14**, 624–637

Transient depolarisation and action potential alterations following mechanical changes in isolated myocardium

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SUMMARY The effects of induced changes in muscle length on the action potential of frog ventricular strips and cat papillary muscle have been studied. When the frog preparation was stretched near the onset of contraction, the action potential duration shortened whereas a stretch during peak activity produced minimal change. Action potentials of cat papillary muscle do not alter with stretch at any time. By contrast, release of both preparations at a time when tension was near its peak, prolonged repolarisation or produced a transient depolarisation. The ECG changes corrobrated the action potential changes. The release produced a deactivation of contraction which correlated with the transient depolarisation when the contraction and potential were expressed as ratios of the undisturbed measurements. Possible explanations for the results are discussed in terms of active and passive mechanisms that can relate to mechanical and electrical phenomena simultaneously. The mechanically induced transient depolarisations are clinically relevant, for regional ischaemia produces electrical and mechanical inhomogeneities which would cause contraction-excitation feedback interactions and thus electrophysiological abnormalities.

In "contraction-excitation feedback" alterations in contractile activity as well as extraneous imposed mechanical disturbances of myocardium result in changes in the accompanying action potential.[1] Regional myocardial ischaemia causes prominent mechanical disturbances and if contraction-excitation feedback operates under these conditions it should cause electrical changes with important clinical consequences. One possible example has been briefly reported[2] and is related to the fact that mechanical changes can induce prolongations of the action potential sometimes appearing as transient depolarisations.[1] These depolarisations can take the form of after depolarisations which are relevant to the generation of arrhythmias in pathological heart conditions. In view of its potential clinical bearing, the role of contraction-excitation feedback appears worthy of further study. However the precise mechanism of this feedback link is unknown, partly because it probably uses the components of excitation-contraction coupling and in mammalian cardiac muscle this process is a complex series of events, and partly because few investigations have been concerned with the effects of mechanical events during muscle activity on membrane phenomena. A preparation with a more rudimentry excitation-contraction coupling process than in mammalian heart was sought. Frog ventricle has a less complicated and slightly different internal structure and excitation contraction coupling mechanism from mammalian muscle[3 4] and there is evidence to suggest that mechanical changes in intact frog ventricle can affect the action potential.[5] The initial part of the study was to demonstrate and characterise mechanoelectric or contraction-excitation feedback in frog ventricular strip and to compare the type of coupling found previously in cat papillary muscle. The final part of the study was to extend some of the experiments to a mammalian preparation and define the relationship between the transient depolarisation observed and the mechanical event inducing it.

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Methods

A. FROG VENTRICULAR STRIP

(i) Preparation for monophasic action potential and ECG

The preparation was that of Niedergerke[6] namely a strip of frog ventricle (1 $mm^2 \times 7$ mm approx) set up in a double-chambered bath so that it was clamped in a narrow canal passing through a partition which separated the two chambers. The canal was formed by the apposition of two sliding plates (fig 1).

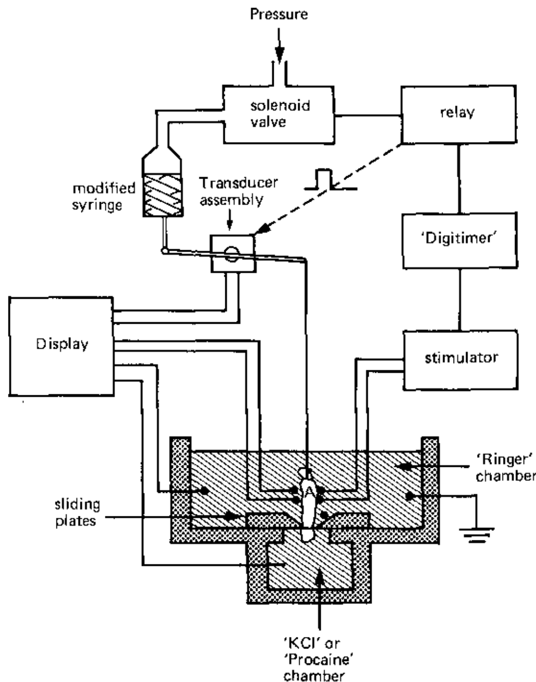

FIG 1 *Diagram of the overall recording and mechanical control system. In some of the experiments (horizontal preparation for microelectrode recordings) the Relay via a dc current source, controlled the transducer assembly (dashed line) which then consisted of a modified galvanometer.*
(A=frog ventricular strip).

Silicone grease served to assist in sealing the bottom from the top chamber. The part of the muscle in the lower chamber was immersed in either 18.33 mmol·litre^{-1}. Procaine-7% sucrose or 121 mmol·litre^{-1} KCl solution while the upper chamber contained approximately 50 cm^3 Ringer's solution (NaCl 111.3 mmol·litre^{-1}, KCl 1.8 mmol·litre^{-1}, $CaCl_2$ 1.08 mmol·litre^{-1}, $NaHCO_3$ 2.4 mmol·litre^{-1}) continuously bubbled with 95% oxygen and 5% carbon dioxide. The average length of muscle protruding in the upper chamber was 4.97 mm (SD 1.25).

The monophasic action potential was obtained from the potential difference between upper and lower chambers sensed by Ag/AgCl electrodes. The second recording in this arrangement was similar to a bipolar electrocardiogram from the upper part of the muscle, derived from two Ag/AgCl electrodes situated in the upper chamber but not actually touching the preparation. This record was thus obtained with the upper chamber as a whole acting as a volume conductor.

If procaine was used in the lower chamber rather than KCl, a dc potential (procaine chamber positive) of 36.5 mV (SD 5.4) was set up within the first half second. This potential was due to the liquid junction between the procaine-sucrose solution and the NaCl in the Ringer's solution as it depended on the concentration of sodium chloride in the upper chamber and reversed sign when NaCl was zero. There was virtually no standing potential with the KCl solution. However the action potentials were somewhat smaller and less stable over long periods than with procaine — presumably because KCl diffused across the gap faster. The procaine technique was used for many of the experiments but in case the standing potential influenced the results, confirmatory studies were performed using KCl. Both KCl and procaine produced the same results.

Although this technique has inherent limitations it was sufficient to establish the existence of mechano-electric coupling. Other techniques and preparations were used to measure the time resolution and absolute amplitude of action potentials in these circumstances.

(ii) Microelectrode recordings

The strip of ventricle was prepared as above. The muscle was then clamped horizontally in a perfusion chamber and a conventional microelectrode system for cardiac muscle used. The electrodes were of the floating type with resistances typically about 20 MΩ. The signals were recorded via a high input impedance field effect transistor amplifier and stored on magnetic tape for later analysis. Tension was measured with an RCA transducer valve. The imposed changes in length were usually made in steps from slack length (L_i) to near L_{max}. Because of the difficulty in maintaining impalements in the small cells of the frog no attempt was made to put the muscle through a range of accurately predetermined changes of length varying with amplitude, time or duration.

(iii) Mechanical recording

In the vertical preparation (fig 1) the top end of the muscle strip was tied with cotton thread and attached to one end of a pivoted lever to which

strain gauges had been glued for recording tension.[7] The other end of the lever was connected to the spring loaded plunger of a modified syringe[8] and was pneumatically activated. Movements of the plunger thus caused changes in the muscle length. The pivoting movement of the lever was recorded photoelectrically by means of a graded-density film, whose movements varied the intensity of a light beam falling on a phototransistor.[9] Thus muscle tension and movement were simultaneously monitored.

The lever was horizontally mounted and the vane adjusted on the shaft so that the output was linear when the tip of the lever where the muscle was attached moved through at least 4 mm. The calibration was done with a micro-manipulator moving the pneumatically controlled device described below. The system was arranged so that 1 mm downward movement of the micro-manipulator or pneumatic device would stretch a muscle 1 mm.

The compliance of the tension transducer lever was less than 0.05 mm·g^{-1}. The frequency response of the arrangement was not measured but the resonant frequency of a similar but more compliant lever (0.1 mm·g^{-1}) has been quoted as being 100 Hz.[7]

(*iv*) *System controlling length changes*

Stepwise changes in muscle length were obtained by appropriate movements of the spring loaded plunger. These movements were programmed using a Digitimer (Devices), controlling a relay which acted through a pneumatic system onto the plunger. The plunger was a modified plastic 5 cm^3 syringe with the back end of its piston rod extended so as to move the lever system. Downward movements of the piston rotated the system so that the muscle was stretched. As a consequence of the downward movement of the plunger a spring was compressed which later returned the plunger to its original position when the compressed air was released. The muscle thus returned to its pre-stretched length. A 5 mm muscle could be stretched to 8 mm in less than 15 ms which was fast enough for the purposes of this study. The compressed air was controlled via a solenoid valve (Dewrance Controls Limited) which was mains operated and switched into the circuit by a relay (Devices) triggered from pulses obtained from the Digitimer. By this means, changes in the length of the muscle were accurately timed during any particular contraction cycle.

The muscle was electrically stimulated at room temperature (19° to 20°C) with 1 to 5 ms duration supramaximal stimuli at 30 per min and the resultant contractile and electrical activity examined under various mechanical conditions. The length of the muscle was first set so that it developed some tension (*eg* 1 G) and the muscle made to contract at 30 per min for 15 min while the action potential was observed over this period.

The potentials were recorded with a multichannel pen recorder through a Devices DC pre-amplifier. The differential input leads were connected one to each chamber with the signal input lead to the bottom chamber. When procaine was used this method of recording gave rise to an action potential of 36.5 mV $\pm$14.3 (mean $\pm$ SD).

The length and tension transducer outputs were also connected to the pen recorder via a DC pre-amplifier. The outputs of this amplifier were thereafter fed into a storage oscilloscope (Tetronix).

The preparation was discarded in any of the following circumstances: development of spontaneous activity or tension alternans, a fall in action potential amplitude faster than 10% of its original height per hour, the appearance of a deep notch in the action potential immediately after the spike, or a biphasic response with a relative hyperpolarisation at completion of repolarisation greater than 5% of the total action potential height, any other atypical shape of the monophasic action potential.

Before beginning experimentation the initial muscle length was set so that the muscle just began to develop tension when contracting (*ie* the muscle was just slack). This length was chosen so that the effects on different parameters of a complete range of muscle extensions could be studied. Even though the resting tension in the heart *in situ*, might be the most relevant starting tension, it could not be conveniently determined.

At the end of each experiment L_i was measured from just above the point of clamping in the sliding plates, to where the cotton was tied. The muscle was then cut at these two points and weighed after removal of excess Ringer with tissue paper.

(*v*) *Histology*

At the end of each of 8 length-tension experiments lasting 3 to 4 h the muscle strip was fixed in buffered formalin. Three of the preparations were first left to soak for 12 h in Ringer containing Trypan blue for the parts of the muscle which were dead to autolyse and take up the stain before fixing. These muscles were then cut longitudinally and stained with haematoxylin and eosin for normal viewing or van Giemsa for looking at collagen tissue. The Trypan blue was for identification of dead tissue.

B. CAT PAPILLARY MUSCLE

The method used for this part of the investigation, microelectrode recordings, isotonic lever system, force transducer, load control unit and sucrose gap chamber, has previously been partly described by

Hennekes *et al*,[10] and Hennekes *et al*,[11] and therefore will only be briefly mentioned. Papillary muscles were dissected from the right ventricle of cats to include part of the muscle's attachment to the ventricular wall. The tendinous end of the muscle was pulled either through a small ring in the isometric lever to prepare for microelectrode recordings, or through the hole of the modified isometric lever in the sucrose gap chamber. The portion of the ventricular wall was thus wedged in the ring and force was measured when the other tendinous end of the muscle was fixed to an isotonic lever made of a coil type galvanometer. A dc current through the coil generated the force used to stretch the muscle. Muscle shortening was measured using the same photoelectric assembly as used with the frog experiments or as previously described. The initial muscle length (L_i) was adjusted to correspond to L_{max}.

Timing of releases and stretches of the preparation was achieved by the methods previously described as well as with the system used with the frog preparation. In the latter system the relay (fig 1), controlled by the Digitimer, switched the dc current into the galvanometer coil at variable times and durations. The isotonic lever, attached to the coil, thus provided quick-release followed by quick-stretches at the desired intervals.

The modified Tyrode solution used in these experiments had the following composition (mmol·litre^{-1}): NaCl, 136.9; KCl, 2.68; $NaHCO_3$, 11.9; $CaCl_2$, 2.5; NaH_2PO_4, 0.42; glucose, 10.0; $MgCl_2$ 1.0; (EDTA 2×10^{-5} mmol·litre^{-1} was also used in the solution in some cases). The solution was oxygenated with 95% O_2 and 5% CO_2 at 31°C with the pH at 7.4. The muscle was stimulated at 20 per min using stimuli with twice threshold intensity.

Microelectrode recordings were obtained using the identical method to the frog experiments or to that previously described by Kaufmann *et al*[1]. The modified sucrose gap chamber used for transgap potentials was also used for obtaining extracellular electrograms. This was done by eliminating the "chamber" separation, (partly achieved with liquid/liquid interfaces) by perfusing all three chambers with Tyrodes solution. This procedure produced a biphasic action potential or electrogram.

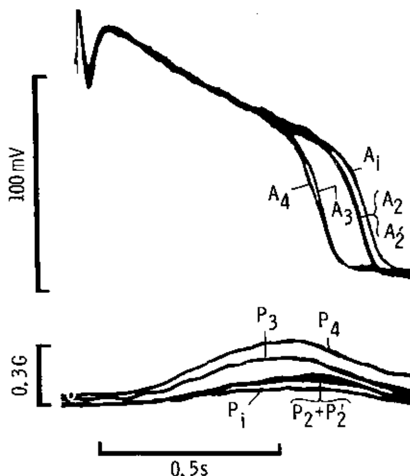

FIG 2 *Action potentials, recorded with microelectrodes, from a frog ventricular strip at different lengths. The action potentials were obtained during a single impalement while the muscle was gradually stretched.*

As the tension increased from P_1 to P_4 the action potential duration decreased from A_1 to A_4.

Results

A. FROG VENTRICULAR STRIP

(i) Length-tension relations

Since the changes in action potential studied here are mechanically induced some mechanical characteristics of the preparation used are worth summarising. They are described in more detail elsewhere.[12]

Two different points of L_{max} could be obtained for each preparation. One variety was found when lengthening the muscle by 1.0 mm increments. Change in length was expressed as a ratio, L_f/L_i where L_f and L_i were final and initial lengths respectively. This gave rise to a mean L_{max} of 2.06 L_i (SD 1.25, n=9) showing a very ill defined maximum tension in some of the experiments, with a wide scatter: 5 ± 4 nM·mm^{-2} (mean and SD).

The second L_{max} was found when the muscle was stretched briefly to each new length, from L_i, while stimulated. It was then returned to L_i before the next, larger, stretch. With this method L_{max} was 1·65 L_i (SD 0.13) with a maximum tension of 3 ± 1.4 mN·mm^{-2}. Many of the experiments with action potentials involve the latter transient stretch and release type of mechanical perturbation.

The length-tension relation of many of these preparations, determined by gradually extending the muscle, showed that the passive tension began rising relatively early and steeply compared with the developed tension. The question therefore arose as to whether this disproportionately large passive tension (or small active tension) was due to an excess of collagen tissue inadequately trimmed away, to muscle tissue having died during the dissection or from anoxia, or to the unfavourable orientation of muscle fibres within the strip. The histological studies showed that the most likely explanation was that the muscle fibres in any one preparation did not all run in the same direction, some were longi-

tudinal and others transverse. This inhomogeneous arrangement of the fibres in a given strip, varying from preparation to preparation, could also account for the wide scatter in the magnitude of developed tension.

(ii) Effect of stretch on the action potential

A simple experiment is shown in fig 2 in which the muscle was gradually stretched from 1.2 L_i to L_{max}. Small extensions were used to avoid disturbing the microelectrode impalement. Action potential duration is clearly seen to reduce as the muscle was stretched. The heights of the plateau at 50% and 70% repolarisation were also reduced. This figure was typical of 16 observations in two preparations. In order to study the effects of stretch on the preparation systematically in all the experiments in the subsequent figures the muscle was stretched after the spike of the action potential. This was done because there may be a depolarisation with larger stretches of a resting muscle which could sometimes produce a contraction.[5] This would make the interpretation of the length influence on the plateau potential difficult, for depolarisation can itself influence the action potential.[13] In over 100 experiments on 40 preparations the insulated gap technique was used and in each case increases in length were accompanied by reductions in amplitude and duration of the plateau phase of the action potential. The duration was measured at 75% repolarisation and the amplitude at 50% of the duration. The greater the length change the greater the change in action potential. Fig 3 shows a representative experiment and the reduction in amplitude of the monophasic action potential with increasing length is plotted in fig 4 for another preparation. From experiments on six preparations the change in voltage on stretching to 1.75 L_i was 9.36% of action potential height (SD 4.2). The mean correlation coefficient (10 preparations) was 0.91 (SD, 0.07, $P<0.001$ for each correlation) with a mean slope of 0.02 (SD 0.02).

(iii) Relationship between action potential shortening and Q-T interval of the ECG

Although both microelectrode recordings and external monophasic action potential recordings have shown a reduction in the duration of repolarisation with stretch, the electrocardiogram was also used because, unlike the preceding methods, the ECG samples all cells of the preparation. Furthermore, the mechanical distortion of the preparation in and around the gap across which the extracellular recordings were taken, could cause changes in the passive electrical properties of the experimental arrangement leading to recording artefacts, which would not be the case with the ECG. In the ECG

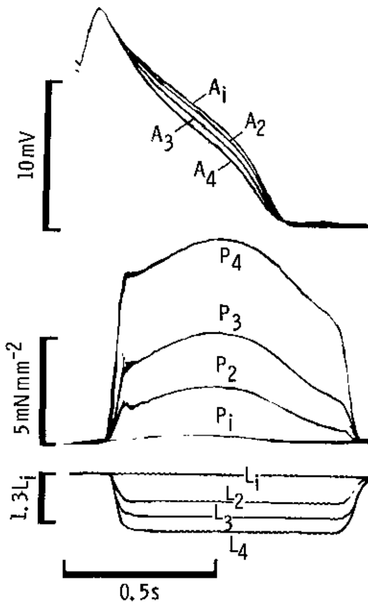

FIG 3 *Effect on active tension and repolarisation phase of action potential (recorded across an insulated gap) of stretching frog ventricular strip.*

Stretching the muscle just after the onset of activity, from L_i through to L_4 results in progressively larger active tensions (P_i-P_4). The amplitude, and duration, of the repolarisation phase is progressively reduced (A_i-A_4).

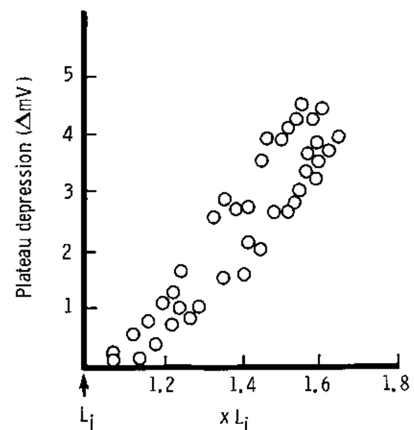

FIG 4 *Graph of effect of increasing the length of frog ventricular strip, expressed as multiples of initial muscle length, L_i, on the plateau depression, expressed as the induced change in millivolts (△mV). See text for further discussion.*

record the T wave represents the combined electrical vectors of all the terminal repolarisation phases of the cells in the upper chamber and the Q-T interval roughly corresponds to the time taken for all the cells to repolarise after being stimulated. Thus if the apparent shortened action potential duration in the stretched muscle really is representative of accelerated repolarisation of the whole strip, this should be detected as a shortening of the Q-T interval of the electrocardiogram. This shortening is clearly seen in fig 5. The percentage reduction in duration in action potential at T_{75} was of the same order as the reduction in duration of Q-T interval. In seven experiments a change in length of 1.6 L_i (SD 1.2), mostly constituting an extension from slack length to near L_{max}, led to a reduction in duration in action potential of 4.4% (SD 1.5) and a reduction in Q-T interval of 4.8% (SD 2.0).

