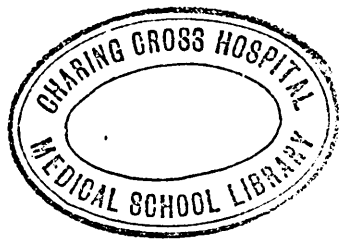


REGIONAL DIFFERENCES IN ELECTRICAL ACTIVITY
IN THE LEFT VENTRICLE UNDER DIFFERENT
MECHANICAL CONDITIONS OF CONTRACTION

by

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I dedicate the work of this thesis to
my father, mother and my brother Samir, who
made the achievement of an ambition possible

Thank you very much.

ABSTRACT

The purpose of my project was to investigate the effects of changes in the mechanics of contraction on the electrophysiological behaviour of the intact mammalian left ventricle.

The experimental preparations used were the isolated rabbit heart, and the heart of the intact open-chested pig. Monophasic action potentials and the epicardial ecg were recorded from the left ventricle while the ventricle contracted under free loaded or isovolumic conditions. Under these conditions action potential durations were measured at steady state and during changes in rate and the durations were used to construct electrical restitution curves. Over most areas of the left ventricle of the rabbit, the basal steady state action potential duration, measured at 70% repolarization, was shorter under isovolumic as compared to free loaded (normal) contraction. This was a regional effect since the posterior middle region, between the base and apex, was not generally affected. The influence of a change in the mode of contraction on the time course of recovery of action potential was also regional and, following the examination of the restitution curves, could be divided into effects on the plateau, and on the time course. There was a consistent mechanically dependent downward shift of the plateau, and depending on the region of recording, either an acceleration or slowing of the time course of the restitution curve. The effects of changing the mechanics of contraction on the restitution curve was abolished with low extracellular Ca^{++} (0.5mM) perfusion. Under high K^{+} (8mM) perfusion the mechanically dependent downward shift was maintained but the effect on the time course was significantly reduced. At low temperature (24°C) isovolumic

contraction shifted the plateau of the restitution curve down but had little effect on the time course of the curve compared with free loaded contraction.

The effects on the action potential of changing the mode of contraction in the intact open-chested pig, is in agreement with the observation in the isolated rabbit heart. Isovolumic contraction, produced by clamping the aorta, shifted the plateau of the restitution curve down, and slowed its time course.

The effects of changing the mechanics of contraction from free loaded (normal) to isovolumic on the epicardial ecg were also consistent with the action potential changes. This manoeuvre depressed the T-wave and reduced the Q-T interval. The consistency also applied with rate changes and different ionic perfusion in the rabbit heart. The behaviour of the T-wave was different under isovolumic from that under free loaded contraction when increasing the rate; and the T-wave change was greater under high K^+ perfusion but abolished by low Ca^{++} perfusion. There is thus a concordance of the electrophysiological findings; first with local action potentials and ecg; and second in two types of preparation, isolated solution-perfused heart and intact heart insitu.

The results suggest that the mechanically dependent downward shift of the restitution curve under isovolumic contraction could be due to intracellular accumulation of Ca^{++} and possibly extracellular accumulation of K^+ . The mechanical effect on the time course of recovery is consistent with some change in ionic exchange currents, e.g. the $Na^+ - K^+$ ATPase pump activity or $Na^+ - Ca^{++}$ exchange. But other transmembrane currents need consideration.

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ABBREVIATIONS

AP	Action Potential
APD	Action Potential Duration
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanine monophosphate
E-C coupling	Excitation-Contraction coupling
[X]	Concentration of substance X.
$[Ca^{++}]_i, [Ca^{++}]_o$	Intracellular and Extracellular (in the perfusing Krebs) concentration of calcium respectively.
$[Na^+]_i, [Na^+]_o$	Intracellular and extracellular sodium concentration (respectively).
$[K^+]_i, [K^+]_o$	Intracellular and extracellular concentration of potassium respectively.
$[K^+]_e$	Potassium concentration in the extracellular spaces (clefts).
E_m	Membrane potential
I_{Na}, i_{Na}	Sodium current
I_{Ca}, i_{Ca}	Calcium current
I_{si}	Slow inward current
I_k, I_x	Potassium currents
ecg	Electrocardiogram
wt.	weight
DNP	Dinitrophenol
TPI	Test Pulse Interval

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Chapter 1

INTRODUCTION

Regional differences in action potential duration were first suggested 100 years ago by Bayliss and Starling to explain the genesis of the T-wave of the electrocardiogram and its positivity in mammalian hearts. In the last two decades regional differences in action potential duration have been established in the left ventricle of dog, sheep, pig, rabbit and rat heart, and this could be common to all mammalian and amphibian hearts. Also regional differences in the electrophysiological behaviour have been reported in isolated strips of sheep ventricular muscle (Cohen et al 1976) and isolated rabbit heart, as well as in insitu pig heart (Lab and Yardley unpublished data). These differences were manifest by the regional differences in the degree of action potential shortening or prolongation in response to a change in rate, e.g. in the first beat following a change in frequency the change in action potential duration was greater at the apex than at the base of the left ventricle in an intact insitu pig heart (Lab & Yardley 1979). Also the electrical restitution curves constructed from different areas were shown to be different at the rising phase as well as at the level of the plateau (Lab & Yardley unpublished data).