(iv) Effect of stretches at different times on the action potential

The change in action potential seen with stretch may be somehow directly due to the mechanical change, or be indirectly related via some other mechanism. If simply due to the former then no matter when the stretch is given the same type of response should be

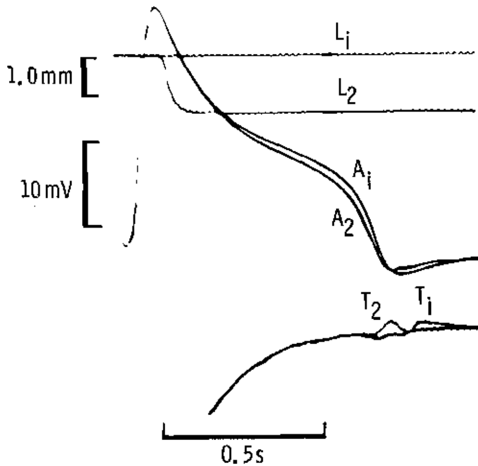

FIG 5 *Effect of changes in length on electrocardiogram of frog ventricular strip. Recordings (from above down) of two different lengths (L), action potentials (A), and electrogram which mainly show the T-waves. (The QRS complex is obliterated by the stimulus artefact). Note that the T-wave (T_2) associated with the longer length (L_2) and the shorter duration plateau (A_2) occurs sooner than (T_i) which accompanies (L_i). The T-wave usually terminates when the muscle as a whole has repolarised, hence the time lag between the repolarisation phase of the action potential and the T-wave: the former represents the repolarisation of fibres near the insulating plates whereas the T-wave is an extracellular recording of the repolarisation of the whole strip.*

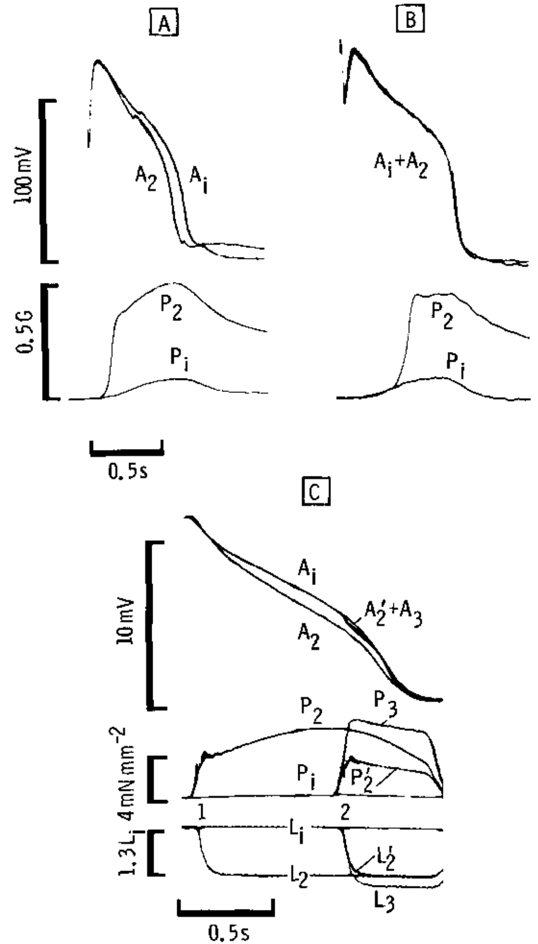

FIG 6 *Action potentials from frog ventricular strip during stretches at different times. A. The muscle is stretched from about 40% L_{max} to just under L_{max} soon after the onset of activity. The tension rises from P_i to P_2 and is accompanied by an alteration in time course and amplitude of action potential, A_i to A_2. B. The stretch is delayed and in this case no change in action potential is observed (action potentials in A and B recorded with micro-electrodes). C. The figure shows the effect of an early sustained stretch at 1, from L_i to L_2 with the depression of the plateau previously observed. The stretch 2 is delayed and the tension thus produced (P_2') is smaller than tension P_2, and the action potential deviation, A_2', is smaller than A_2. When the muscle is given a larger stretch (L_3), with the corresponding tension P_3 greater than P_2, the potential deviation (A_3) is still smaller than A_2 (action potentials recorded with insulation gap technique).*

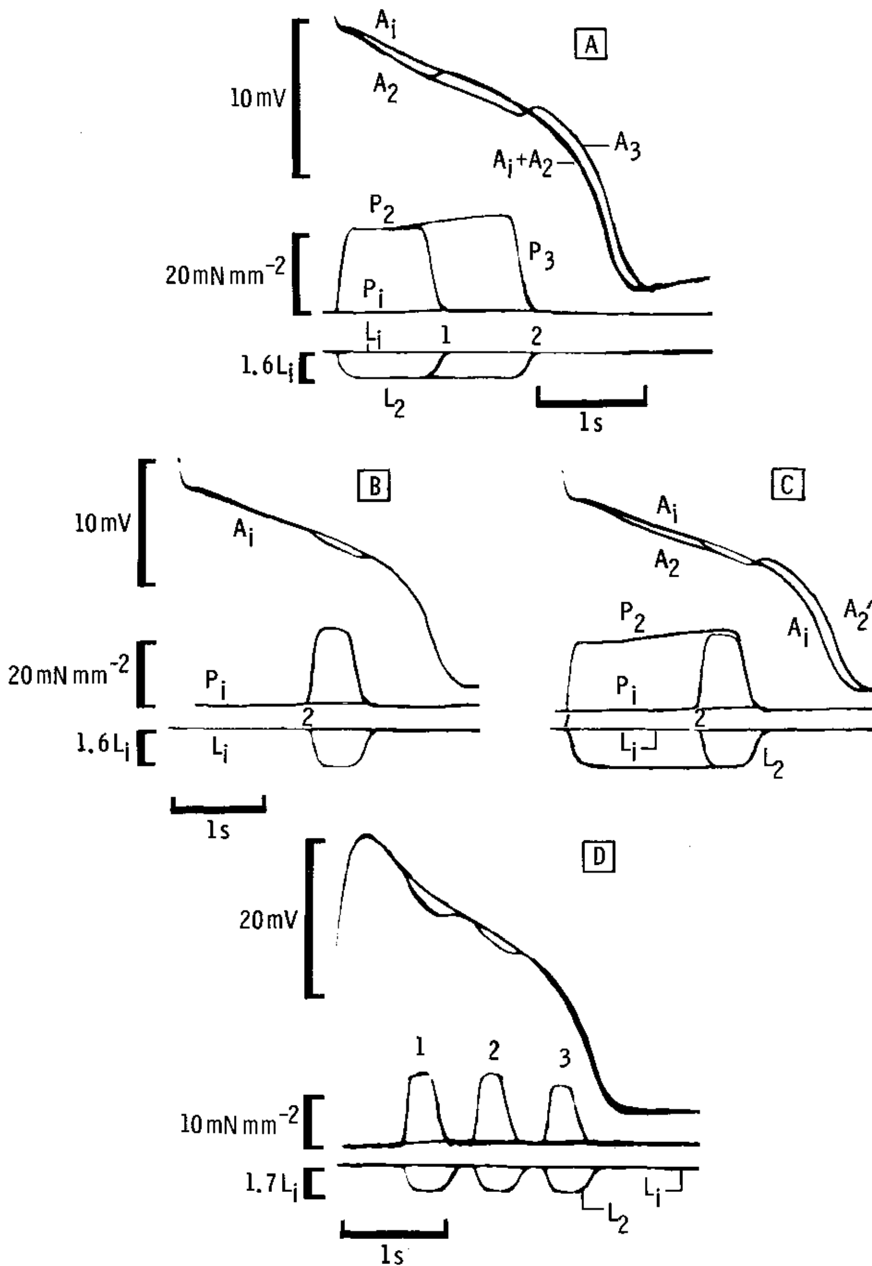

FIG 7 *Effect on frog ventricular action potential, recorded with insulated gap technique, of releases at different times and after stretches of different duration. A Releases at different times. Initial muscle length (L_i) is associated with tension (P_i) and normal action potential (A_i). Stretching the muscle to L_2 increased the tension to P_2 and reduced the plateau amplitude to A_2. With an early release, at (l), initial mechanical and electrical conditions are restored. With a later release, at (2), original conditions are restored but the action potential (A_2) crosses over A_i to delay the rapid repolarisation phase (A_3). This delay is referred to as a transient depolarisation. B The muscle is briefly stretched at 2 then released just before the rapid repolarisation phase of the action potential. On release electrical and mechanical conditions are restored. (P_i and A_i). C Shows superimposed on part B, a longer duration stretch, at 1, beginning sooner, but terminating at the same time as stretch 2. This is associated with a sustained tension rise (P_2) and plateau depression (A_2). On release the mechanical conditions return to prestretch conditions but the potential A_2 overshoots so that there is a delay in the rapid repolarisation phase of the action potential (A_2'): the transient depolarisation. D Briefly stretching the muscle from L_i to L_2 results in increases in tension. Stretch 1 produces a temporary depression in the action potential plateau, stretch 2 a smaller depression whereas stretch 3 has no effect. At no stage does the release cause a transient depolarisation.*

observed in the action potential. In order to differentiate between the two broad groups of possible mechanisms the stretches were progressively delayed after the muscle was activated. Impalements were difficult to maintain in experiments using microelectrodes but fig 6a and b, show two successful recordings. Stretching the muscle soon after the onset of activity reduces the amplitude and duration of the action potential (fig 6a), but when the stretch was delayed to 50% of the total duration of the action potential (fig 6b) there was no effect on the action potential. The possibility that delayed stretch required a greater amplitude to produce an effect was tested using the insulated gap recordings. This experiment (fig 6c) also showed that when the delayed stretch was applied at a time more than halfway through the action potential the change in plateau amplitude was negligible compared with that produced by the early onset stretch. Any deviation in the action potential produced was not maintained, and it returned to its prestretch time course. The failure to influence the action potential was not a function of the degree of stretch as a delayed stretch of larger amplitude than the preceding delayed stretch resulted in a larger total tension, but no further significant plateau depression.

(v) Effect of releases on the action potential

An early stretch shortens the action potential and a stretch at mid contraction has no effect. Will a release now restore the original time course of the action potential, and what are the electrical effects of release? In this series of experiments the muscle first contracted at control slack length and was then

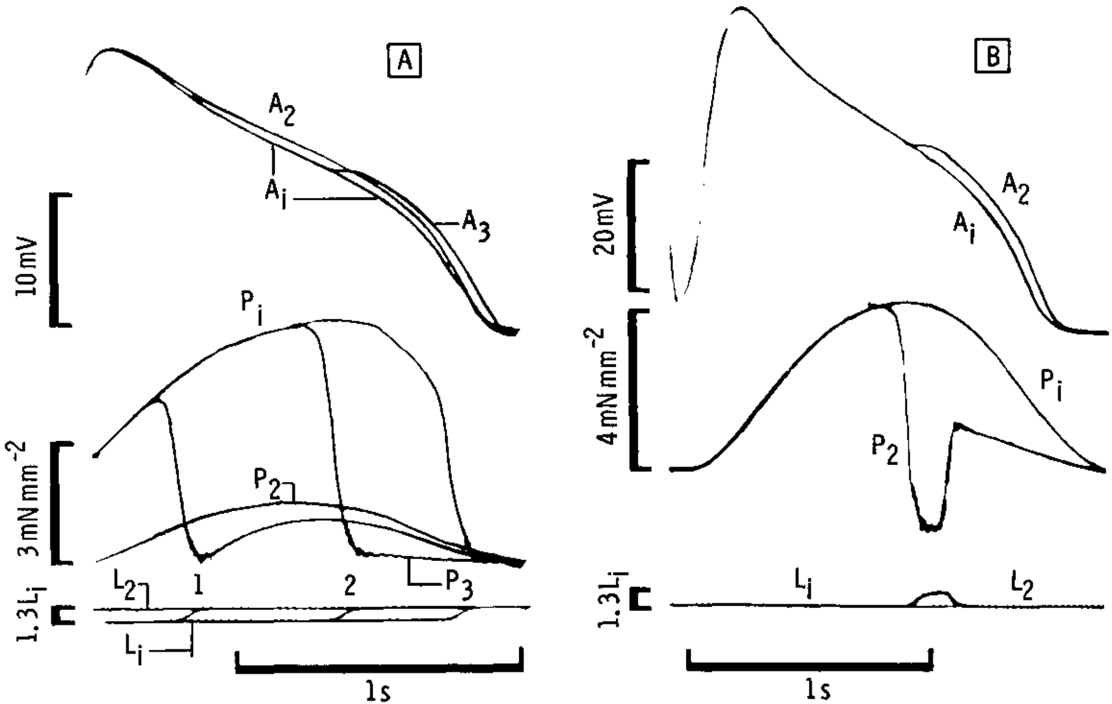

FIG 8 *Effect on frog ventricular action potential and developed tension of releases at different times. A The muscle is set so that an active tension is clearly seen when the muscle is unstretched (P_2). At the beginning of the next two action potentials the muscle is stretched to L_i giving rise to tension P_i and action potential A_i. The first release, at 1, results in an immediate reduction in active tension, but which redevelops almost reaching the tension P_2. The second release, 2, is not followed by any redevelopment of tension at all (P_3). The effect on the action potential of these two releases are similar to those seen in releases 1 and 2 in fig 7A, namely the first release results in the action potential returning to its unstretch time course A_2 whereas the second, later, release results in a depolarisation (A_3). B Effect on the plateau of transient release. The muscle at length (L_i) is released near the peak of the developed tension to L_2. The tension rapidly decreases from P_i to P_2 and the time course of the plateau of the action potential alters from A_i to A_2. On restretching the strip to L_i neither tension nor action potential return to the previous amplitudes. (The initial muscle length in these cases was not the slack length).*

stretched just after the spike of the action potential. One example of 10 such experiments is seen in fig 7a. A muscle contracting isometrically was stretched at the beginning of its contraction. This resulted in the depression in the voltage of the early repolarisation phase of the action potential described earlier. Thereafter the muscle was released at two different times during the contraction. In both releases the mechanical records returned to the prestretch level. After the early release the action potential also returned to its original time course but a later release resulted in a delayed repolarisation. Release No 2 gave rise to an "overshoot" of the return of the action potential and to a greater voltage at the instant of release than before the muscle was stretched, *ie* a transient depolarisation was produced. The time course of the remainder of the repolarisation was the same as it would have been without the stretch, but the overall time to complete repolarisation was prolonged. The relative importance of the duration of the stretch or its timing to the transient depolarisation, was examined in 10 experiments on five preparations. The duration of the stretch was clearly important, as a short duration resulted in the action potential returning to its original time course on release of the stretch (fig 7b). However, when a long duration stretch was terminated at the same time (fig 7c) a transient depolarisation occurred. It is worth noting that the tension during the short duration stretch was very little below that during the long duration stretch. Timing was investigated by applying a transient stretch of constant but short duration at different times after stimulation (fig 7d). The duration of the stretch was clearly more important, for at no stage was a transient depolarisation observed on release of the short duration stretch. However, the later the stretch the smaller the reduction in the plateau amplitude during the stretch, indicating that timing also plays a role in this case.

(vi) Relationship to deactivation

A release is accompanied by a reduction in the ability of the muscle to redevelop the tension it would have developed had it not been released. This phenomenon has been attributed to an uncoupling of the active state by Brady[14] and by Kaufmann *et al*[15] or, as has been aptly described by Julian and Moss,[16] a deactivation. The delay in repolarisation seen with the releases in fig 7 may somehow be related to a change in this tension bearing ability. This possible association was investigated by studying the relationship between the transient depolarisation, time and the ability to redevelop tension. Experiments similar to those shown in fig 7, were performed but the muscle was adjusted to an unstretched, or "release" length that was accompanied by an easily observed active tension (*ie* greater than the slack length). This enables the time course and amplitude of the tension following the release to be readily followed. Fig 8a demonstrates the effect of two releases on tension redevelopment and action potential at two different times. The first release from about halfway up the rise of tension just after the spike of the action potentials, resulted in a fall of tension followed by significant tension redevelopment. The action potential associated with this release resumed its unstretch time course. A release occurring later, however, resulted in virtually no tension redevelopment and this was accompanied by a transient depolarisation. In fig 8b, an experiment is shown where a release near peak tension was followed immediately by restretching. This reduced redeveloped ten-

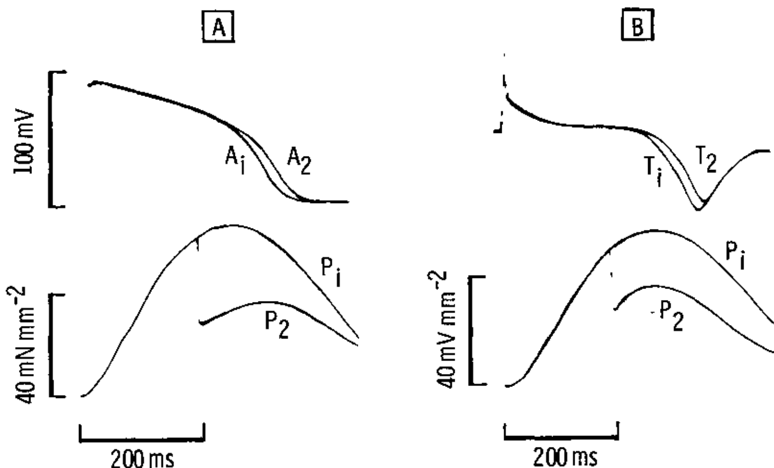

FIG 9 *Effect of release on electrical recordings from cat papillary muscle. A Following a release of the muscle, the action potential, recorded via a sucrose gap, is prolonged (A_2) (transient depolarisation) when compared with the action potential (A_i) of an undisturbed isometric contraction (P_i). The release is accompanied by a reduced tension development (P_2). B The extracellular electrogram shows a prolonged Q-T interval, (T_2) following a similar mechanical manoeuvre as in Part A, when compared with the undisturbed contraction* ie T_i.

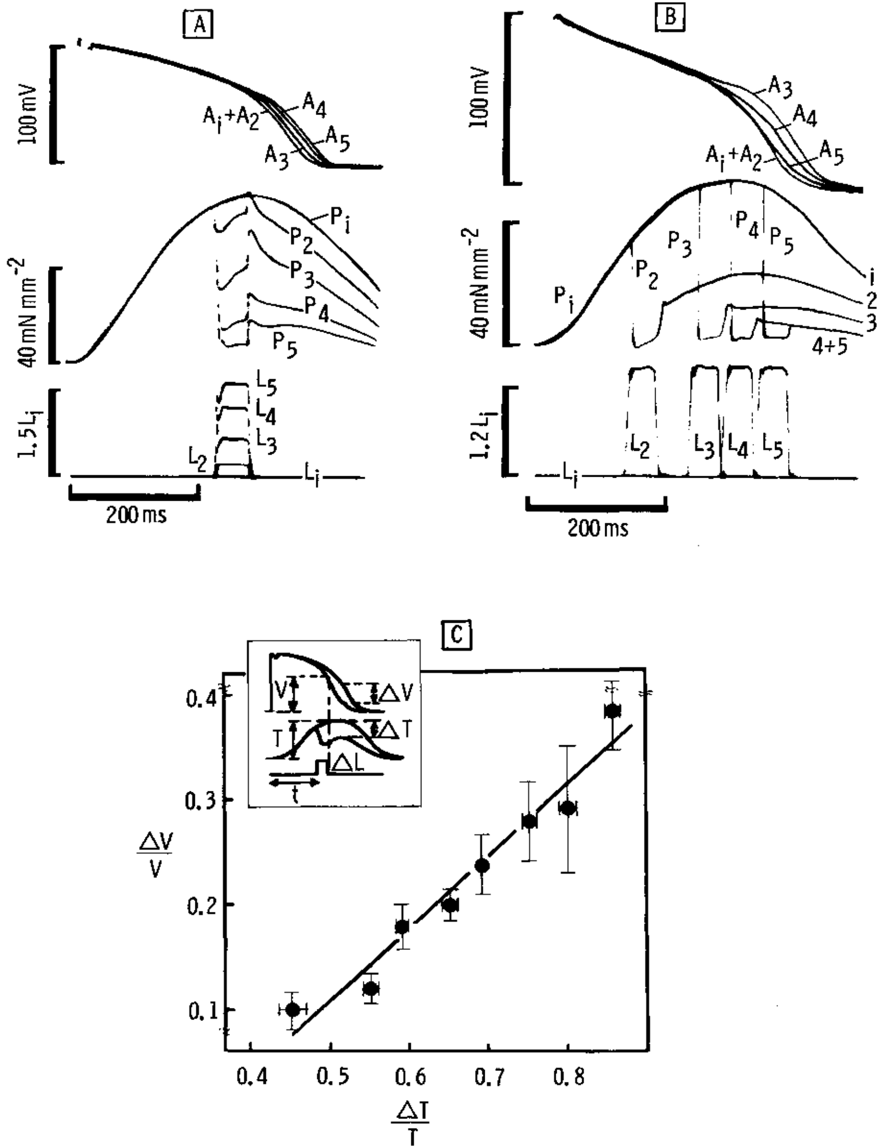

FIG 10 *Effect on action potential of transient releases of cat papillary muscle. A An isometrically contracting muscle,* P_i *contracting at* L_i *is transiently released to different lengths* L_2 - L_5 *for 50 ms each. This procedure deactivated the muscle, despite the restretch, to produce the reduced tensions,* P_2 - P_5. *Associated with the mechanical change, depolarisations are produced A3 - A5: the greater the deactivation the greater the depolarisation. B An isometrically contracting muscle (*P_i*) is transiently released to the same length for the same duration, but at different times, 2, 3, 4, and 5. Increasing deactivations,* P_2-P_5, *are produced and are associated with depolarisations* A_3-A_5. *No apparent relationship between deactivation, voltage or time emerges. C The degree of deactivation, and amplitude of depolarisation are expressed as ratios of the control measurements (see insert) and plotted in this graph. Records similar in nature were originally obtained in Professor R Kaufmanns Laboratory in collaboration with Dr R Hennekes.*

Insert. *The voltage and tension ratios, following transient releases of varying amplitude and time t are derived as follows. At the end of the intervention, control measurements T and V are made. The maximum reduction in redeveloped tension, or deactivation* $\triangle T$, *is obtained for the ratio,* $\triangle T/T$. *Maximum* $\triangle T$ *may be found just after the tension overshoot on restretch, eg* P_2 *in A, or some time later, eg* P_2 *in B. At roughly the equivalent time, the maximum amplitude of the after depolarisation* $\triangle V$ *is obtained, to produce the ratio* $\triangle V/V$.

sion[14 15] and was found to be accompanied by a transient depolarisation. The muscle was deactivated and a transient depolarisation which was not aborted by the following restretch accompanied the release. Although mechanically induced transient depolarisations were found in all the frog preparations, most of the changes in action potential were small. The relationship between transient depolarisation and deactivation was therefore further investigated using cat papillary muscle.

B. CAT PAPILLARY MUSCLE

It is known that stretching cat papillary muscle produces no change in action potential[10] whereas a quick-release does.[1] The extracellular electrogram of cat papillary muscle confirmed this. Most, if not all, the cells in the preparation could have their electrical activity altered by a quick-release which prolongs the Q-T interval (fig 9). A quick-release followed immediately by a quick-restretch was used in subsequent investigations, similar to the experiments on the frog (fig 8). By varying the amplitude of this mechanical perturbation at a given time the degree of muscle deactivation was varied and the extent to which transient depolarisations developed could be observed. The recordings in Fig 10a show that the greater the drop in redeveloped tension (deactivation) after the mechanical intervention, the greater the amplitude of the transient depolarisation. The correlation was linear ($r = 0.88$) and statistically significant ($P < 0.001$, $n = 13$). An alternative way of using release restretch cycles to vary the degree of deactivation is to alter the timing of the intervention rather than its amplitude. Examples of such recordings are shown in fig 10b. Clearly no linear relationship between deactivation and depolarisation is immediately evident. The late intervention produced a large deactivation but the amplitude of the depolarisation was small. By comparison the early intervention produced a small deactivation but a larger depolarisation. The membrane response to the mechanical perturbation appeared to be related to some factor(s) which became less effective as the muscle repolarised. This can be taken into consideration by dividing the amplitude of the depolarisation by the amplitude of the undisturbed action potential at the time of the intervention. If this ratio is plotted against the ratio, reduction in redeveloped tension: maximum undisturbed isometric tension, the relationship shown in fig 10c emerges. This composite graph includes measurements from several different preparations using microelectrodes as well as the insulation gap technique, and was derived from mechanical perturbations that varied in amplitude and/or time. The correlation coefficient (r) of the 80 points used was 0.63 with $P < 0.001$. A higher correlation coefficient was obtained when the data was used from one preparation or when one recording technique was used, *ie* the scatter is a consequence of deriving the voltage ratios from several preparations using both microelectrode and sucrose gap recordings. The latter method also introduced a series elasticity in the preparation which influenced the tension ratio.

Discussion

The results show that under certain circumstances the action potential is influenced by mechanical changes in frog ventricular strips and cat papillary muscle. The effect of the changes in length on the membrane can be unidirectional in that a release produces an effect whereas a stretch at the equivalent time does not. The release induced depolarisation can be far more consistently and prominantly produced in the papillary muscle than in the frog strip. The best correlation between the mechanical and electrical effects appears to be between the muscle deactivation, which is release induced, and the membrane depolarisation. In general the results are in keeping with the experiments of Lab[5] using the intact frog ventricle, and Kaufmann *et al*[1] using cat papillary muscle in which an isometrically contracting muscle had a short duration action potential compared with an isotonically contracting one, which had a longer duration. The different effects on the action potential of stretches and releases in the frog are also compatible with some briefly reported observations by Hennekes *et al*[10] on cat papillary muscle. It is unlikely that the mechanically induced electrical changes in the present experiments were artefactual for several reasons. First there is a general concordance of the results in different preparations. Second, there are three types of electrical recording techniques showing the electrical changes. Thirdly, stretching frog ventricular muscle during peak activity produced little or no effect whereas releasing the muscle at a similar time could have a marked effect on the action potential duration. Finally an electrical deviation caused by a particular length change of the muscle was not instantaneously reversed by restoring its original length, and a larger total tension and/or length was not necessarily associated with a larger voltage change.

In proposing a suitable explanation perhaps the most important observation is the directionality of the mechanical effect on the action potential: *ie* stretches can produce little change in the action potential whereas a release at an equivalent time usually has a dramatic effect. It has been previously proposed that the velocity of shortening of the con-

tractile elements determines the time course of repolarisation in cat papillary muscle when changing from isometric to isotonic contraction.[1] It is difficult to account for the present results in the frog heart using this explanation, as the muscle was kept isometric, although some internal shortening must occur. There are several other ways in which changes in contraction may influence membrane phenomena and these include three "passive" processes and two "active" ones (a) A mechanical distortion of the muscle could alter some of its electrical constants[17–19] to influence the recordings obtained. (b) A conformational change in internal or external membranes could change their ionic permeabilities resulting in the membrane potential moving towards the relevant equilibrium potentials. (c) There could also be some change in the extracellular space *eg* narrow clefts or T-tubules such that ionic diffusion may be affected. This in turn could alter the diffusion potentials across the sarcolemma. Potassium might be important here, for an increase in extracellular potassium is associated with an increase in potassium conductance which, if it occurred during an action potential, would accelerate repolarisation.[13] Clefts and spaces in cardiac muscle are small enough to influence potassium diffusion under certain conditions.[20–22] (d) An "active" mechanism needing brief mention is one in which action potentials and tension development compete with a common biochemical or energy mediator. Thus the deactivation following a release could be related to an altered phosphorylation concerned with contractile protein interaction or calcium uptake.[23] This could affect a compound which alters surface membrane properties thus prolonging the action potential. Cyclic-3-5 AMP is a likely contender as it is known to cause changes in both membrane potential and muscle contractility.[24 25]

Although one cannot totally exclude from the realms of possibility the foregoing "passive" hypotheses, it would be difficult to invoke them to explain the unidirectionality of the mechanical influence on the action potential. However, the clear correlation between the amplitude of transient depolarisation and the extent of the mechanically induced deactivation (fig 10) suggests a role for Ca^{2+}, the second "active" mechanism. This correlation may be a manifestation of a causal relationship mediated via the calcium ion since this ion is implicated in tension development and membrane phenomena in cardiac muscle.[26 27] The changes in the length-tension relationship in ventricular muscle could produce alterations in sarcoplasmic calcium $[Ca]_s$, which then influences membrane repolarisation. There is evidence to support the two aspects of the proposal. Experiments have demonstrated or suggested a length dependence of calcium release and/or uptake in cardiac muscle during muscle activity, with long muscles having greater activity (see Jewell[28] for a review). Moreover an increased internal calcium concentration can augment an outward current, possibly potassium[29 30] which would therefore accelerate repolarisation. Further, this outward current correlates with twitch amplitude in situations that alter the amount of calcium released.[31] The different effects on the action potential of releases and stretches could be explained by the observation in cardiac muscle that a release near peak activity causes deactivation of contraction[14–16] which is likely to be associated with a reduction in the concentration of contractile dependent calcium.[15] Allen[32] has shown in fact that when tetanised frog skeletal muscle is stretched there is little change in free internal calcium. By contrast, when the muscle is released calcium concentration is suddenly and significantly reduced. Thus release during peak activity would reduce $[Ca]_s$ (deactivation) to reduce the outward potassium current and prolong the action potential. Recently, however Allen and Kurihara[33] have provided evidence that may not be in keeping with this [Ca] hypothesis.

This calcium hypothesis, and perhaps indirectly the c-AMP mechanism, receives indirect support in possibly explaining some discrepant observations concerning mechanically dependent changes in action potential in cat papillary muscle and frog ventricular strip; since there are differences between their structure and function related to internal calcium kinetics.[3 4 20] When a relatively large change in action potential duration is produced in mammalian cardiac muscle on changing between isotonic and isometric contraction, there follow clear, directional, staircase-like changes in active tension. These "staircases" probably bear some relationship to the simultaneously observed alterations in action potential duration[1] since similar tension changes can be produced by electrically changing action potential duration.[34–36] Some "mechanical staircases" were observed in the frog but are more likely to be the result of stress relaxation than change in action potential duration, for the staircases were very small, and also, electrically induced changes in action potential duration in frog ventricle appear to affect only the immediately accompanying contraction and not the subsequent ones.[35] Moreover Brutsaert *et al*[37 38] found a far greater load dependence of relaxation in mammalian than in frog myocardium, which disappeared when the Ca^{2+} sequestering system in mammalian muscle was restricted. Incongruencies in mechanically dependent changes in action potential between the two

types of preparation are thus not surprising if they are indeed related to alterations in internal calcium kinetics. As any length-dependent calcium change in the frog preparation would be expected to be closely related to the sarcolemma, calcium could still provide a mechanism whereby an early stretch reduces the action potential duration in this situation. In this case the mechanically induced increase in $[Ca]_s$ via the membrane is unlikely to be through an augmented inward calcium current, as this would initially prolong the action potential. However, other, additional, mechanisms of inward calcium movement in frog ventricle have been proposed by Anderson *et al*[39] and could provide a mechanism for the required increase in $[Ca]_s$. While contrasting the structure/function in frog and cat myocardium as related to contraction excitation feedback, it is as well to note that there are differences in internal organisation of the two preparations apart from the SR. Stress or strain coupled changes in any electrical contribution of the T-System to the action potential could also produce a variation in the expression of contraction-excitation feedback in these preparations and could also provide a mechanism for the required changes in $[Ca]_s$.

These mechanically induced depolarisations may have clinical relevance. This is related to the fact that the mechanical behaviour of the wall of the intact left ventricle is normally inhomogeneous[40 41] and regional ischaemia exaggerates this to produce "paradoxical" wall motion classically described by Tennant and Wiggers.[42] This dyskinesia develops with time in a distinctive pattern when related to the intraventricular pressure.[43] On coronary occlusion shortening of the ischaemic segment still occurs during the ejection phase but lengthens in early relaxation, only to shorten a second time late in relaxation. Plotting pressure — length loops a figure of eight is described the first minute or so following ischaemia. Ischaemia also causes the now well-documented decrease in action potential duration described by Samson and Sher.[44] However if the contraction-excitation feedback model holds under abnormal conditions, the shortening phase during relaxation predicts an initial transient *increase* in action potential duration associated with the ischaemia (see fig 10b). In fact such transient increases have been recently observed by Douglas *et al*[45] and Lab and Woollard.[46] These biphasic changes in action potential duration (increase/decrease) may bear some relationship to the curious biphasic changes in fibrillation threshold and conduction velocity, reviewed by Elharrer and Zipes,[47] which also appear in the first 2 min of ischaemia. Finally, with progressive ischaemia the action potential duration decreases while the segment still shortens during relaxation. This means that the mechanically induced depolarisation, causing the initial prolongation of the action potential, now maintains a defined relation to the relaxation phase, while the action potential ends sooner. The mechanically induced depolarisation should thus reveal itself as a discrete transient depolarisation, and an observation in keeping with this prediction has been reported.[2] This type of transient depolarisation may be accompanied by extrasystoles[1 2] and in consequence myocardial arrhythmia.[2 47]

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Pflügers Arch (1981) 392:22–28

Pflügers Archiv
European Journal
of Physiology
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The Dependence of Cardiac Membrane Excitation and Contractile Ability on Active Muscle Shortening (Cat Papillary Muscle)*

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Abstract. 1. A quick release of an isometrically contracting cat papillary muscle results in a depression of the ability to redevelop tension (deactivation) and an increase in the duration of the accompanying action potential (prolonged depolarization). The nature of the mechanical perturbation influencing both phenomena was investigated.

2. The prolongation of the action potential depends on the amplitude of the release and the time it is applied and, provided quick release-quick restretch cycles of less than 50 ms are used, on the duration of the cycle.

3. No change in action potential duration is observed, if initial muscle length, or the velocity of shortening is altered, or if the muscle is stretched at any time during contraction.

4. Although stretches and releases both have a "deactivating" effect on the muscle the effect is more pronounced with releases. This difference in "deactivation" is related to the prolongation of the action potential in so far as it is also controlled by the time and extent of release and release-restretch cycle duration, and is independent of shortening velocity.

5. Caffeine (8 mmol/l) in the bathing solution prolongs isometric tension development whilst the duration of the action potential is relatively unchanged. Under these conditions release-restretch cycles applied at times when the membrane has apparently repolarized, produce a deactivation and an afterdepolarization which can reach threshold to elicite an action potential.

6. If the membrane is partially depolarized by increasing extracellular potassium to 20 mmol/l, release-restretch cycles still induce deactivation but no change in the action potential.

7. The results are in keeping with the hypothesis that shortening during contraction partly contributes to the deactivating effect by reducing the concentration of internal free ionic calcium. This change in $[Ca]_i$ decreases the outward potassium current to produce a prolongation of depolarization which can take the form of an increase in action potential duration or an afterdepolarization wave.

Key words: Cardiac muscle – Electro-mechanic coupling – Mechano-electric recoupling – Contractile deactivation – Active state

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* This work has been supported in part by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 30, Kardiologie

0031-6768/81/0392/0022/$01.40

Introduction

The notion that excitation contraction coupling in ventricular myocardium is not strictly unidirectional, has been demonstrated fairly recently (Kaufmann et al. 1971; Hennekes et al. 1977; Lab 1979). Thus releasing an isometrically contracting muscle results in a delayed repolarization. This prolongation of the action potential is then thought to partly determine the tension development over the next few beats.

Apart from some preliminary speculation, the precise mechanism by which the mechanical change alters the action potential has received little attention. While investigating this problem further it became necessary to extend aspects of some previous purely mechanical studies (Brady 1966; Edman and Nilsson 1971; Bozler 1972; Kaufmann et al. 1972; Reggiani et al. 1980). Accordingly we have set out first, to determine the mechanical responses of the preparation which follow various mechanical interventions, secondly, investigate the nature of the relationship between mechanical and electrical changes, and finally perform some experiments to allow some reasonable speculation about the mechanism of the feedback loop between contraction and excitation.

Materials and Methods

The method used for this investigation (isotonic lever system, force transducer, load control unit, sucrose gap chamber) has been described in detail (Hennekes et al. 1977, 1978).

For the systematic study of the electrical changes, recordings of action potentials were obtained with the sucrose gap technique. For the experiments in which mechanical changes only were measured, a simple test chamber was used because the sucrose gap introduced a compliance which interfered with the mechanical recordings. In the latter chamber, confirmatory microelectrode recordings were obtained for virtually all the sucrose gap studies.

In all experiments the initial muscle length (L_i) was adjusted to a standard preload of $0.007\,Nmm^{-2}$ and the average muscle length attained corresponded to l_{max}. Rectangular stimuli of twice threshold intensity and 0.3 ms duration were applied at 3 s intervals throughout the experiments.

The study was carried out on 49 preparations and each of the experiments in the figures is one example of more than ten similar observations in not less than five different preparations. Each result demonstrated is a consistent finding in all the experiments and there were no exceptions.

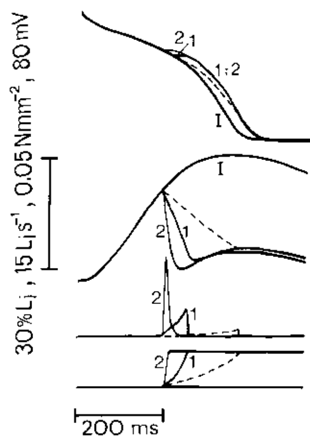

Fig. 1. Effect of different shortening velocities on action potential duration and tension redevelopment. Traces from above down: action potential, tension, shortening velocity, displacement. Although the maximum velocity of a release (200 ms after stimulus) is changed from 4 (trace 1) to 12 (trace 2) $L_i s^{-1}$, the effects on action potential prolongation and on tension redevelopment are almost identical in the two releases. For comparison isometric contractions are also superimposed (I). If, however, the release velocity is slowed down further (dotted trace), the action potential prolongation starts later

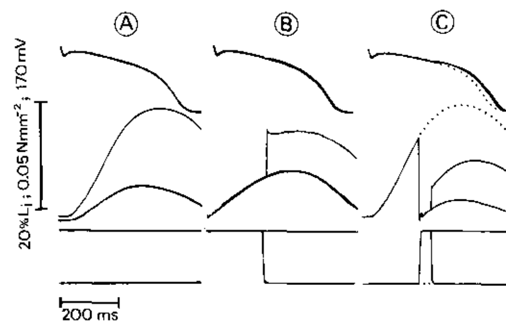

Fig. 2. Three examples in which changing the mechanical conditions of contraction of cat papillary muscle produce no detectable influence on the excitation process. Traces from above down: action potential, tension, length. *Part A* demonstrates two isometric contractions at two different muscle lengths (L_i and 90 % L_i resp.). *Part B* shows an isometric contraction at 90 % L_i and another contraction which starts at 90 % L_i and which is stretched to 100 % L_i 200 ms after stimulus. *Part C* compares two isometric contractions one following a release from 100 % to 90 % L_i 200 ms after stimulus, and another at 100 % L_i but which has been interrupted 200 ms after stimulus by a release-restretch (QR-QS) cycle 50 ms in duration. The dotted lines indicate uninterrupted isometric conditions with accompanying action potential and tension. In each of the three situations compared the excitation process remains unaltered

Results

A. Effects of Changing Mechanical Conditions on the Action Potential

1. Velocity of Shortening. Previous experiments have shown that shortening of cat papillary muscle altered the configuration of the action potential (Kaufmann et al. 1971; Hennekes et al. 1977). To test under more defined conditions the meaning of the velocity of shortening in particular we changed the maximum shortening velocity of a quick release from 4 – 12 $L_i s^{-1}$ [Fig. 1, trace (1) and (2)] keeping shortening amplitude constant (at 10 % L_i) and applying both release interventions at about the same time after stimulus (200 ms).

Both release interventions induced the same action potential prolongation. If the difference between the shortening velocities compared exceeded the range of velocities demonstrated here (4 – 12 $L_i s^{-1}$, i.e. using values less than 4 $L_i s^{-1}$), there is a difference in the action potential configuration (dotted trace in Fig. 1). The slower the release velocity the later the action potential prolongation starts. In each case the action potentials, measured at 90 % repolarization, are prolonged to the same duration as soon as the final length is attained.

2. Initial Length (L_i) Changes and Stretches. In contrast to the effect of a release it is of interest to see if stretching the muscle results in an equivalent abbreviation. First, changing the initial muscle length of an isometrically beating cat papillary muscle significantly varies the amount of tension development but the action potential configuration remains unaltered (Fig. 2A). Secondly, stretching the muscle at any time during contraction also does not alter the action potential although it is accompanied by a significant length and tension change (Fig. 2B and Hennekes et al. 1977; Fig. 2). Even (re)stretching the muscle after a series of preceding releases [i.e. switching from quick release (QR) to quick release-restretch (QR-QS) cycles] has no additional effect on the action potential configuration (Fig. 2C). The action potentials are similarly prolonged by the release as well by the release-restretch as compared with an action potential under purely isometric conditions (indicated by dotted lines).

3. Effect of the Duration of Quick Release-Quick Stretch (QR-QS) Cycles on Action Potential and Tension. In order first to test the degree to which the duration of a QR-QS cycle influences the prolongation of the action potential, a series of experiments was performed in which the duration of a QR-QS cycle, 10 % L_i in amplitude, was varied between 10 and 500 ms (Fig. 3A). The action potential and tension development are compared with those obtained under isometric conditions. A graph of these experiments is shown in Fig. 3B, in which the change in action potential duration measured at 50 % repolarization level and expressed as a percentage of the "isometric" action potential duration, is plotted against the duration of the QR-QS intervention. Brief QR-QS cycles are less effective in prolonging the action potential duration than longer ones. The maximum effect is reached with transient releases of more than 50 ms duration. (It is of interest to note that a similar behaviour applies for QS-QR cycles, i.e. the longer the QS-QR duration the more pronounced is the accompanying release-related AP prolongation until a maximum effect is reached with interventions of more than 70 ms in duration.) The finding implies that the action potential is similarly prolonged whether it is induced by a sole quick release or by a quick release-restretch cycle, provided the latter is more than 50 ms in duration (see also Fig. 2C). A 50 ms QR-QS is the intervention used in the following experiments.

4. Effect of Time, the Stimulus-Intervention Interval, on the Action Potential. In Fig. 4 it is shown that the degree of action potential prolongation depends on when the release occurs: QR-QS cycles of 50 ms duration were applied during isometric contractions at various times after the stimulus. The

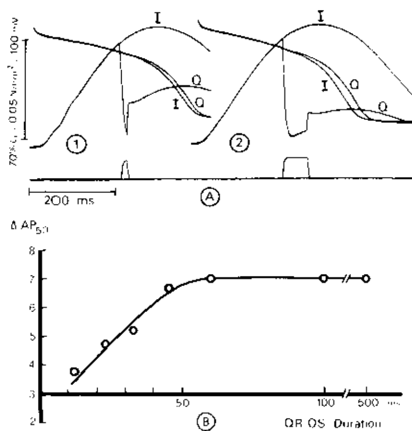

Fig. 3. The effect of varying the QR-QS cycle duration on tension and action potential. *Part A:* two QR-QS cycles 200 ms after stimulus are applied during isometric contraction [10 ms duration in (1) and 70 ms duration in (2)]. The duration of the intervention determines how much the action potential is prolonged and tension redevelopment is diminished after restretch. Traces from above: action potential, tension, displacement. The records labelled with subscript Q and I are related to the QR-QS cycle and pure isometric contraction respectively. *Part B:* Plot of QR-QS duration (intervention at 200 ms after stimulus) against the accompanying action potential prolongation (expressed as a percentage of the undisturbed isometric action potential measured at 50% repolarization level). Note the graph flattens out at a QR-QS duration of just under 50 ms

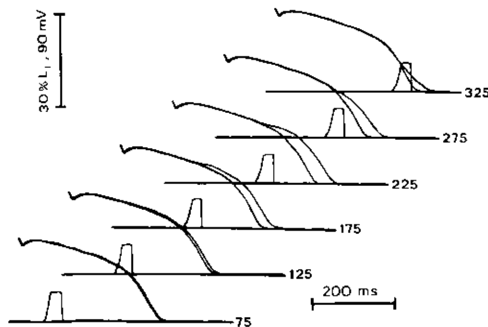

Fig. 4. Effect of quick release – quick stretch (QR-QS) cycles at different times after stimulus on action potential duration (stimulus intervention interval). During isometric contraction (shortest action potential in each pair of superimposed action potentials) QR-QS cycles of 50 ms in duration are applied at stimulus intervention intervals of 75, 125, 175, 225, 275 and 325 ms. These interventions induce different degrees of action potential prolongation. The optimum is reached with releases applied at about 225 ms after stimulus

concomitant action potentials were recorded and superimposed on the reference "isometric" action potential. Clearly, releases occurring prior to or early during contraction (until about 75 ms after stimulus) do not change the action potential. However, with releases applied 100 ms after the stimulus or later, the action potential is progressively prolonged. A maximum is reached at about mid-plateau (225 ms after stimulus) after which the prolongation declines.

5. Effect of Changing the Amplitude of Shortening on the Action Potential. The experiment in Fig. 5A demonstrates the effects on the action potential of varying the amplitude of QR-QS cycles, 50 ms in duration and applied 225 ms after

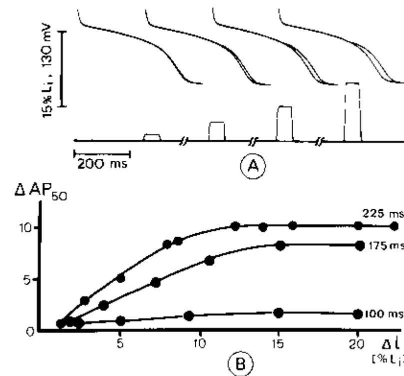

Fig. 5. The effect of varying the amplitude of QR-QS cycles of constant duration on action potential prolongation. *Part A:* QR-QS cycles (lower trace) 225 ms after stimulus are varied in amplitude between 2 and 11% L_i. The accompanying action potentials which are prolonged according to the extent of the release, are superimposed on the undisturbed "isometric" action potential. *Part B:* graph of variation of the extent of shortening (Δl) plotted against the prolongation of action potential derived as in Fig. 3. Three curves are presented using QR-QS cycles 100, 175 and 225 ms after stimulus. The curves form functions which flatten out at shortening amplitudes between 10 and 15% L_i

stimulus. The action potential was progressively prolonged with increases in amplitude of the release until a maximum was reached with releases of more than 15% L_i (Fig. 5B). In the graph of this figure the QR-QS amplitude is varied at three different times during contraction. The resulting prolongation of the action potential duration are plotted against the amplitude of the intervention. Each curve represents a function which reaches its plateau at about the same shortening amplitude (i.e. between 10–15% L_i).

B. Differences in Mechanical Deactivation Produced by Stretches and Releases

It appears so far that the mechanism underlying mechanically induced changes in action potential is unidirectional in that lengthening (stretch) at any time during contraction does not change the action potential configuration but releases do. After a release during contraction, the muscle does not reach the active tension it should develop according to its new position on the length active tension curve (Brady 1965, 1966, 1968; Kaufmann et al. 1972): an "uncoupling" effect (Brady 1968) or deactivation. A consistent observation during these experiments was that whenever a mechanical intervention induced a prolongation of the action potential this deactivation of contraction accompanied the intervention. Some mechanical changes, however, do not produce an action potential change yet they can cause deactivation: a stretch for example (Brady 1965, 1966, 1968; Kaufmann et al. 1972). So one could suggest that perhaps there are differences between the deactivation produced by the mechanical interventions which change the action potential duration and those that do not. The following experiments try to test this possibility by the example of a muscle as a whole.

1. Stimulus Intervention Interval. In Fig. 6 experiments are shown in which the muscle first contracted at 90% L_i under steady state conditions (upper part of panels A and B). It was

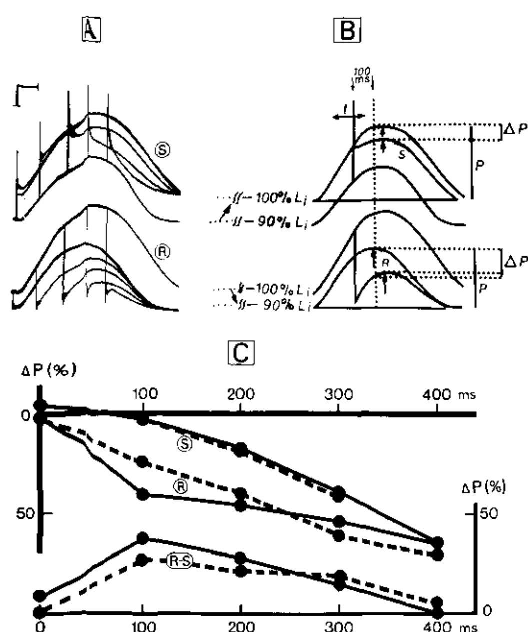

Fig. 6. The effect of stretches and releases on tension redevelopment as depending on the stimulus intervention interval. *Panel A and B* demonstrate recordings and a schematic drawing, labelled to clarify the procedure (for detailed information see text). *Panel C:* the drop in tension (ΔP) induced by either intervention (R and S) is expressed as a percentage of the tension (P) developed by the muscle in the undisturbed contraction [$\Delta P/P \times 100 \triangleq \Delta P(\%)$] and is plotted against the time after stimulus, the intervention took place. The curve (R-S) represents the difference between R and S thus representing the augmented deactivation brought about by releases as opposed to stretches. The maximal difference in deactivation induced by the 10% L_i displacements ranges between 25 and 35% (no sucrose gap in this preparation). *Solid lines:* ΔP measured 100 ms after the intervention (see B). *Dotted lines:* ΔP measured at peak isometric and peak post-intervention tension (as indicated by arrows in the scheme B)

then stretched at different times (t), during different beats, to 100% L_i. After each stretch the muscle was allowed to regain a steady state at 90% L_i. This avoids tension transients (Hennekes et al. 1977) which make mechanical measurements unreliable. Each stretched contraction was compared with a control contraction at 100% L_i, the length to which the muscle is stretched 300 ms before stimulation. Stretches applied at the time of stimulation or at various intervals until about 100 ms after stimulation, result in tension curves that superimpose or even slightly exceed the "control curve" (upper part Fig. 6A). From about 200 ms after stimulation stretches become progressively effective in deactivating the muscle.

The reverse operation is shown in the lower parts of panels 6A and B. A muscle contracting under steady state conditions at 100% L_i is released to 90% L_i at the time of stimulation and at different times thereafter. Control reference in this case is an undisturbed isometric contraction when initial muscle length is changed from 100% to 90% L_i 300 ms before stimulus. A contraction released at the time of stimulation superimposes on the control contraction. But with releases applied only a few milliseconds later the deactivating effect of the release appears.

In the graph of Fig. 6C an attempt is made to quantify the efficacy of both, stretches and releases as related to the stimulus-intervention interval, and to calculate their difference. To obtain a simple quantitative measure of the deactivating efficacy of either intervention we measured i) the peak force redeveloped after either intervention and compared it with the peak force developed in the undisturbed contraction at the same length (dashed lines in the graph) and ii) the redevelopment in tension at an arbitrarily chosen point of time of 100 ms after the intervention and compared it with the corresponding control contraction as defined above (solid lines in the graph). ΔP is expressed as a percentage of the tension developed in the control contraction at peak force or at the corresponding time resp. Clearly at any given time after stimulus the release operation is more effective in producing deactivation than is the same amount of stretch. Graph (S) relates to stretch interventions and (R) to release interventions. The difference in the deactivating effect of stretches and releases may be calculated and is indicated by (R-S). The difference is most prominent with interventions applied between 100 and 200 ms after stimulus. Although the deactivating efficacy of the release may be overestimated as compared to that of a stretch by the two rather arbitrary methods of evaluation and although a stretch may be a rather complex molecular mechanism as compared to a release (Housmans and Brutsaert 1976; Edman 1980a, b), it is worth to note, that the "difference-curve" (R-S) starts at zero, reaches its maximum with interventions applied at already 100 ms after stimulus and declines to zero again at times of peak isometric tension. A similar relationship applies to the action potential prolongation as depending on the stimulus-intervention interval (Fig. 4).

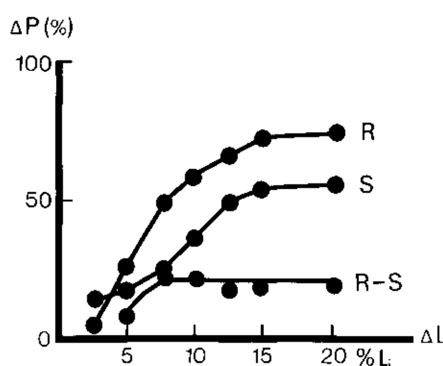

Fig. 7. The effect of stretches and releases on deactivation as depending on the amplitude of displacement. Releases and stretches of different amplitudes are applied 250 ms after stimulus and are plotted against the reduction in tension redevelopment (= deactivation) they induce 100 ms later. Note that the difference between the curves becomes constant with displacements greater than about 8% L_i. A similar relationship is obtained by relating post-intervention peak force to control peak force like in Fig. 6 (no sucrose gap in this preparation)

2. Amplitude of Intervention. In the graph of Fig. 7, data are collected on experiments in which the amplitude of releases and stretches applied at a fixed time of 250 ms after stimulus is varied between 2.5% and 20% L_i. This stimulus intervention interval is chosen because at 250 ms after stimulus not only the deactivation efficacy of the stretch as well as release intervention is clearly seen, but also their difference is most prominent (cf. Fig. 6C). The experimental procedure and data evaluation is similar to that described in Fig. 6. For the "release curve" the muscle is released, in each case, after a series of isometric steady state contractions at 100% L_i to

various new lengths. As in Fig. 6, the tension trace of the released contraction is compared with a control curve which is an undisturbed isometric contraction released 300 ms before stimulus to the same new lengths. Conversely, for the "stretch curve" in the figure the muscle is stretched from a steady state at various initial lengths to the final length, 100 % L_i. Post-stretch tension is compared with the control curve which is an isometric contraction at 100 % L_i. The muscle is stretched to this length, 300 ms before stimulus during steady state conditions, from the various initial muscle lengths. Thus stretches and releases may be regarded as reverse operations covering the same range of absolute muscle lengths (e.g. release from 100 % to 90 % L_i and stretch from 90 % to 100 % L_i: $\Delta l = 10\%\ L_i$). From the graph in Fig. 7 it is clear that the efficacy of both types of intervention (releases, curve R; stretches, curve S) on the deactivation depends on the extent of the displacement. However, a release always produces a greater deactivation than does a stretch, provided the amplitudes of interventions compared exceed 4 % Li_i. Also the calculated difference between both curves [curve (R-S) in this figure] demonstrates the greater deactivation induced by releases. This curve also bears some resemblance to the curves in Fig. 5, relating the action potential prolongation to the extent of shortening.

It is a general finding that at least in the length range between 115 and 80 % L_i, a release is always more effective in deactivating the remainder of the contraction than a stretch of comparable amplitude. It turns out consistently that if applying a stretch or release of the same deactivating efficacy (i.e. of different amplitude), only the release is able to influence the AP.

C. Changing the "Active State": The Effect of Caffeine on the Contraction-Excitation Feedback Phenomenon

If the time course of activation might be a determinant of the contraction excitation recoupling loop a possible approach in testing this hypothesis is to dissociate – for instance by caffeine – the time course of activation and that of the action potential the two of which roughly coincide in normal ventricular myocardium. Caffeine is known to increase and prolong the activation of cardiac muscle (Blinks et al. 1972) while leaving the action potential duration relatively unaffected.

1. The Effect of Releases 200 ms after Stimulus. As shown in the preceding sections, a QR-QS cycle applied 200 ms after stimulus under normal conditions is highly effective in both prolonging the action potential and deactivating the muscle (Fig. 8A). After the addition of caffeine, however, the same intervention cannot produce an action potential alteration (Fig. 8B) and, at the same times, is much less effective in causing mechanical deactivation.

With caffeine the onset of contraction is slow but tension development is augmented in amplitude and duration (Blinks et al. 1972). Therefore, a QR-QS cycle occurring 200 ms after stimulus, if related to the time course of this particular active state, may be considered as a comparatively early intervention, and, it is not unexpected that there is less deactivation in the caffeine contraction as compared with the same intervention in a normal contraction.

2. Stimulus Intervention Interval. In Fig. 9 recordings are shown from a caffeine treated muscle in which QR-QS cycles are applied at progressively later times after stimulus.

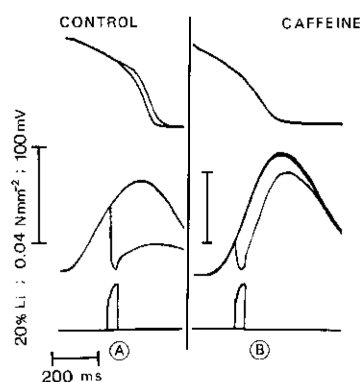

Fig. 8. The effect of 8 mmol/l caffeine on the release induced prolongation of action potential. *Part A:* control conditions. Changing from a purely isometric contraction to QR-QS conditions (10 % L_i, 200 ms after stimulus) results in an immediate prolongation of the action potential duration. *Part B:* The same mechanical procedure when applied with caffeine in the solution, does not alter the action potential duration. Note that under caffeine the isometric contraction is prolonged in relation to the action potential duration. The latter is nearly the same as the control action potential. The QR-QS intervention produces little deactivation. (The late onset of the "Caffeine-contraction" is overestimated in the figure due to the series elasticity of the sucrose gap region and the comparably high force developed by the muscle thus giving rise to some unregistrable shortening within the active part of the muscle)

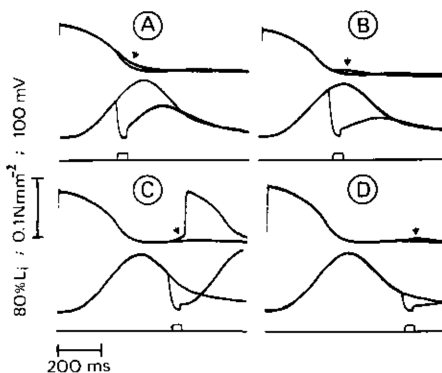

Fig. 9. The effect of 8 mmol/l caffeine on the changes in action potential and tension produced by varying the stimulus intervention interval. Traces from above down in each panel: action potential, tension, displacement. QR-QS cycles (10 % L_i) applied 200 ms after stimulus and later, prolong the repolarization process (e.g. *panel A*, 280 ms after stimulus) or produce a depolarization wave (*panel B and D*, 400 and 700 ms after stimulus). In *panel C*, 525 ms after stimulus, a depolarization is seen which reaches threshold to elicit an extra action potential

If we take into account the prolonged time course of the "caffeine contraction", the deactivation potency of the QR-QS cycles increases with time (the stimulus-intervention interval) in a similar fashion as compared to control conditions. But the release induced alterations of the membrane potential now reappear and have a similarly extended time course as compared to the deactivation mentioned above. However, we have the interesting situation that mechanical and electrical "recoupling efficacy" reach a maximum when repolarization is complete: the action potential has terminated and the membrane potential has attained almost its resting value. In this situation the QR-QS cycles induce late afterdepolarizations lasting about 200 ms, whereas under

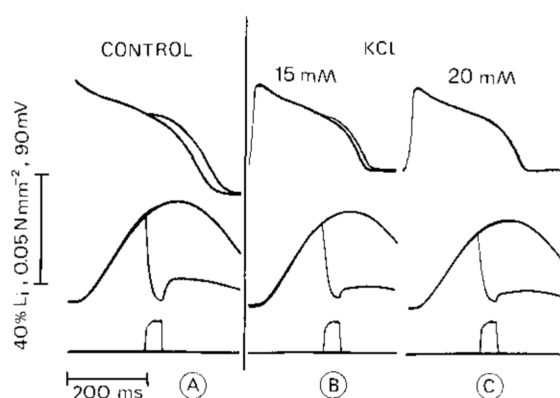

Fig. 10. The effect of increasing the potassium concentration in the perfusing solution on the contraction-excitation recoupling loop. Traces in each panel from above down: action potential, tension, displacement. *Part A:* shows the control experiment, in which a QR-QS intervention (10% L_i, 200 ms after stimulus) induces a prolongation of action potential duration. *Part B:* after increasing extracellular [KCl] to 15 mmol/l, the action potential prolongation produced by the same QR-QS cycle as in Part A is reduced. *Part C:* the prolongation is abolished when $[KCl]_0$ is increased to 20 mmol/l. The tension redevelopment in all three cases is similarly reduced

control conditions 500 ms after stimulus there is no detectable effect of releases on the membrane. The magnitude of these depolarizations vary with the time after stimulus when the QR-QS cycle is applied. Under the particular conditions described in Fig. 9 (8 mmol/l caffeine), the optimal amplitude of the depolarization induced by 10% L_i QR-QS cycles, is attained with releases applied 500–600 ms after stimulus. In this case, the afterdepolarization reaches threshold to elicit an extra action potential (Fig. 9C).

3. Amplitude of Displacement. Increasing the extent of the release increases the amplitude and the rate of the induced depolarizations. The threshold for the new action potential is reached sooner. The latency period i.e. the time between the beginning of the release and the first detectable change in membrane potential is less than 10 ms in all the experiments.

D. Changing the Action Potential: Increasing Extracellular Potassium Concentration

The two ions mainly contributing to the transmembrane currents at the time the mechanical interventions are most effective are Ca^{2+} (Beeler and Reuter 1970, 1977) and K^+ (cf Noble 1975). The mechanically induced prolongation of the action potential could thus either be due to an increase in inward calcium current or to a decrease in outward potassium current. Tritthart et al. (1976) showed that partial depolarization of the membrane with potassium produced an action potential with characteristics suggesting it was predominantly calcium mediated. If the action potential prolongation induced by a QR-QS cycle is due to an augmented inward calcium current then the prolongation should appear when applying a QR-QS cycle during this type of action potential. We investigated the effect of increasing extracellular potassium on release-induced changes in action potential (Fig. 10A). Under normal Tyrode solution ([KCl] = 2.68 mmol/l) a QR-QS cycle 200 ms after stimulus produces a prolongation of action potential of about 11% of the duration under isometric conditions. Increasing extracellular potassium concentration to 15 mmol/l reduces resting potential, action potential upstroke velocity and overshoot (Tritthart et al. 1976).

In the present experiments the change in [KCl] also reduced the ability of the QR-QS cycle to prolong the action potential to about a third of that under control conditions (Fig. 10B). At a [KCl] of 20 mmol/l, no change in action potential configuration is elicited by the QR-QS cycle (Fig. 10C), regardless of the extent of the release or the time after stimulus it occurs. Yet under these conditions, tension development and the mechanical response to the intervention (= deactivating effect) is virtually unaffected compared with control.

Discussion

The results of this study show that in cat papillary muscle shortening during the later phases of contraction influences both the membrane excitation process and the redevelopment of tension. They also show that there is a quantitative but most probably also a qualitative difference in the mechanical deactivation produced by either a stretch or a release. It appears that mechanical deactivation contains a unidirectional component such that at least in the muscle as a whole releases are more effective in producing deactivation than stretches of equal amplitude. The "surplus in deactivation" with releases and the effects on the membrane excitation, manifest as a prolongation of the action potential (a depolarizing effect in essence) are strikingly parallel. They both appear to depend on the same mechanical parameter (shortening during a certain phase of the contractile cycle). The two phenomena may either condition each other or may be two independent expressions of a common basic event. For reasons discussed below, we think the second possibility is the more likely one.

Many of the changes in contraction presented here are in keeping with those of Edman 1971, 1975, 1976 observed in skeletal muscle. He suggested that "the depressant effect is based on a structural change in the myofilament system". However, with cardiac muscle we cannot exclude the possibility, as Edman does for skeletal muscle, that changes in activator calcium concentration might be involved. A change in the internal free calcium concentration may be a reasonable way of explaining why the mechanical events are so closely related to the electrical events.

It has to be considered whether the effects on action potential configuration observed might simply be the result of alterations in cellular architecture, accompanied by changes in membrane properties. However, besides the objections raised already earlier (Kaufmann et al. 1971; Lab 1978) this hypothesis appears unlikely because the mechanism underlying the mechanically induced action potential alteration is unidirectional[1] (Fig. 2). This argument also applies if the mechanisms were related to changes in architecture leading to localized potassium accumulation (cf Kline and Morad 1976; Attwell et al. 1977). In this case a stretch should induce a repolarizing effect: an abbreviation of the action potential. It appears further, that a prerequisite for this mechanism to operate is the muscle to be in the active state (see Caffeine experiments).

The experiment shown in Fig. 10 suggests that a potassium outward current rather than an inward Ca^{2+} current

1 That means it cannot be elicited or counteracted by stretches

during repolarization may somehow be involved. There is, in fact, evidence that an outward current, probably carried by potassium, is influenced by internal free calcium (Isenberg 1975; Bassingthwaighte et al. 1976; Siegelbaum et al. 1977). Augmenting internal free calcium results in an accelerated repolarization of the action potential, or even in a hyperpolarization. Reducing internal calcium reduces this outward K^+ current and will prolong the action potential. Since a delayed repolarization is observed after a release, one may ask whether shortening might somehow reduce the amount of internal free calcium. Indeed, a preliminary observation in keeping with this possibility has been made by Allen (1978).

Also part of an augmented deactivating effect of releases, as compared with stretches (Fig. 7), could be interpreted as a consequence of such a diminution of activator calcium.

From the above, especially from the exclusively shortening dependent effects on the action potential, one may deduce the hypothesis that active shortening in heart muscle induces transient $[Ca^{2+}]_i$ changes at least during a certain period of the contractile cycle. These changes in turn may cause a reduction in outward potassium current and thus by sustaining the depolarization will prolong the action potential. This concept, of course, is highly tentative but could not only explain the parallel effects of releases on both, the action potential duration and the deactivation, but also the delayed afterdepolarizations which occur with late releases in Caffeine treated muscles.

The phenomena appear not to be related to length dependent changes in calcium release or affinity (Allen et al. 1974; Fabiato and Fabiato 1975; Gordon and Ridgway 1976; Jewell 1977) because the process feeding back from contraction to excitation is not dependent on the initial and final muscle length (see e.g. QR-QS cycle and stretches, Fig. 2). Therefore we suggest that shortening itself might be a mechanism which sequestrates free internal Ca^{2+}. This might also explain the differences in deactivation when comparing the effects of stretches and releases on tension redevelopment.

Under our experimental circumstances the mechanism by which stretches and releases produce deactivation could perhaps be separated into two components: a structural change in the myofilament interaction as depending on the displacement in both directions including non-uniform movement and a reduction of activator calcium brought about by releases (shortening) only. Releases amongst other would be more effective than stretches in inhibiting reactivation, or causing deactivation, because both mechanisms are involved in the former manoeuvre.

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Received December 29, 1979/Accepted July 28, 1981

5

Stress-strain-related Depolarisation in the Myocardium and Arrhythmogenesis in early Ischaemia

Max J. Lab

5.1 INTRODUCTION

There is no entirely satisfactory explanation for the premature excitation that initiates ventricular fibrillation in the very early stages of myocardial ischaemia. The electrophysiological disturbances which accompany the ischaemia, such as differential changes in conduction velocity, re-entry and enhanced automaticity, have been invoked as causing ventricular fibrillation (chapters 3 and 4 in this book). However, correlation does not imply causality. There are severe mechanical disturbances during regional ischaemia and extrasystoles can accompany physical stresses and strains in the normal myocardium. A critical consideration of mechanical causes for extrasystoles during ischaemia is therefore appropriate. This chapter briefly discusses the mechanical generation of threshold excitations and considers the possibility that mechanical changes may induce ectopic impulses in early ischaemia.

5.2 MECHANICALLY INDUCED DEPOLARISATION IN THE NORMAL MYOCARDIUM

Several studies show that three types of mechanical intervention can produce depolarisation in myocardium.

Sustained passive stretch

Although Ling and Gerard in 1949 first successfully used micropipettes for recording intracellular potentials and found no changes in resting membrane potential when they stretched isolated skeletal muscle, Ishiko (1956, 1958) produced small but

clear depolarisations with this manoeuvre. Further, Bülbring *et al.* (1956) stretched calcium-deficient skeletal muscle and caused spontaneous activity. In heart muscle mechanically induced changes are more easily demonstrated. Stretch of several isolated preparations of cardiac muscle produce depolarisation, steeper diastolic depolarisation associated with spontaneous activity, and /or threshold depolarisation (Dudel and Trautwein, 1954; Penefsky and Hoffman, 1963; Deck, 1964; Kaufmann and Theophile, 1967; Lab, 1974). Figure 5.1 shows examples of sus-

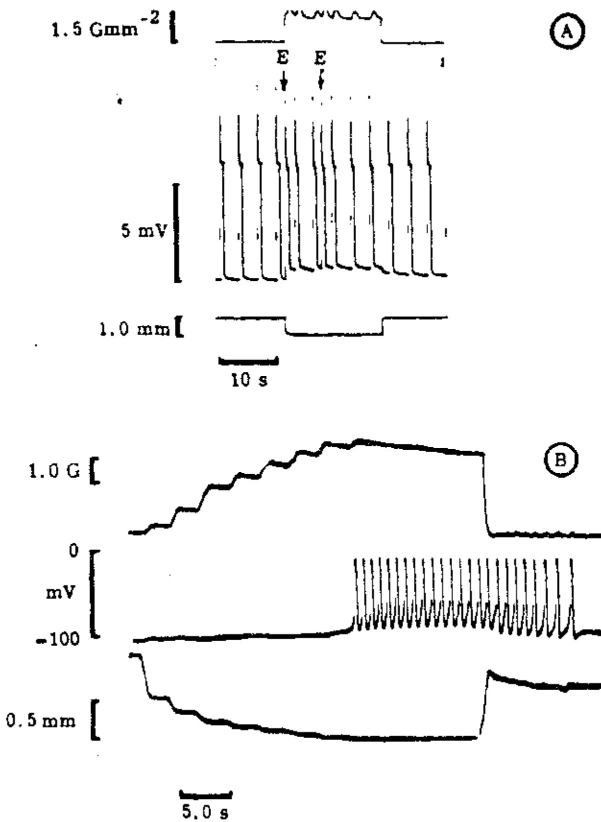

Figure 5.1 Effects of sustained stretch on action potentials from isolated ventricular myocardium. Traces from above down: tension (increase is upward deflection), action potential and length (increase in downward deflection). A, Records from unstretched frog ventricular strip which is regularly stimulated. A stretch causes depolarisation which is accompanied by an extrasystole (E). The sustained increase in length produces a sustained depolarisation and commensurate reduction in spike height. Action potentials recorded via insulation gap technique (From Lab (1974).) B, Preparation of quiescent cat papillary muscle. Stepwise increases in length produce a small but perceptible depolarisation in the resting membrane potential until pronounced diastolic depolarisations develop to produce spontaneous activity. Action potentials recorded with microelectrodes (After Kaufmann and Theophile (1967).)

tained stretch producing depolarisation in ventricular muscle which can then cause threshold excitation. Increases in muscle length of intact ventricles have also produced analogous depolarisations (Lab, 1969, 1978a,b; Boland and Troquet, 1980), sometimes associated with extrasystoles (Lab, 1969, 1978a,b; Covell *et al.*, 1981).

Rapid transient stretch

Rapid transient stretch of frog ventricular strips after the repolarisation phase can induce transient depolarisations associated with extrasystoles (Lab, 1974). These have also been seen in the intact frog ventricle following sudden increases in ventricular volume (Lab, 1978a). Figure 5.2A shows a depolarisation with stretch, which produces an extrasystole when the stretch is delayed beyond the mechanical refractory period. Kluge and Vincenci (1971) have mechanically provoked extrasystoles in Langendorff-perfused rabbit hearts and have also shown refractory periods to mechanical interventions, as did a previous study by Brooks *et al.* (1964). The extrasystoles never produced fibrillation but could do so easily in the presence of acetylstrophanthidin, which also reduced the 'mechanical' refrac-

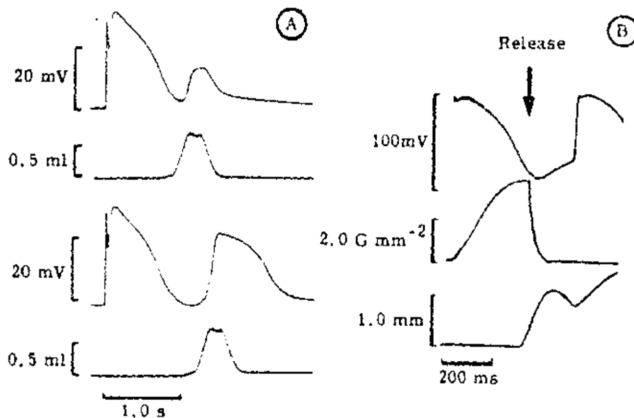

Figure 5.2 Threshold depolarisations produced by different, rapid, mechanical manoeuvres in two preparations of ventricular myocardium. A, An isolated intact frog ventricle is rapidly and transiently injected (stretched) near the end of the repolarisation phase of the action potential (the top trace in each panel). In the upper panel intraventricular injection, given just before repolarisation is complete, produces discrete depolarising potential. An injection after completion of repolarisation (lower panel) produces a depolarisation associated with threshold excitation. Action potentials recorded with suction electrodes (After Lab (1978a).) B, Records from isolated cat papillary muscle. The muscle begins its contraction isometrically, that is, develops force (middle trace) with no length change (lowest trace). Near the end of the repolarisation phase of the action potential (top trace) the muscle is 'released' and allowed to shorten. This manoeuvre causes the membrane to depolarise to reach threshold for a propagated action potential. Action potentials recorded with micro-electrodes (After Kaufmann *et al.* (1971).)

tory period. In patients, mechanical perturbations, such as a blow to the chest (Hurst and Logue, 1966), and cardiac catheterisation can also induce threshold depolarisations. Moreover Zoll *et al.* (1976) were able systematically and mechanically to stimulate the heart externally and non-invasively with a 'mechanical thumper'. This device has been used in patients with cardiac arrest.

Mechanical changes during the action potential

In contrast to the foregoing, stretch during the action potential has no effect on the membrane potential of cat papillary muscle. However, the opposite mechanical perturbation, a sudden shortening or 'release' of isometrically contracting muscle at the equivalent time, does produce a depolarising potential. If the release is near the end of the repolarising phase of the action potential, this change in potential resembles a discrete transient depolarisation or early after-depolarisation (Cranefield, 1977). Figure 5.2B is an example of such a transient depolarisation which was accompanied by a propagated extrasystole. In Kluge and Vincenci's study the electrical impulse followed the mechanical impulse by about 40 ms, a latency which they were unable to explain. A release-induced depolarisation is just discernible within 5–10 ms and reaches threshold within 40 ms (Kaufmann *et al.*, 1970, 1971). The latency measurements would be in keeping with the possibility that the myocardium was being released following the compression of the heart with the 'thump'. However, one cannot exclude the mechano-electric link being via the stretch described in the two preceding subsections.

5.3 MECHANICALLY INDUCED DEPOLARISATION IN ISCHAEMIC MUSCLE

The factors predisposing to extrasystoles in ischaemia are thought to be related to altered membrane characteristics which are directly due to the consequences of the reduced blood flow (Hauswirth and Singh, 1978; Cranefield and Wit, 1979; and Rosen, 1981). However, premature excitations may be observed purely with sudden changes in the mechanical conditions of contraction of heart muscle. Tennant and Wiggers (1935) were the first to describe inhomogenous wall motion with coronary occlusion. In this situation ischaemic ventricular muscle is stretched during systole and shortens (that is, is released) during diastole. There are thus analogous mechanical situations to those described in section 5.2, for example the stretch or late release of an isometrically contracting papillary muscle. If this is the case then the mechanical changes which affect still viable muscle during ischaemia would cause depolarisations. Janse *et al.* (1980) have already suggested a role for diastolic depolarisation of viable myocardium, rather than re-entry in ischaemic muscle, in generating the first ectopic beat during early ischaemia. The questions are whether conditions predisposing to the 'contraction-excitation feedback' pertain in ischaemic myocardium and whether this feedback is a hitherto unsuspected cause of early ectopic beats in myocardial ischaemia. Figure 5.3 shows

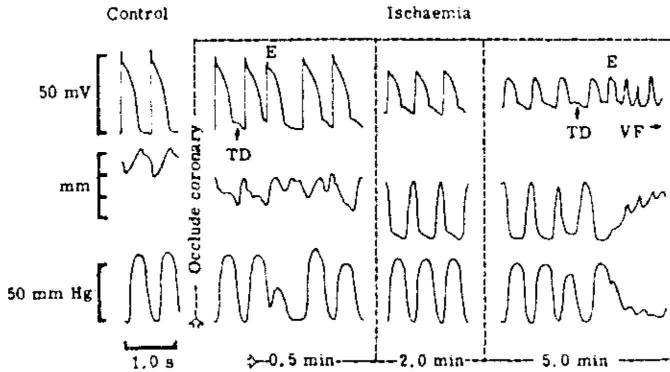

Figure 5.3 Effect of ischaemia on action potential (upper trace), ventricular segment motion (middle trace) and intraventricular pressure (lower trace). In the control panel segment shortening (upward movement of trace) occurs during the action potential, that is, mainly during systole; the configuration of the control action potential was stable over several minutes. After coronary occlusion the shortening occurs predominantly when repolarisation is over (that is, during diastole) and a transient depolarisation (TD) appears, which sometimes seems to reach threshold for an extrasystole (E). The transient depolarisation of the ectopic action potential is not pronounced but the length changes are also small. Continued ischaemia progressively reduces the action potential amplitude and duration while the segment shows holosystolic bulding. The final panel demonstrates an extrasystole precipitating ventricular fibrillation (VF). See text for further discussion. Monophasic action potentials and segment length records as in Lab and Woollard (1978).

monophasic action potentials and epicardial segment motion before and soon after occlusion of a small coronary artery. After occlusion the area becomes dyskinetic and demonstrates paradoxical wall motion; it lengthens during ventricular contraction and actually shortens when the ventricle relaxes. These movements are associated with a transient depolarisation which can reach the threshold for a new propagated action potential. If ventricular fibrillation does not ensue, the multiple extrasystoles disappear within an hour of the onset of ischaemia. It is of interest in this respect that Pirzada *et al.* (1976) showed that ventricular ischaemia produced systolic stretching at the affected segments which reached a maximum after 15 min, remained high during the first hour and thereafter declined towards normal. The time course of these compliance changes roughly parallels the frequency distribution of ectopic beats. The complex inhomogenous intramural stresses and strains hinder definition of the relationship between mechanical changes and depolarisation in the intact beating ventricle *in situ*. Further, the re-entry mechanism could explain the depolarisation as being the result of electrotonic spread of a delayed action potential in an adjacent area of the heart, the retardation a consequence of the conduction defect because of the ischaemia. In fact, similar transient depolarisations to those reported here have been observed using micro-electrodes, and re-entry mechanisms were invoked to explain these (Czarnecka *et al.*, 1973;

Downar *et al.*, 1977). The nature of suction electrode recording from a large number of cells increases the difficulty in interpretation. One could strengthen the credibility of a stress-strain cause of arrhythmia by demonstrating mechanically induced transient depolarisations and accompanying premature beats in intact hearts while minimising the possibility of re-entry circuits due to ischaemia. In fact this demonstration should be a prerequisite of 'contraction-excitation feedback' as a mechanism causing extrasystoles during ischaemia. The specific requirement still would be 'abnormal' segment motion associated with extrasystoles. This situation appears during ventricular outflow constriction in the frog ventricle (Lab, 1978a) and analogous experiments in the intact dog produce comparable findings. Figure 5.4 (Covell *et al.*, 1981) demonstrates that pulmonary artery occlusion

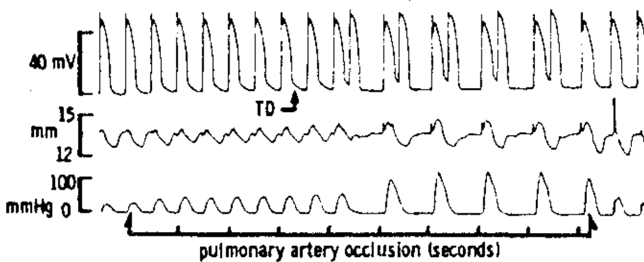

Figure 5.4 Changes in monophasic action potential (top trace), segment length (middle trace) and right intraventricular pressure (bottom trace) during pulmonary artery occlusion. Occlusion increased ventricular pressure and induced coupled extrasystoles. See text for further discussion. TD = transient depolarisation. (After Covell *et al.* (1981).)

increases right ventricular pressure, disturbs segment motion and produces afterdepolarisations (transient depolarisations) on the action potential. The depolarisations progressively increase in size during the occlusion and are associated with right ventricular extrasystoles, which in this case are coupled. The extrasystoles occur too suddenly to be explained by external reflexes or by imbalances in oxygen supply and demand. The results not only support the findings in the ischaemic ventricle but also largely confirm some of the micro-electrode studies in isolated perfused preparations described in section 5.2, indicating that changes in myocardial mechanics may be accompanied by transient depolarisations which can reach threshold.

5.4 MECHANISMS

Proposals for the molecular mechanisms for mechanically induced potentials have been presented (Kaufmann *et al.*, 1971; Lab, 1978a) and discussed in a recent review (Lab, 1982). It is of interest to see how these mechanisms relate to the changes found in ischaemia and to other hypotheses explaining early ectopics.

First, passive mechanical alterations could distort the internal and external membranes and produce permeability changes so that, for example, the membrane potential would move closer to the relevant equilibrium potentials. A non-specific increase in permeability could depolarise the membrane despite the probability that the final potential reached would be more negative than zero (Takeuchi and Takeuchi, 1960). This increase in permeability could augment the ischaemically induced potassium leak. The changes in tension could also distort intracardiac spaces. Changes in potassium movement or accumulation could follow rapidly (Kline and Morad, 1976) and this influence potassium conductance to alter membrane repolarisation (Weidman, 1956). However, these hypotheses do not easily explain some of the experimental observations described in section 5.2. For example, a release can induce a transient depolarisation but a stretch at the equivalent time does not produce the expected and opposite change in potential (a repolarisation). Another possibility relates to intracellular calcium. Mechanically induced changes in intracellular calcium (Allen and Kurihara, 1981) show analogous alterations to the mechanically induced changes in action potential, and figure 5.5

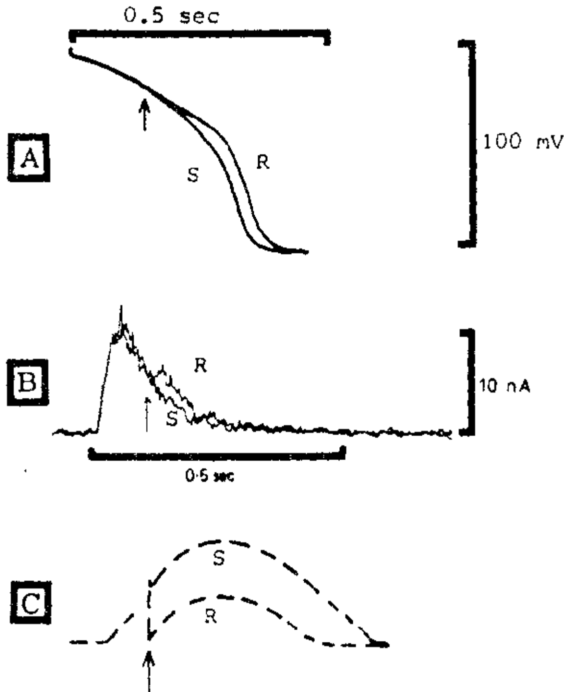

Figure 5.5 Analogous effects on action potential (A) and free sarcoplasmic calcium (B) of mechanical perturbations diagrammatically presented in C as dashed lines. Both records were from cat papillary muscle, but were obtained in different laboratories. Sarcoplasmic calcium, $[Ca^{2+}]_s$, is indicated by the light output (in nanoamperes) of intracellularly injected aequorin. A stretch (S) halfway up developed tension affects neither the action potential nor $[Ca^{2+}]_s$. However, a release (R) at the equivalent time delays the repolarisation and also the fall in $[Ca^{2+}]_s$. (Panel B is after Allen *et al.* (1981).)

demonstrates this similarity. These calcium variations have been invoked to explain the mechanically induced changes in potential (Lab, 1982). Further, lowered pH can increase sarcoplasmic Ca^{2+} in cat papillary muscle (Allen *et al.*, 1981) and this is expected in ischaemia. Myoplasmic calcium can affect outward currents (Isenberg, 1975; Bassingthwaighte *et al.*, 1976), the electromechanical gradient for the inward calcium current (Reuter, 1979) and an electrogenic Na/Ca exchange (Mullins, 1979). The transient depolarisation observed with strophanthidin (Kass *et al.*, 1978) and low potassium (Eisner and Lederer, 1979) may also have similar common explanations.

It appears that during the critical first hour or so of ischaemia there are coincident wall motion disturbances, after-potentials, and changes in cyclicAMP (chapter 11) as well as in intracellular Ca^{2+}. It is tempting to speculate that all these findings are causally related to the extrasystoles observed in this period. Pollack (1977) has suggested that stretch accelerates diastolic depolarisation via a cyclicAMP-mediated mechanism, and there are interactions between Ca^{2+} and cyclicAMP pertinent to transmembrane ionic currents and to contraction (Harary *et al.*, 1976; Schneider and Sperelakis, 1975; Reuter and Scholz, 1977; Tsien, 1977; Chapman, 1979; Katz, 1979). Notwithstanding which mechanism is responsible, stress-strain-related ectopics via some sort of contraction excitation feedback may occur regularly in very early myocardial ischaemia. This is a subject that needs further investigation as a triggering cause of the premature beat that, in the appropriate ionic, metabolic and electrophysiological milieu, precedes potentially lethal arrhythmias.

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1.7. TIME COURSE OF CHANGES IN ACTION POTENTIAL DURATION AND EJECTION SHORTENING DURING REGIONAL TRANSIENT ISCHAEMIA OF PIG VENTRICLE IN SITU

MAX J. LAB, KEITH V. WOOLLARD

1. INTRODUCTION

The duration of the action potential is normally one of the determinants of the strength of contraction of cardiac muscle. The longer the cardiac cell membrane is depolarized per unit time the greater the inward calcium current and the greater the tension (1). When cardiac muscle is made ischaemic the action potential duration (APD) shortens (2), and this will contribute to the deterioration in contraction during ischaemia (3). Some evidence has also been presented suggesting that the time course of mechanical recovery from oxygen deprivation may be related to changes in APD (4). To our knowledge no systematic study has been carried out in the intact heart correlating action potential duration measurements and myocardial dynamics during regional ischaemia. The object of this study is therefore to determine the time course of these electromechanical measurements on a beat-to-beat basis during transient occlusion of small branches of the coronary arteries in pig hearts, and to see if they may be causally related.

2. METHODS

Pigs of about 25 kg were anaesthetized with 1% halothane and a mixture of nitrous oxide and oxygen (1:1). The animals were ventilated and, during the experimental phase, the halothane was discontinued and a 1% solution of chloralose administered intravenously as necessary.

The chest was opened and a pericardial cradle was fashioned to support the heart. A catheter was inserted through the apex of the ventricle and intraventricular pressure was measured. Arterial pressure was measured via a catheter inserted into the proximal aorta. A small branch of the left anterior descending branch of the left coronary artery was selected so that when a catgut snare was tightened around the vessel a cyanotic area about 1.5 cm × 2.5 cm was produced.

A tripodal device (5) was attached, by vacuum through the legs, to the area of epicardium to be made ischaemic. This device provided three

J. Baan, A.C. Arntzenius, E.L. Yellin (eds.), Cardiac Dynamics, 61–67.
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outputs due to strains on gauges attached to the legs. The distance between each leg and the central point of the triangle they formed was about 7 mm. The movement of the legs along the axes from the centre to the corners of the base plane was recorded and taken to represent length changes in the underlying segments of epicardium. This assumption depends on the compliance of the instrument which was about 0.1 mm/g along the axis of movement of each leg. Little movement was possible in any other direction. Even though the device was somewhat stiffer than mercury-in-silastic gauges (6) our results are similar to those obtained with these gauges and also to the results obtained from ultrasonic crystals (7). In particular, marked elongation during isovolumic contraction was not observed.

The three outputs from the legs of the tripod, representing length changes in three directions over a small area, were fed into an analog computer for on-line analysis (8). Briefly, this first involved continuous arithmetical summing of the three outputs to produce a "summed segment length" which was used to represent the behaviour of the underlying area of epicardium. The aortic and ventricular pressure signals were used to define the phases of the cardiac cycle, and the instantaneous values for pre-ejection length (PEL), segment length at the beginning, and end-ejection length (EEL), segment length at the end of the ejection period, were displayed. The difference between these two values represents the length change during systole (ejection shortening).

A monophasic action potential (9) was obtained with a suction electrode which was capable of following changes in duration due to ischaemia (5). The indifferent electrode consisted of a thin wick of cotton wool which completely surrounded the suction electrode and from which the epicardial ECG was also recorded. The monophasic action potential and epicardial ECG were obtained either using one of the legs of the tripod as an electrode or from an area as close to the tripodal device as possible.

All the signals were passed to a Devices M19 pen recorder, displayed on paper and on a Tektronix storage oscilloscope. The signals were also stored on magnetic tape for later analysis. The action potential duration could be automatically measured with a device constructed for this purpose (10).

3. RESULTS

Figure 1 is an example of a mechanical recording (ejection shortening) during an episode of transient regional ischaemia, as obtained with the tripodal device. The outputs of the changes in each direction of movement of the legs of the tripod have been summed to provide an indication of the mechanical behaviour of the underlying myocardium. The changes observed were seen to be in keeping with those obtained with other techniques

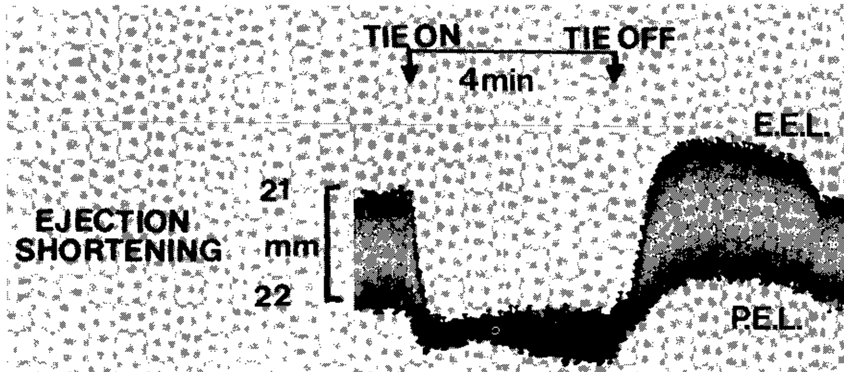

Figure 1. Continuous display of instantaneous segment length at the beginning (PEL) and end (EEL) of the ejection phase during a period of ischaemia. The width of the band represents the magnitude of the shortening during the ejection phase. On occluding the artery a reduction in ejection shortening may be detected within a few beats. The rapid mechanical deterioration is nearly complete in 30 sec. With reperfusion ejection shortening recovers over a minute reaching values greater than control, for several minutes, before pre-ischaemic levels are reattained.

and during ischaemia the area showed early systolic expansion and reduced overall shortening.

A rapid reduction in inward calcium current could conceivably explain the rapid loss of the ability of the myocardium to contract during the onset of ischaemia. An abbreviation in action potential duration (APD) could be a cause of the reduction and it was pertinent to follow the time course of changes in APD from this area on a beat-to-beat basis. On occlusion the APD actually slightly increased transiently and we could not detect a significant reduction in APD until two minutes after the tie (Figure 2). Apart from the increase in APD, there was no change in action potential amplitude or configuration during this period. The duration and amplitude thereafter steadily declined.

There was a close relationship between the action potential duration and the T wave of the epicardial ECG. As the action potential shortened the T wave became more positive (inset of Figure 2), and correspondingly, lengthening of the action potential was associated with increasing negativity of the T wave. The S-T segment however did not alter in a biphasic manner. Some elevation could be detected within the first minute and it slowly increased over the next 3–4 minutes.

The question now arose as to the precise relationship between the time course of the mechanical deterioration and the APD alterations. This relationship is clearly seen in Figure 2 where ejection shortening and APD were plotted during transient ischaemia. The early, rapid reduction in ejection shortening corresponds in time to the slight transient increase in

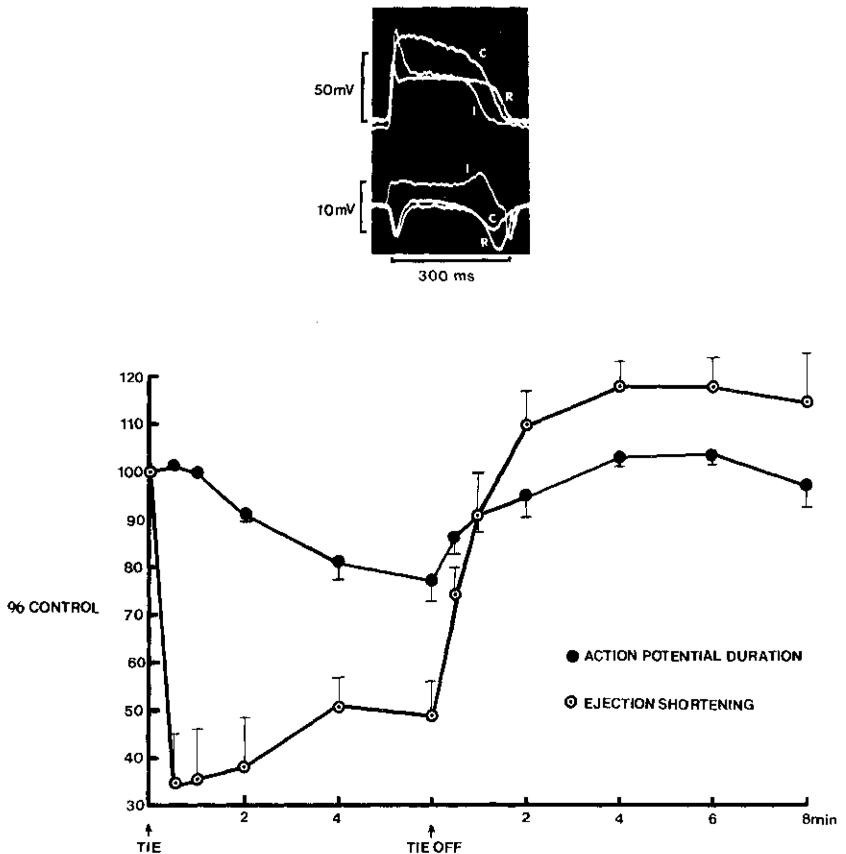

Figure 2. Graphs to show time course of changes, produced by ischaemia and reperfusion, in ejection period shortening and action potential duration (mean ± S.E., $n = 15$). Ejection shortening deteriorates much more rapidly than the action potential duration which actually first increases over the first minute ($P < 0.05$). There is a transient improvement in ejection shortening 2–4 minutes after the tie, which is also not accompanied by a comparable electrical change. However, on reperfusion electrical and mechanical changes recover at a similar rate and both exceed control values for a short period. *Inset*: Effects of ischaemia on monophasic action potentials (upper traces) and epicardial electrocardiograms (lower traces). Compared to the control situation (C), ischaemia (I) produces a reduction in duration of action potentials and a raised S-T segment. The base-lines of the epicardial ECG are superimposed. There is in fact significant T-Q depression. The sharp downward deflection at the end of T-waves are conducted atrial pacing spikes. (they are not detected in the action potential).

APD. Thereafter the ejection shortening showed some improvement, although the APD now steadily declined, and this improvement was a consistent finding with the type of preparation we have studied. The action potential rarely disappeared with these small ischaemic areas. However, with longer or larger infarcts, areas could easily be found with the suction

electrode that were electrically quiescent, but where the epicardial ECG was completely monophasic in character, showing a marked S-T elevation (5).

We wished to answer three questions concerning reperfusion. First, do the electromechanical changes follow the same time course, during recovery as on occlusion? On removing the occlusion, electrophysiological measurements and the mechanical measurements show a rapid parallel recovery towards control values contrasting with the opposite biphasic behaviour at the onset of ischaemia. The next question was whether we could observe values of ejection shortening greater than control, during the reperfusion period, as previously reported for other mechanical measurements and preparations (4, 11). Such an overshoot is clearly demonstrated in both Figures 1 and 2. Finally, we wished to see whether in the intact heart in situ an increase in APD above control levels occurred in parallel with this ejection shortening overshoot. An overshoot above control values was observed in the electrophysiological as well as the mechanical recordings, both having roughly the same time course (Figure 2).

4. DISCUSSION

Correlations between epicardial electrocardiograms, action potentials, and mechanical performance in intact hearts in situ have not been studied before. Part of the difficulty in attempting to correlate mechanical changes and alterations in APD in epicardial segments during ischaemia and reperfusion on a beat-to-beat basis lies in the continuous recording of action potentials with microelectrodes while normal mechanical changes are unhindered. Recording with microelectrodes alone without measuring mechanical change is difficult enough, frequently necessitating immobilization of the area studied. However, the immobilization itself may influence the recorded potentials (12, 13). Monophasic action potentials obtained with suction electrodes can overcome some of the difficulties and give an indication of transmembrane events, particularly the time course of repolarization. Action potentials thus obtained may show the same changes in duration observed with microelectrodes during anoxia (5).

On release of the tie after 5 to 10 minutes of ischaemia, there was a dramatic restoration of contraction and action potential duration over approximately the same time course. During this period of reperfusion ejection shortening was greater than control and there was also a transient increase in action potential duration above control values. These observations on epicardial segments support those on isolated perfused preparations in which there was a prolongation in tension (particularly a delayed relaxation on re-oxygenation) (4, 11).

Simultaneous microelectrode and mechanical studies have previously

shown an increase in action potential duration above control on reoxygenation (4) although this has not been confirmed by others (11). These supranormal mechanical and electrical changes could be causally related in that a prolongation in action potential duration by electrical means can increase tension development in isolated muscle (1). The increase in tension is thought to be mediated by an increase in calcium-inward current (14). However if this mechanism operates it may only be part of the explanation. Figure 2 shows that recovery of the ejection shortening is slightly faster than the recovery of the APD and, furthermore, the overshoot in ejection shortening lasts a little longer than the overshoot in APD.

Although electromechanical recovery during reperfusion is rapid and parallel in time, and the changes in APD and segment shortening may be causally related, our results clearly show that no such relationship exists during the onset of acute ischaemia. The mechanical deterioration is faster than the change in APD. It is thus unlikely that the rapid reduction in contractility is mediated via a reduced inward calcium current and thus a fall in contractile dependent calcium. However, the interruption of the energy-dependent chain governing intracellular Ca^{++} has a sound basis (3) and this mechanism probably operates later. A small but consistent and hence potentially significant observation is that the action potential duration actually increased over the period of rapid mechanical deterioration. Cooling of the myocardium can prolong the APD (5, 15) and qualitatively this change is in the right direction. However, we feel that this is not the explanation because our temperature measurements from the centre of the small ischaemic zones show very small reductions (0.1°C). Furthermore, other electrophysiological measurements during the first 2 min of ischaemia have been known to follow a biphasic pattern similar in time course to the changes in APD observed here (16).

What common denominator could exist between the increase in APD and reduction in contractility? One interesting possibility is the calcium ion, for it has been suggested that an increase in internal calcium may increase the outward current which would reduce the APD (17). If, therefore, the reduction in contraction is due to a reduction in available free intracellular calcium in the case under discussion, it would be associated with a reduction in outward current and thus a prolongation in APD. It is thus conceivable that the electromechanical changes during ischaemia and reperfusion may both be calcium-mediated, but with different mechanisms.

ACKNOWLEDGEMENTS

K.V. Woollard is in receipt of Trustee Fellowships of Charing Cross Hospital (Clinical Research Committee). We thank Mr. R. Price for constructing the tripodal device and Mrs. R. Kingaby for her technical assistance.

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Circulation Research JUNE 1982 VOL. 50 NO. 6
An Official Journal of the American Heart Association

BRIEF REVIEWS

Contraction-Excitation Feedback in Myocardium

Physiological Basis and Clinical Relevance

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EXCITATION-CONTRACTION coupling in ventricular myocardium has received exhaustive investigation. Feedback processes in the reverse direction between contraction and excitation are less conspicuous and, not surprisingly, have attracted little notice despite their potential physiological and clinical importance. For the present, contraction-excitation feedback is inferred when changes in mechanical stress or strain cause or precede changes in membrane potential. For example, reduced force development or increased shortening can induce greater depolarization. Contraction-excitation feedback, according to the type and timing of the mechanical change producing it, can appear either as a prolongation of an action potential, or as a transient depolarization. This review will describe the circumstances under which contraction-excitation feedback occurs, discuss possible explanations for the phenomenon, and speculate on its potential importance in selected clinical situations.

The durations of the action potential and of contraction in the heart are of the same order of magnitude. This relationship allows mechanical activation to influence the initiating, concurrent action potential. However, the term "feedback" may be a misnomer, as the process may comprise initiating factors that cause both electrical and mechanical changes. These factors may also be common to excitation-contraction coupling. Accordingly, a precis of the relevant aspects of excitation-contraction coupling is given in Figure 1. There are comprehensive reviews elsewhere (Huxley, 1974; Noble, 1975; Fozzard, 1977; Tsien, 1977; Coraboeuf, 1978; Carmeliet, 1978; Nayler and Williams, 1978; Vassalle, 1979; Reuter, 1979; Chapman, 1979; Fabiato and Fabiato, 1979; Katz, 1979).

The action potential normally causes a rise in sarcoplasmic calcium concentration, $[Ca^{++}]_s$ via the mechanisms outlined in Figure 1. This instigates and mainly controls actin and myosin interaction. Relaxation is induced by processes that lower $[Ca^{++}]_s$. Active tension is also modulated by cyclic AMP and changes in binding constants of Ca^{++} for troponin. The configuration of the action potential can be modified by three basic mechanisms: the degree of inactivation or recovery of ionic currents, accumulations, involving, for example, internal Ca^{++} and external K^+, and ionic exchange such as Na^+/K^+ and Na^+/Ca^{++}.

I. Prevalence of Contraction-Excitation Feedback

Electrocardiographic studies by Stauch (1960) provided the first evidence for a feedback between contraction and excitation. He demonstrated shortening of the Q-T interval of the ECG in an isovolumic contracting frog ventricle as compared with the auxotonic beating heart, allowed to empty. Later corroborations used monophasic action potentials in a similar experimental preparation (Stauch, 1966; Lab, 1968). The plateau phase of the action potential was steeper during isovolumic than during auxotonic contraction. Stretch can also induce resting membrane potential changes (Penefsky and Hoffmann, 1963; Kaufmann and Theophile, 1967). Because of this, as well as the length-tension relation and length-dependent changes in activation (Jewell, 1977), this review will briefly include the effects of length changes on membrane potentials.

Before proceeding, we should note that the experimental protocols used in the studies produce results which need cautious interpretation because the mechanical maneuvers can disturb the electrical relationships between the biological signal generator and the recording electrode. In addition, isolated preparations can show internal inhomogeneity in contraction, as well as relaxation (Krueger and Strobeck, 1978). This inhomogeneity is exacerbated by damaged and compliant ends associated with anchoring the preparation. Mechanically induced changes in potential may thus vary within and between preparations because of nonrepresentative sampling. However, reasonably

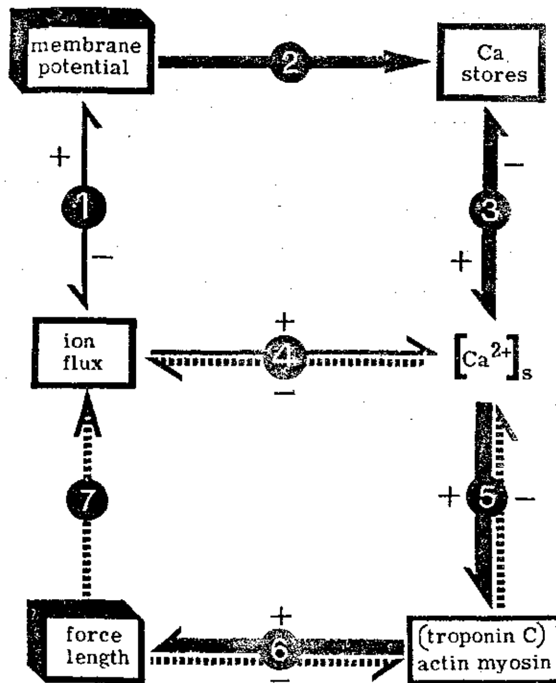

FIGURE 1. *Schematic diagrams of some interactions betweem membrane potential and contraction in heart. The sequence in excitation-contraction coupling may be followed via the heavy black arrows (mainly clockwise). Ion fluxes across the sarcolemma [1+] determine the membrane potential, which itself can provide a driving force for ion movements [1−]. The changes in membrane potential are a function of the ionic equilibrium potentials and conductances (g). At rest, g_{Na} is low and g_K is high. The latter mainly contributes to the negative resting potential which is maintained by the exchange of intracellular Na^+ for extracellular K^+ by an ATP-ase dependent Na/K pump, that maintains internal Na^+ concentration, $[Na^+]_i$ low and $[K^+]_i$ high. Extracellular Ca^{++}, $[Ca^{++}]_o$, is much higher than sarcoplasmic Ca^{++} concentration, $[Ca^{++}]_s$. During the upstroke of the action potential, g_{Na} is rapidly increased, generating a fast inward sodium current, i_{Na}, that reverses the transmembrane potential. Despite the resulting increase in the outward driving forces for potassium, g_K decreases, causing the outward repolarizing current to be less than expected, thereby prolonging the cardiac action potential. A slow inward current, i_{Ca}, carried mainly by Ca^{++}, also prolongs the action potential. [i_{Ca} probably generates action potentials in the sinus node and partially depolarized muscle (Cranefield, 1975)]. The channels for i_{Ca} are influenced by cAMP-dependent protein kinase. Depolarization [2] results in a rise in sarcoplasmic calcium derived from intracellular stores [3+] directly, possibly by a calcium-induced calcium release. In mammalian muscle, i_{Ca} does not normally raise $[Ca^{++}]_s$ immediately to any significant degree unless the action potential is long [4+]. The calcium within the sarcoplasm combines with troponin-C [5+] which causes troponin-I to allow an actin and myosin interaction. These interactions, which require ATP, result in force development [6+]. As, or probably before, the membrane repolarizes, the sarcoplasmic reticulum sequesters Ca^{++} [5−; 3−] causing relaxation [6−]. Ca^{++} can also leave the sarcoplasm by a metabolically dependent calcium pump or by Na/Ca exchange [4−]. The exchange system is important for contraction in the frog. Greater binding to troponin can also lower $[Ca^{++}]_s$, but this is associated with increased force rather than faster relaxation. Length dependent activation (Jewell, 1977) is portrayed in [6±]. Force and length changes could influence membrane events (contraction-excitation feedback) by processes depicted by the dotted lines. For example, mechanical changes could change ionic fluxes across the sarcolemma by affecting permeability or diffusion gradients directly [7]. Indirectly [6−; 5−], force and length changes could influence the membrane by altering $[Ca^{++}]_s$. This may influence ionic flux [4−], and hence membrane potential [1 +], by modulation of the electrochemical gradient for Ca^{++}, outward potassium currents, "leak" currents, and the electrogenic Na/Ca exchange.*

consistent results have been found using a variety of preparations and recording techniques, some of which sample many if not all the cells. Further, several predictions based on the existence of this "feedback" have been fulfilled.

(i) Mechanical Changes in Resting Muscle

(a) *Effect on Resting Potentials.* In several different ventricular preparations, a stretch during diastole, to about L_{max} (the length at which maximum tension is produced) can result in an immediate reversible depolarization (Penefsky and Hoffman, 1963; Lab, 1978a; Boland and Troquet, 1980). These depolarizations may produce action potentials (Lab, 1978b) or spontaneous activity (Kaufman and Theophile, 1967). Boland and Troquet (1980) attributed their results in the intact rat ventricle to ischemia, but no blood flow measurements were made and their results were in keeping with those from the superfused isolated preparations cited above. In all these cases the depolarization was sufficiently fast to operate on a beat-to-beat basis. Dudel and Trautwein (1954) found depolarization with large stretches in cat papillary muscle but interpreted this as muscle damage. Two studies conflict with the foregoing results by showing no change in resting potential with length change (Spear and Moore, 1972; Allen, 1975).

(b) *Effect on Action Potentials.* When stretched muscle is stimulated, the action potential shows a reduced *spike amplitude* (Dudel and Trautwein, 1954; Penefsky and Hoffman, 1963; Lab, 1978a), as expected with a partially depolarized membrane (see previous section). Spear and Moore (1972) also found reductions in action potential amplitude, and conduction velocity, recorded from stretched rat ventricle. However, the latter changes were slowly reversible and were unlikely to operate on a beat-to-beat basis. This preparation also showed graded and asynchronous electromechanical behavior, which was absent in guinea pig, cat, and frog.

Several reports show that lengthening can also alter the *duration* of the action potential, although not all studies are in agreement. Shortening of action potential duration closely follows stretch in several studies (Dudel and Trautwein, 1954; Allen, 1975; Lepeschkin, 1976b, in his Fig. 3; Lab, 1978a; Lab, 1980). Allen showed a small but significant initial shortening during the first few beats after stretch, that was followed by prolongation as steady state was reached many beats later. Two investigators, however, reported no effect on the action potential duration (Gennser and Nilsson, 1968; Hennekes et al., 1977), whereas Nomura (1963), using non-vertebrate cardiac muscle,

found a prolongation of action potential duration with stretch.

A stretch, then release, of turtle ventricle perfused with 40 mM Ca^{++} solution was reported to cause small mechanical and electrical oscillations (Bozler and Delahayes, 1971). These authors considered the transmembrane potential changes to have been the consequence of the mechanical changes. Stretch of normally perfused resting muscle (normal [Ca^{++}]) was reported to produce only small changes in membrane potential if the extension was large (see also Lab, 1978a, where large stretches were needed to produce an electrical effect). Bozler and Delahayes also saw oscillations after a twitch in 20 mM Ca^{++} that occurred with only moderate reliability. The oscillations were absent in some frog ventricles and in all frog atria. Lab (1978a) reported no mechanical induction of a membrane potential change in one intact frog preparation. Possible explanations for these discrepancies already mentioned at the beginning of Section I are outlined in context below.

In general, moderately large passive length changes in ventricular muscle can induce changes in membrane potential. Discrepancies in a few preparations may be species related and/or be due to varying experimental conditions. Changes in sarcomere length with mechanics are inhomogeneous in any one preparation (Krueger and Pollack, 1975), and difficult to control between preparations. This inhomogeneity is enhanced by the variable damage to the ends of the muscle produced in anchoring them at their ends during different experiments. The differing electrical effects of stretch can thus be the result of partially damaged and compliant ends that could take up most of a relatively small length change. In such a situation, electrical recording from a distant area would show no mechanically induced effect, whereas recording sites closer to the stretched region could demonstrate a variable electrical effect or even oscillation due to electrotonic spread. A change in internal *milieu* might also account for some of the discrepant observations. Isolated and electrolyte superfused cardiac preparations change with time (Reichel, 1976). In keeping with this possibility, Bozler and Delahayes (1971) observed a reduction in amplitude of oscillation after several hours' study, and that storage for an unspecified time and under unspecified conditions prior to experimentation reduced the oscillations.

(ii) Mechanical Changes in Active Muscle

(a) *Comparison of Action Potentials in Isometric and Isotonic Contraction.* In contrast to the somewhat confused situation regarding the effect of length changes in membrane potential of resting muscle, alteration of the mode of contraction of cat papillary muscle affects the action potential clearly and reproducibly. Kaufmann et al. (1971) found that the action potential duration during an isometric contraction was shorter than that associated with an isotonic contraction. Similar results were found when the action potentials of an isovolumically contracting frog ventricle were compared with those of an isotonic (auxotonic) contraction (Stauch, 1966; Lab, 1978a). These results were not identical to those in the papillary muscle in some aspects, probably because isovolumic contraction of the whole ventricle does not imply isometric contraction of the wall.

(b) *Effect of Imposed Perturbations during Action Potentials.* A quick stretch of cat papillary muscle at any time does not affect the action potential (Hennekes et al., 1977; Hennekes et al., 1981). A typical example is seen in Figure 2A—(s). Release during the early rising phase of contraction (first perturbation in Figure 2B) also does not affect the action potential. In contrast (Fig. 2, A and B), a quick release near the peak of developed force prolongs the action potential (Kaufmann et al., 1971; Hennekes et al., 1977). Concordant changes are found using microelectrodes, insulation gap, or electrograms (Lab, 1980). After the onset of delayed repolarization, immediate restretch of the muscle cannot restore control electrical conditions (Fig. 2B). The prolongation of action potential following a late release (Fig. 2C) can be similar in configuration to an early afterdepolarization, as characterized by Cranefield (1977), that can produce a propagated action potential (Fig. 2C). This phenomenon may be relevant to some pathological arrhythmias [see Section III (iii)]. In the pig ventricle in situ, a disturbance of segmental wall motion analogous to the foregoing mechanical perturbations, produced by occluding the aorta, can cause both a transient depolarization (Fig. 2D) and an extrasystole (Lab, 1978b).

The precise relationship between the mechanical intervention and the depolarization is unclear. There is no simple correlation between the change in potential and the extent of the mechanical alteration measured as tension, velocity of shortening, and length (Lab, 1980; Hennekes et al., 1981). Further, for any given length change, the change in potential is crucially dependent on time: both early and late release can produce a small depolarization, whereas release of an intermediate timing yields a large depolarization (Fig. 2B). Mechanically induced "uncoupling of the active state" is also a time-dependent process (Brady, 1965; Kaufmann et al., 1972). In this process, a release of the muscle to a short length during contraction is incapable of producing an active tension appropriate for the new length: this phenomenon is called "tension deactivation" (Julian and Moss, 1976). That the release-induced depolarization is related to the release-induced deactivation (Lab, 1980) is supported by some experiments using caffeine, which is known to prolong contraction while leaving the action potential relatively unaffected. In caffeine-treated muscles, release did not produce a voltage change until significant deactivation occurred after repolarization of the action potential (Hennekes et al., 1981): i.e., a very late release produced both a significant deactivation and membrane depolarization. Extrasystoles followed more frequently under these conditions than during normal perfusion.

Notwithstanding some inconsistencies and difficul-

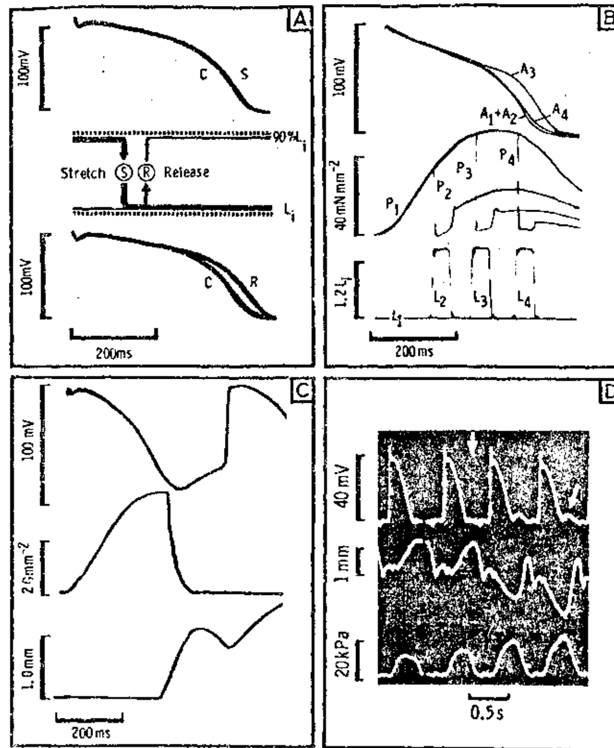

FIGURE 2. *Action potential, length, and force changes following imposed mechanical changes in four muscle preparations. A–C, cat papillary muscle; D, intact ventricle. A: The muscle contracts at two initial muscle lengths (dashed lines L_i and 90% L_i), producing upper and lower control action potentials (C) with similar time courses. A stretch, (s), from 90% L_i to L_i, does not affect the action potential (upper traces) and S and C superimpose. This negative finding is obtained regardless of the timing of the stretch. However, when the muscle shortens (release —R), the action potential is prolonged (lower trace R). B: The undisturbed isometric muscle (P_1/L_1) has the shortest action potential duration (A_1) Transient quick releases (L_2–L_4) inactivates force development (P_2–P_4). With forces P_3 and P_4, the action potential is prolonged by depolarizing potentials (A3–A4). Intermediate timing of the intervention (L_3/P_3) produces the greatest action potential change (A_3), whereas the early release (L2/P2) has no effect (modified with permission: Lab, 1980). C: A release near the end of repolarization produces a discrete depolarization which is prominent and reaches threshold for a propagated action potential (modified with permission: Kaufmann et al., 1971). D: Recordings from pig left ventricle in situ. Monophasic action potentials from the epicardium (top trace) show a transient depolarization (inclined arrow) after the aorta was constricted (vertical arrow). This is apparently related to the rapid stretch then shortening of the epicardial segment (middle trace). Shortening is an upward movement. Lowest trace is intraventricular pressure. (Unpublished record, see Lab, 1978b.) Unit conversions 0.1 g ≈ 1 m N; 1kPa ≈ 7.5 mm Hg.*

ties in interpretation, the available evidence indicates that mechanical changes can affect the action potential. High tensions, e.g., in an isometric contraction, are associated with a short action potential, while a lightly loaded muscle allowed to shorten has a longer action potential. There is also a unidirectional component in contraction-excitation feedback in that muscle lengthening during activity produces no electrical change, whereas muscle shortening does.

II. Explanatory Mechanisms

When we consider explanations for contraction-excitation feedback, three important properties need to be borne in mind: the phenomenon operates mainly when the muscle is activated, it occurs rapidly, with a time lag of only 10–20 msec (Kaufman et al., 1971), and it can be unidirectional.

(i) Passive, Physical Mechanism [Fig. 1 (7)]

An architectural change could passively affect the relationship between the electrical signal and monitoring electrodes; for example, some studies show changes in the cable properties and electrical constants of muscle with mechanical change (Deck, 1964; Potapova and Chailakian, 1965; Dulhunty and Franzini-Armstrong, 1977; Dominguez and Fozzard, 1979). A permeability change that depends on membrane stress or strain could also move the membrane potential closer to the relevant equilibrium potentials. Finally, mechanical changes could distort intercellular spaces, which might alter K^+ movement or accumulation (Weidman, 1956; Kline and Morad, 1976) to influence membrane potential.

It is difficult to invoke these passive changes to explain all of the experimental observations. A release and a stretch at a given time should produce the appropriate distortions to depolarize and repolarize, respectively, the membrane of cat papillary muscle, whereas stretch—in fact—has no effect on the action potential. Further, a release-restretch maneuver returns the muscle to the passive mechanical state preceding the intervention, but the electrical change is not aborted. These arguments therefore weaken the credibility of a purely passive mechanism.

(ii) Active Indirect Mechanism

Mechanically induced deactivation of tension (Brady, 1965; Kaufmann et al., 1972; Julian and Moss, 1976) has properties similar to those outlined in Section II above: it occurs during muscle activity, is rapid, and directional. Further, Gordon and Ridgeway (1976) found a Ca^{++} mediated length-dependent change in membrane potential in skeletal muscle. Also, the mechano-electric effects in cardiac muscle are most prominent at a time when internal calcium $[Ca^{++}]_s$ [measured as light emitted when Ca^{++} reacts with microinjected aequorin], is declining, i.e., just before peak developed tension (Allen and Blinks, 1978). It seems reasonable to implicate the calcium ion in contraction-excitation feedback, because this ion influences transmembrane currents as well as developed tension. The crucial questions are whether a mechanically dependent $[Ca^{++}]_s$ change occurs in mammalian cardiac muscle under these circumstances, and whether it does so in a directional manner. Allen and Kurihara (1981) clearly showed an increase in light output of aequorin, $[Ca^{++}]_s$, with release, but no change with stretch. How release enhances $[Ca^{++}]_s$

while reducing developed tension remains speculative. Allen and Kurihara suggested a tension-mediated change in the Ca^{++} binding constant of troponin (Fig. 1 [5±]) possibly related to the number of attached cross-bridges (Bremel and Weber, 1972). However, because stretch has no effect on $[Ca^{++}]_s$ and action potential, this suggestion probably is not the whole explanation, suggesting that cross-bridge interaction during tension *deactivation*, in particular, may also be important. Although they may prove related, the load-dependent changes in relaxation observed by Brutsaert et al. (1978a, 1978b) remain enigmatic.

These observations suggest that any explanation for contraction-excitation feedback has to account for a rise in $[Ca^{++}]_s$, together with a reduction in tension and prolongation of action potential, in addition to the three properties listed above. Modulation of $[Ca^{++}]_s$ can affect transmembrane movement of ions in several ways (Fig. 1 [4-]). Whereas it can alter i_{Ca} (Reuter, 1979), there is no release-induced change in a calcium-mediated action potential (Hennekes et al., 1981). Modulation of $[Ca^{++}]_s$ also can influence outward currents (Isenberg, 1975; Bassingthwaighte et al., 1976; Di Francesco and McNaughten, 1979). On first inspection, neither of these hypotheses explains the observed phenomena because the hypothesis requires that a rise in $[Ca^{++}]_s$ should shorten the action potential and reduce force. More evidence is needed to exclude these explanations because Ca^{++} compartmentalization is not fully understood. The $[Ca^{++}]_s$ variation could also modulate an electrogenic Na^+/Ca^{++} exchange in the appropriate direction (Mullins, 1979). Kass et al. (1978) considered this mechanism for their transient inward current. A mechanically induced alteration in Na^+/K^+ exchange may also be involved, because this ion exchange is also electrogenic (Thomas, 1972; Isenberg and Trautwein, 1974; Schwarz et al., 1975). Calcium could also act as an internal "transmitter substance" and modify a non-specific leak current (Kass et al., 1978; Eisner and Lederer, 1979).

Finally, $[Ca^{++}]_s$ may affect ionic currents by Ca-dependent phosphorylation of sarcolemmal sites (Kakiuchi and Yamazaki, 1970; Hazary et al., 1976). In the context of phosphorylation processes, it should be noted that cAMP can also modulate i_{si} (Schneider and Sperelakis, 1975; Reuter and Scholz, 1977), and increases in $[Ca^{++}]_i$ can reduce cAMP levels (see also Tsien, 1977; Chapman, 1979; Katz, 1979, for reviews). Furthermore, there is evidence that concentrations or activities of cyclic nucleotides may vary in the heart, on a beat-to-beat basis (Brooker, 1973; Wollenberger et al., 1973) and with length (Flitney and Singh, 1981). These findings introduce the intriguing possibility that mechanically dependent changes in phosphorylation of membrane sites could mediate in contraction-excitation feedback. This mechanism bears some resemblance to the one proposed by Pollack (1977) for accelerated diastolic depolarizations produced by stretch, and can also account for the changes described in Section I (i) (a).

III. Role and Context

Few investigators have specifically studied contraction-excitation feedback, but any electrophysiological study that entails some primary mechanical change should show elements of the feedback. A number of experiments that provide circumstantial evidence relating to the existence and importance of contraction-excitation feedback were selected from the literature during the period beginning in 1969 to date, and the mechanical analogues to those producing contraction-excitation feedback extracted. The characteristics shown in Figure 2 were used to predict the electrophysiological and other consequences of the mechanical changes: viz mechanically induced changes in repolarization phase, transient, and/or threshold depolarizations. Finally, the expected observations and interpretations were compared with the ones actually obtained.

(i) Possible Effects of Feedback at a Cellular Level

Relation between Mechanically Induced Action Potential Change and Ensuing Mechanical Change. Electrically induced changes in action potential duration (Antoni et al., 1969; Wood et al., 1969) initiate transient changes in tension over several beats—presumably by changing $[Ca^{++}]_s$. Similar tension transients may also follow a mechanical change (Parmley et al., 1968; Jewell and Rovell, 1973) and subsequently alter direction with a slower time course (Parmley and Chuck, 1973; Maisch et al., 1975; Suga and Sagawa, 1978). It is probable that mechanically induced changes in action potential contribute to the initial tension transients (Kaufmann et al., 1971). Hennekes et al. (1977) later presented evidence to show that there may be additional contributions, an interpretation endorsed by Suga and Sagawa (1978).

At present it is not easy to reconcile the feedback with the force changes in homeometric autoregulation ("Anrep phenomenon") in the intact heart. Anrep (1912) showed that an increase in developed pressure accompanies an increase in afterload in the 2 minutes following the change. However, the feedback requires an increased afterload to be accompanied by immediate reductions in force transients (and action potential duration), ostensibly the wrong direction for the Anrep phenomenon. Regional variation in myocardial blood flow has been suggested to explain the latter (Monroe et al., 1972), but it could also be related to the slow action potential changes described by Allen (1975).

(ii) Intact Normal Ventricle

Action Potential Duration and Ventricular Repolarization (Q-T) Interval of ECG and Left Ventricular Shortening. One study in ventricle as a whole is a direct corroboration of the initial experiments in excitation-contraction feedback. Isolated myocardium that shortens substantially and rapidly against a small afterload prolongs the action potential duration (Fig. 2 A and B). An analogous situation exists in intact

human hearts. Ford and Campbell (1980) used amyl nitrite to produce a reduction in afterload, and this speeded wall shortening, reduced systolic time intervals, and prolonged the Q-T interval of the ECG. The second heart sound (S_2) occurred earlier and the T-wave later: i.e. S_2 and T times moved in opposite directions and the S_2-T interval lengthened. Other interventions that changed heart rate only, moved S_2 and T times in the same directions.

Other studies relate to differential intramural shortening during contraction in intact ventricle (Fischer et al., 1966; Dieudonne and Jean, 1968; Rushmer, 1970; Streeter, 1979), and relaxation (Krueger and Strobeck, 1978). These mechanical inhomogeneities should provide a background for intramural feedback interactions. Further, the ECG is generated by electrical inhomogeneities (Schlant and Hurst, 1976); therefore the mechano-electric interactions should cause predictable alterations in the ECG. Three examples will be considered in relation to the T-wave (repolarization changes—Fig. 2, A and B), the U-wave (transient depolarization—Fig. 2, C and D), and extrasystoles (Fig. 2C).

Action Potential Duration and Ventricular Repolarization Gradient (T-wave of the ECG). During normal ventricular contraction, the epicardial circumference shortens proportionately less than the endocardial circumference (Rushmer, 1970), and the tension distributions are also different (Law of Laplace). According to contraction-excitation feedback, this length/tension distribution implies that epicardial repolarization (short action potential) will precede endocardial recovery (long action potential). Such a repolarization gradient, curiously opposite in direction to depolarization, is and in fact normally found. The vector contributes to the concordancy of QRS and T-wave vectors of the ECG. An increase of ventricular volume causes the inner and outer circumferences to approach each other. Under these circumstances, if mechanical conditions of the endo- and epicardial musculature become similar, so should the electrical conditions. This mechanical maneuver produces the expected, similar, endocardial and epicardial action potentials, and consequent flattened T-wave (Lab, 1971). Figure 3A illustrates a remarkable T-wave change with increase in ventricular volume. Analogous mechano-electric changes can be found in the intact pig ventricle in situ (Fig. 3B), and similar maneuvers can alter regional electrical differences in isolated rabbit hearts (Khatib and Lab, 1982). Finally, the flattened T-wave commonly observed in pathologically dilated hearts is in keeping with the proposal just illustrated. The relevance of these observations to steady state conditions or changes in rate (Lab and Yardley, 1979) remains to be shown, but the possibility that the mechanics of intramural contraction modulate the repolarization gradient needs further investigation, especially as the generation of this gradient is not clear (Lepeschkin, 1976a; Burgess, 1979), and Nobel and Cohen (1978) have demonstrated electrophysiological differences in ventricular preparations, isolated from the endocardium and epicardium.

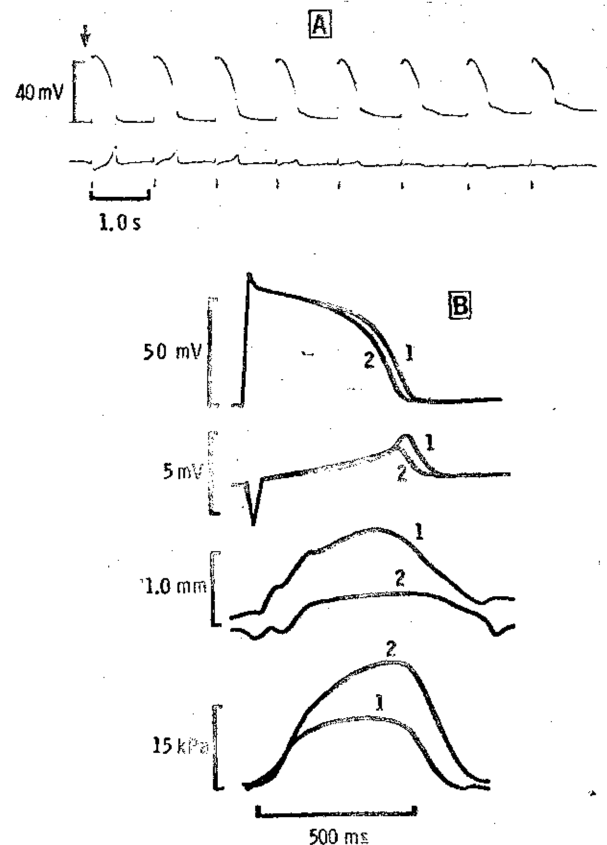

FIGURE 3. *Monophasic action potentials and ECG from the surface of intact ventricles in situ, on aortic occlusion. A: Action potentials (top trace) and electrocardiogram (bottom trace) from frog. After aortic occlusion (arrow) the T-wave becomes inverted within 6–7 beats, whereas QRS complex changes are small by comparison. The action potential duration (at T_{70}) shortens and a depolarizing potential appears on the terminal repolarization phase. (Unpublished record. Action potentials similar to those reported in Lab, 1971, 1978a). B: Electrical and mechanical records taken immediately before (1) and after (2) aortic constriction in the pig. Superimposed traces from above down: action potentials, epicardial ECG, epicardial segment motion (shortening is upward), intraventricular pressure. The rise in intraventricular pressure reduces both epicardial segment motion and the action potential duration. The epicardial ECG shows a reduction in Q-T interval and a smaller T-wave. The QRS amplitudes superimpose. (In other records, the QRS with the isovolumic beat is smaller than with the auxotonic beat.) Data obtained from 28 kg pig, that had a constant heart rate of 75 beats/min. All records except pressure were from the antero-lateral surface of left ventricle. (Unpublished record. Experimental preparation and recording techniques described in Lab and Woollard, 1978).*

Transient Depolarization and U-wave of ECG. If mechanical inhomogeneities in the intact ventricle occur after repolarization is complete, discrete depolarizations (Fig. 2, B and C) could generate current flow after the T-wave, and thus form or influence the U-wave. It has already been proposed that the U-wave may be associated with late afterdepolarizations (Lepeschkin, 1941; Lepeschkin and Surawicz, 1964), and Lepeschkin (1976b) suggested that U-wave amplitude is related to contraction. Variable diastolic intervals and/or extracellular potassium affect con-

traction (Hennekes et al., 1981), afterdepolarizations (Cranefield, 1977), and U-waves (Lepeschkin, 1976b), in accordance with this hypothesis. The electrical vector responsible for the U-wave may thus originate from potential differences between muscle regions with different transient depolarizations that may arise from different degrees of contraction-excitation feedback due to inhomogeneous wall contraction.

Threshold Depolarization and Ventricular Extrasystoles. Mechanical perturbations near or during diastole can induce threshold depolarization (Figure 2C; Kaufmann and Theophile, 1967; Kaufmann et al., 1971; Lab, 1978a, 1978b). Analogous situations should exist if extraneous mechanical perturbations were to be imposed on intact hearts. Mechanically induced depolarizations were found in isolated rabbit hearts, which also demonstrated refractory periods appropriate to the mechanical intervention (Brooks et al., 1964; Kluge and Vincenzi, 1971). Acetylstrophanthidin reduced this "mechanical" refractory period and could precipitate fibrillation. Strophanthidin alone can generate afterpotentials or transient inward currents (Kass et al., 1978), perhaps facilitated by the mechanically induced depolarization. Clinical analogues to the extraneous mechanical perturbations also exist. A blow to the chest (Hurst and Logue, 1966) and cardiac catheterization (McIntosh, 1968) are well known to precipitate extrasystoles, and Zoll et al. (1976) were able to stimulate the heart non-invasively with a "mechanical thumper."

(iii) Intact Abnormal Ventricle

During ischemia, contractile and electrical performance at the cellular and gross levels deteriorate (Samson and Scher, 1960; Coraboeuf et al., 1976; Kubler and Katz, 1977; Elharrar and Zipes, 1977; Lazzara et al., 1978) and cause abnormal wall motion (Tennant and Wiggers, 1935; Tyberg et al., 1974; Forrester et al., 1976; Theroux et al., 1976). Thus, as described above in normal hearts (Fig. 2), appropriate timing of ventricular mechanical changes during the action potential could affect corresponding deflections in the ECG of the abnormal heart. Several studies indicate that mechanical changes could initiate electrophysiological variation in this and other pathological settings; examples of three abnormalities will be briefly considered.

Reduced Action Potential Amplitude and QRS Complex during Ventricular Dilation. Stretch reduces the amplitude of cardiac action potentials (Penefsky and Hoffman, 1963; Lab, 1969, 1978a; Boland and Troquet, 1980), so that left ventricular distension should reduce the QRS vector by reducing the potential differences across the depolarizing wave front, and, as with the T-wave (Section ii), reduce the electromechanical differences between epicardium and endocardium. Observed reductions in QRS amplitude with dilation have been explained by increases in blood volume (Brody, 1956), or reduced tissue mass under the electrodes (Lekven et al., 1979). However, an alternative possibility is that a length-induced change reduces the amplitude of the action potential.

Altered Membrane Potentials and S-T Segment Elevation. S-T element elevation in the ECG results from abnormal current flow between different regions of the heart and is attributed to different membrane potentials and action potential durations in normal and abnormal myocardium. Varying mechanical conditions could modulate the action potentials differently in the normal and abnormal areas, and thus alter the extent of S-T segment elevation. Lekven et al. (1980) found that ventricular distension during regional ischemia induced S-T segment changes that depended on whether the distension was produced by volume infusion or aortic constriction. Although their plausible explanation was based on differential changes in blood flow, it is of interest that they noted length changes in ischemic segments that would be predicted by contraction-excitation feedback.

Threshold Depolarizations and Ventricular Arrhythmias. A conduction block probably produces some localized contraction abnormality, and thus provides a setting for contraction-excitation feedback. Regular coupled extrasystoles, alternating with irregular parasystoles, may be related to mechanically induced depolarizations. During parasystole, the focus is "protected" from the normal ventricular action potential by an entrance block which may be mechanically transcended for coupling. Such alternating arrhythmias are difficult to explain using the re-entry theory (Schamroth, 1980), and Figure 4A from a preliminary report suggests that contraction-excitation feedback may also provide an explanation. In this experiment, occlusion of the pulmonary artery in an intact heart in situ produced changes in wall motion, transient depolarizations, and regular coupled extrasystoles often alternating with irregular rhythms (Covell et al., 1981).

Finally, early ischemic arrhythmia may also be viewed in the light of contraction-excitation feedback. An ischemic segment is stretched during systole and shortens late in relaxation, i.e., it is dyskinetic (Tyberg et al., 1974; Forrester et al., 1976). As a result, such regions of the myocardium that are still responsive could generate mechanically induced extrasystoles (Figs. 2C and 4A). Preliminary studies show transient depolarizations on the monophasic action potential accompanying segment dyskinesia. These electrical changes can be associated with threshold depolarizations (Lab, 1978b), and Figure 4B is an example in which ventricular fibrillation was precipitated. Within 1 hour of coronary occlusion, ventricular extrasystoles (and often fibrillation) were regularly found. During this period, transient depolarizations were consistently observed, together with segment dyskinesia. Although an ubiquitous electrical artifact must be excluded before contraction-excitation feedback is added to the other membrane factors as a cause of ventricular extrasystoles (Arnsdorf, 1977; Hauswirth and Singh, 1978; Cranefield, 1977; Cranefield and Wit, 1979; Hoffman and Rosen, 1981), this mechanism warrants further study as fibrillation could occur in situations in which there is depressed conduction, re-entry, and altered automaticity.

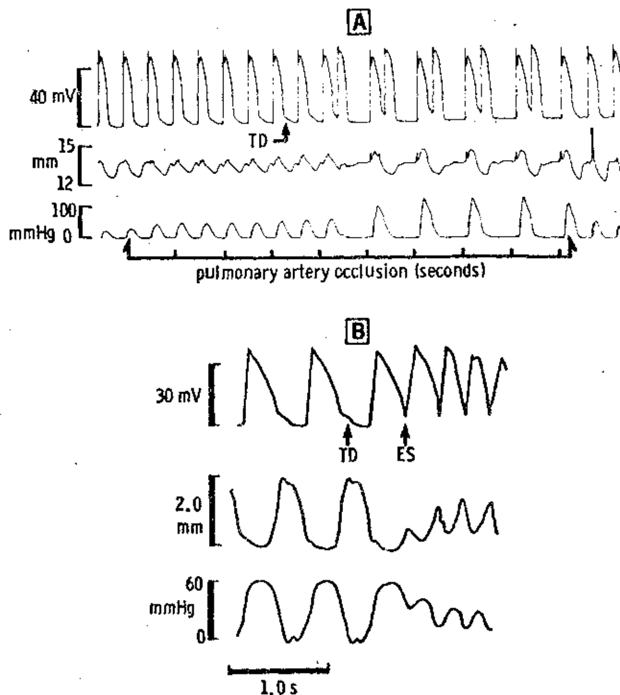

FIGURE 4. *Changes in action potential and segment length from the epicardium of intact ventricles in situ. Traces from above down: monophasic action potential, epicardial segment length, intraventricular pressure. A: Records from right ventricle of an anesthetized dog. Pulmonary artery occlusion increases right ventricular pressure and segment length, causes changes in segment contraction, and produces transient depolarizations (TD) on the action potential. Coupled right ventricular extrasystoles follow. (Modified with permission: Covell et al., 1981.) B: Records from a pig left ventricle during regional ischemia. The action potential shows transient depolarizations (TD) which are related to the stretch and rapid late systolic shortening of the segment (upward is shortening). A premature beat arises during the crest of the depolarization (ES), precipitating ventricular tachycardia and eventual fibrillation within 5 minutes of the coronary occlusion (unpublished record, reported in Lab, 1978b).*

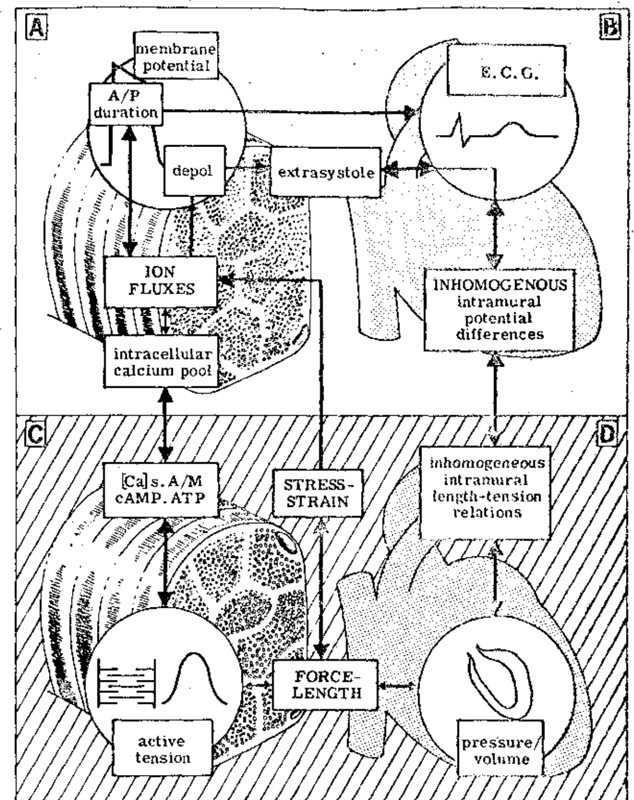

FIGURE 5. *Scheme of some possible interrelationships between excitation-contraction coupling and contraction-excitation feedback in ventricular muscle. The diagram has four quadrants—the lower, hatched, half containing the mechanical, and the upper the electrical events. The lefthand side of the diagram depicts the events at cellular level, and the righthand side indicates their role in the intact ventricle. (In quadrant C A/M = actin and myosin.) See text for discussion.*

Figure 5 summarizes contraction-excitation feedback and its possible ramifications. (1) Contraction and mechanical changes influence myocardial electrophysiologic properties (quadrant C to A). In particular, stretch followed by shortening can produce transient depolarizations which prolong action potentials, or produce discrete depolarizations that can precipitate premature beats. (2) The mechanism is undefined. One or more tenable mechanisms, common to both mechanical and membrane events, may arise from changes in calcium kinetics and perhaps in specific phosphorylation of selected intracellular sites, although an altered architecture cannot be excluded. (3) The normally contracting left ventricle provides a suitable microenvironment for the expression of contraction-excitation feedback. Inhomogeneous wall motion can produce electrophysiological inhomogeneity (quadrant D to B) that can influence the T-wave and, conceivably, produce U-waves in the ECG. (4) Regional ischemia exaggerates mechanical inhomogeneities and magnifies the expression of the feedback. Contraction-excitation feedback can generate "extrasystoles" that may contribute to ventricular arrhythmia in early ischemia.

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[*From the Proceedings of the Physiological Society*, 26–27 *March* 1971]
Journal of Physiology, **216**, 44–45*P*

Comparison of action potentials from endocardial and epicardial surfaces of frog ventricle

BY M. J. LAB. *Department of Physiology, Charing Cross Hospital Medical School, London, W.C.2*

The QRS complex and T wave are usually both positive or both negative-going in a normal electrocardiogram. This stems from the apparent anomaly that, in the intact ventricle, the muscle fibres that depolarize last tend to repolarize first, the epicardium recovering before the endocardium.

To confirm this, three simultaneous records were obtained; the electrocardiogram, and monophasic action potentials from endocardial and from eipicardial surfaces, the latter recorded by suction electrodes as used by Hoffmann, Cranefield, Lepeshkin, Surawicz & Herrlich (1959). The single ventricles of *Xenopus laevis* were perfused with Ringer solution via the aorta with the venae cavae tied off. The endocardial electrode entered the ventricle transmurally or via the aorta, with the outside electrode on the epicardial surface of the same segment. The bipolar electrocardiogram was recorded from outside the heart or with one electrode intraventricular. Repolarization was thus shown to be faster in epicardial than in endocardial fibres (Fig. 1*A*).

A possible mechanism is suggested which may help to explain this curious phenomenon. A feed-back mechanism has been described in ventricular muscle whereby its contraction may influence its time course of repolarization (Stauch, 1966; Lab, 1968, 1969; Kaufmann, Hennekes & Lab, 1971); one feature is an accelerated repolarization following isometric, or limited, contraction. This may be an important contribution to the ventricular repolarization gradient since in a normally contracting ventricle the epicardial circumference shortens proportionately less than the endocardial circumference (Rushmer, 1961). If the inner radius of the ventricle approaches the outer, it can be shown that the percentage differences in shortening of inner and outer muscle layers will decrease. Thus, if the mechanical conditions of the endo- and epicardial musculature approximate, so should the characteristics of the electrical recovery. These mechanical conditions were obtained by operating the ventricle at increased volume (Rushmer, 1961); the anticipated electrical conditions resulted (Fig. 1*A* and *B*).

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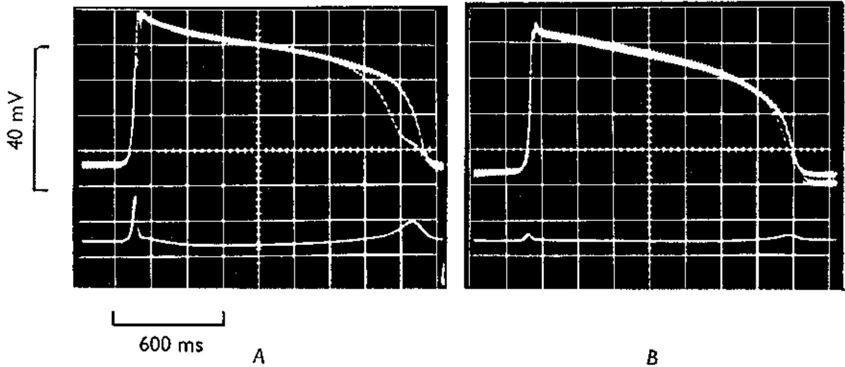

Fig. 1. Traces from above downwards (*a*) action potential from endocardial surface, partially superimposed on: (*b*) trace from epicardial surface; (*c*) electrocardiogram. Part *A* is from a ventricle with a small diastolic volume. The action potential from the epicardial surface has a faster repolarization phase than that from the endocardial surface. The T wave is prominent. Part *B* shows recordings from the same ventricle with a larger diastolic volume. The difference in time course of repolarization between endocardial and epicardial action potentials is less marked and the T wave is flattened.

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[*From the Proceedings of the Physiological Society*, 21–22 *April* 1972]
Journal of Physiology, **224**, 55–56*P*

A calliper for sampling relative movement of tissue surfaces

BY MAX J. LAB and G. A. MORRIS. *Department of Physiology, Charing Cross Hospital Medical School, Chandos Place, London, W.C.2*

During investigations on the ventricles of intact frog hearts (Lab, 1971) it was necessary to record qualitatively the relative movement of two points on either side of an electrode on the endocardium. The usual methods,

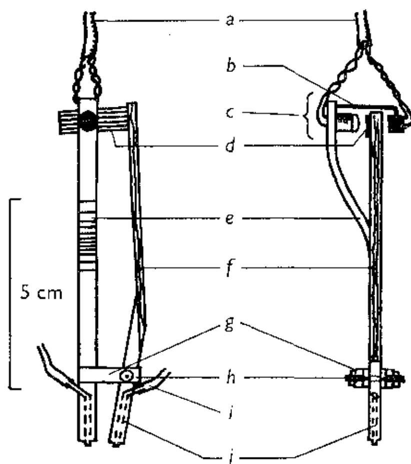

Fig. 1. The calliper is made mostly from 1 × 3 mm Perspex. One of the arms (*e*) has a small double cross-piece (*g*) fixed to it so that the other arm (*f*), weighing less than 200 mg, is gripped by and can pivot between, this cross-piece. The pivot (*h*) consists of two sharp-pointed screws in tapped holes in the Perspex in each branch of the cross-piece. They are directed inwards to a small hole in the pivoting arm. When the screws are adjusted in the hole and secured by lock nuts the pivoting arm can turn freely. The ends of the calliper nearest the heart have 1 mm diameter holes (*j*) drilled longitudinally to meet a length of hypodermic needle of the same diameter pushed into the Perspex near the pivot. This location reduces the drag due to the flexible polyethylene tubing, 1 mm i.d. (*i*), which is pushed over the needles. The tubing is then connected to a vacuum of 40–60 cm Hg. The lamp and a lens of short focal length is held by a small cylinder near the end of the non-pivoting arm. The photo-transistor, on to which the lens focuses, is also fixed to this arm by a piece of stiff, bent wire (*b*). The movement of the pivoting arm is converted to an electric signal by the photo-sensing assembly (*c*). *a*, Leads; *d*, graded density film.

of suturing mercury in rubber and wire strain gauges to the endocardium, were thus unsuitable. There was also the additional difficulty of working with a small heart as well as having to record from different parts of the

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heart at different times. A light-weight calliper was therefore developed which could be fixed to the tissue surface by vacuum.

The Perspex calliper (Fig. 1) is shaped like an elongated letter H with a double 'cross-piece' (*g*) near the lower end. The cross-piece allows one of the arms of the calliper to pivot. To minimize inertia, the greater part of this pivoting arm is of balsa wood (*f*) which is made to incline towards the stable arm. A graded density film (*d*) is glued to its upper extremity. The non-pivoting arm (*e*) is cranked so that a photo-electric transducer assembly (*c*) can be mounted to allow the film (*d*) to move between a light source and photo-transistor. The circuit of the photo-electric system has been previously described (Lewis, 1969). An additional focusing lens is included in front of the lamp and the circuitry is remote from lamp and photo-transistor, except for the 1 K resistor which is glued to the assembly to reduce the number of leads from it.

Longitudinal holes (*j*) at the short ends of the calliper allow it to adhere with suction to the surface of the heart by vacuum from a conventional water pump. The instrument is held by the flexible leads (*a*) and, with the vacuum tubing suitably draped, drag is minimized.

If the calliper tips are close (2–5 mm apart) the readings of tissue surface movement are reasonably quantitative for large hearts or for relatively flat surfaces. For hearts, e.g. frog, with small radii of curvature, however, the discrepancy between the chord length, which the device actually measures, and the arc length becomes greater. The measurements are thus now mainly qualitative.

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XXV INTERNATIONAL CONGRESS OF PHYSIOLOGICAL SCIENCES

CHANGES IN MYOCARDIAL REPOLARIZATION ASSOCIATED WITH CHANGES IN CONTRACTION.

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A feedback between contraction of cardiac muscle and its electrical activity has been demonstrated in intact isolated frog heart, papillary muscle, and mammalian heart in situ. Isometric or isovolumic myocardial contraction shows faster repolarization than isotonic contraction. But in the intact isovolumic ventricle this faster repolarization phase, as recorded by a suction electrode, is interrupted by a hump-like after-potential which may reach threshold and initiate a propagated action potential. Similar electrical events can be obtained when a microelectrode recording is made from an isometrically contracting papillary muscle if it undergoes a "quick release" during the decline of its active state. Out-of-phase movements of the epicardium found during isovolumic contraction may produce mechanical conditions analagous to the quick release. The same mechanism may give rise to changes seen in action potentials of intact or isolated cardiac muscle.

Reprinted from PROCEEDINGS OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES – Volume IX
Printed in the Federal Republic of Germany

XXVI INTERNATIONAL CONGRESS OF PHYSIOLOGICAL SCIENCES

MECHANO-ELECTRIC COUPLING IN FROG VENTRICULAR MUSCLE

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Effects on recorded potentials, of stretching frog ventricular strip at different times, are observed. Monophasic records of muscle action potentials are derived from two sections of a strip mounted in chambers containing Ringer, and procaine-sucrose, respectively. The length and tension of the strip in the Ringer chamber are simultaneously measured. From the strip's slack length, transient stretches of arbitrary magnitude (but not exceeding L_{max}) are imposed. The stretches result in a deviation of the recorded potential. A stretch during the "plateau" (phase 2) of the action potential produces a polarising potential, whereas the identical stretch during quiescence causes a depolarising potential. The depolarisation may reach threshold, initiating a new action potential. There is a phase of transition roughly corresponding to the rapid repolarisation phase, 3, of the action potential, when a stretch causes no observable effect on the potential. Possible mechanisms which could influence the transfer of charge across the membrane, thus effecting a de- or repolarisation, may be in terms of (a) an increase in non-specific membrane permeability; (b) stress and strain, within the muscle, giving rise to Potassium accumulation just outside the cell. But additional mechanisms may operate during active tension development: changes in internal Calcium ion concentration affecting ionic flux; and/or an "energy" link between contraction and membrane ionic flux.

Reprinted from PROCEEDINGS OF THE
INTERNATIONAL UNION OF PHYSIOLOGICAL
SCIENCES—Volume XI—NEW DELHI 1974
Printed in India

[*From the Proceedings of the Physiological Society*, 14–15 *July* 1978
Journal of Physiology, **284**, 143–144*P*]

Depolarization produced by mechanical changes in normal and abnormal myocardium

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I have previously demonstrated that altering the mechanical conditions of contraction of rat ventricle *in situ* changes the recorded monophasic action potential (Lab, 1969). In particular, an afterdepolarization, apparently capable of reaching threshold, was noted on isovolumic contraction as compared to a normal action potential during auxotonic contraction. This mechanically induced change in action potential also occurred in segments of the intact isolated frog ventricle (Lab, 1978), and a possibly related phenomenon has been previously investigated in cat papillary muscle using micro-electrodes; it was described as an excitation–contraction–excitation feed-back loop (Kaufmann, Lab, Hennekes & Krause, 1971). In the latter studies an afterdepolarization was seen, occasionally attaining threshold, when an isometrically contracting muscle was released after peak developed tension was reached.

In the present investigation I wanted to see to what extent the mechano-electric relationship observed previously in the isolated myocardial studies, obtained in segments of normal and ischaemic pig ventricle *in situ*. This relationship may partly explain the afterdepolarization observed in the intact pig ventricle, and have some bearing on ectopic beats found in selected clinical situations. I opened the chests of pigs, anaesthetized with 1 % halothane in a 1:1 mixture of O_2 and NO_2, and studied ventricular segments using a tripodal, suction-operated device for simultaneously obtaining length changes and monophasic action potentials from the epicardium (Lab & Woollard, 1978). Arterial and intraventricular pressure were also monitored to determine the various phases of the cardiac cycle. When the aorta was constricted by a pneumatically operated snare (Jewell, 1969), peak intraventricular pressure increased. With these loaded contractions epicardial segments often showed lengthening, instead of shortening, during much of systole. The segment thereafter rapidly shortened. During this inhomogeneous contraction pattern afterdepolarizations in the action potential may develop which could apparently reach threshold for a new action potential. Occlusion of the coronary artery supplying a segment of the ventricle during normal contraction invariably leads to systolic lengthening, followed by shortening (Tennant & Wiggers, 1935) and which therefore should also be accompanied by afterdepolarization with extrasystoles. Preliminary observations showed that this was indeed so.

The precise relationshp between a stretch, release and the accompanying electrical change is uncertain due to the complex interaction of intramural forces and lengths in the left ventricle. However, it is clear that, as with the isolated preparation, mechanically induced changes in action potential can occur in normal and ischaemic segments of the intact mammalian ventricle *in situ*. Further, the interesting possibility exists that mechanically induced action potentials in ischaemic myocardium may be a mechanism by which potentially lethal ventricular arrhythmias may arise.

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[*From the Proceedings of the Physiological Society*, 17–18 *July* 1981
Journal of Physiology, **320**, 34*P*]

Mechanical induction of paired action potentials in intact heart *in situ*

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The mechanism for abnormal heart rhythms that alternate between irregular beats (parasystole), and paired or coupled beats, is not clear. This communication suggests a mechanical cause for analogous rhythm disturbances and presents a model for studying them.

Mongrel dogs were anaesthetized with Na-pentobarbitone. The heart was exposed, a snare placed around the pulmonary artery, and segments of the right ventricle monitored for: length using ultrasonic crystals (Theroux, Franklin, Ross & Kemper, 1974), monophasic action potentials (Lab & Woollard, 1978), and intramural e.c.g. Intraventricular pressures and normal lead II e.c.g. were also measured.

Pulmonary artery occlusion increased right ventricular pressure and segment length, caused changes in segment contraction, and produced after-depolarizations (transient depolarizations) on the terminal phase of the action potential. The depolarizations progressively increased in size during the occlusion and were associated with multiple right ventricular extrasystoles in about 90 % of seventy-four occlusions in four dogs (Fig. 1). Periods of coupled rhythm occurred in 64 % of the occlusions, often alternating with the irregular rhythms.

The mechanically-induced, transient depolarization may be the causal link between the altered mechanics and the arhythmia (Lab, 1978), but other mechanisms remain. This experimental model provides an interesting tool for use in further investigations.

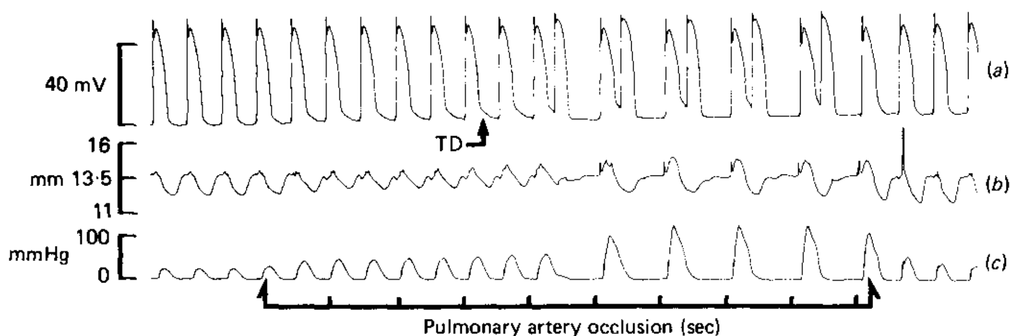

Fig. 1. (*a*) Monophasic action potentials; (*b*) segment length; (*c*) right ventricular pressure. Pulmonary occlusion produced transient depolarizations (TD) and paired beats.

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