Stauch (1960), as cited by Lab (1982), was the first to demonstrate that the mechanics of contraction could effect the electrophysiology as shown by shorter Q-T interval of the ecg in an isovolumically contracting frog ventricle as compared with an auxotonic beating heart. This was followed by the demonstration that action potential duration, in an isolated ventricular muscle (Kaufmann et al 1971) as well as in isolated intact left ventricle, including that insitu (Lab 1982),

was shorter under isovolumic contraction than under normal (isotonic) contraction. These findings suggest that Excitation Contraction coupling is not strictly unidirectional; thus a contraction-excitation feed-back in myocardium seems to exist (Kaufmann et al 1971). Unfortunately this has attracted little attention despite its potential physiological and clinical importance.

Besides regional electrophysiological differences, the evidence is accumulating for the existence of regional biochemical and mechanical contraction differences in the normal left ventricle but it is too early to say whether these differences are responsible for the regional electrophysiological differences.

It was, therefore, of great interest to investigate the effects of mode of contraction on the regional differences in electrophysiological behaviour in the left ventricle by studying the changes in the repolarization phase of the action potential in response to changes in rates. Also an attempt has been made in this investigation to characterise some of the mechanisms that may be responsible for the effect of mechanics on the action potential by constructing electrical restitution curves, which is a powerful tool in studying membrane recovery.

A modified Langendorff rabbit preparation (Quasi-Working) was used to carry out the study, which enabled me to change the mechanics of contraction quickly and easily without interfering with other parameters.

This has several advantages over the working preparation (chapter 6) but to confirm the findings the study was also carried out on insitu pig preparation because the isolated perfused

preparation suffers from limitation in the amount of oxygen that can be delivered to the myocardial cells

Monophasic action potentials were recorded from different areas by suction electrode which has been shown to be reliable for action potential duration measurements (chapter 6).

The results show that the action potential duration, at 70% repolarization, was shorter under isovolumic contraction in both preparations as compared with free loaded (normal) contraction, but this effect was regional in the isolated perfused rabbit heart.

Also the degree of action potential shortening with increases in rate was different under the two mechanical conditions and this effect seemed to be regional, (chapter 7). The plateau of the restitution curve (chapter 8) was shifted downwards but depending on the region of recording either an acceleration or slowing of the time course was produced by changing the mechanics of contraction to isovolumic. These results, therefore, are in support of the suggestion that the regional electrophysiological differences could be attributed to regional differences in the mechanics of contraction.

How changes in the mechanics of contraction produce its effects on the AP is not clear, but the restitution curves results suggest some possible mechanisms involving the handling of Ca^{++} , and probably K^+ . Therefore, it was decided to investigate the effects of lowering the perfusing $[\text{Ca}^{++}]$ and increasing the perfusing $[\text{K}^+]$ on the effects of isovolumic contraction on the restitution curves. Chapters 9 and 10 respectively, describe the results of such studies. The results also threw some light at the possible involvement of the $\text{Na}^+ - \text{K}^+$ pump in producing the effects of isovolumic contraction on

the time course of the electrical restitution curve. An attempt was made to reduce the activity of the pump, by lowering the temperature of the perfusing Krebs to 24°C, and to study the effects of mechanics on the restitution curves in the isolated rabbit heart under such conditions (chapter 11).

As a confirmatory study, the effects of changing the mechanics of contraction to isovolumic on the Q-T interval and the T-wave behaviour in response to rate changes were briefly investigated under different ionic conditions and under low temperature perfusion (chapter 11).

Possible explanations and speculative mechanisms are discussed in chapter 13.

Chapter 2

The Action Potential in Ventricular Myocardial Fibres

The complexity of the cardiac processes in comparison to other tissues, like skeletal muscle, becomes apparent when we examine the form and ionic basis of the cardiac AP. It consists of several phases and varies in its duration, shape and amplitude from region to region within the heart. Despite this variation, the ventricular AP is generally characterized by fast phase of depolarization (phase 0) followed by a long lasting plateau (phase 2) which is largely responsible for the long duration of the AP and finally repolarization phase (phase 3).

What I have studied in this thesis is the effects of changing the mechanics of contraction on the ventricular APD and its recovery. Since it will be seen that a change in the characteristics of some of the currents, that are responsible for the genesis of the AP, can explain the phenomena. I, therefore, will describe only those currents that determine the duration of the AP, i.e. the currents during the plateau (phase 2) and during the repolarization phase (phase 3).

2.1 The plateau of the action potential (Phase 2)

The most distinctive feature of the electrical depolarization of cardiac muscle is the plateau which is responsible for the prolonged cardiac action potential. There is overwhelming evidence to show that a slow inward current carried by Ca^{++} , i_{Ca} , is mostly responsible for the long plateau of the cardiac action potential.

The first evidence for the existence of a slow inward current came from voltage-clamp experiments on Purkinje fibres perfused with Na^+ -free solution (Reuter 1967). The existence of this slow inward current was also demonstrated in cardiac ventricular muscle of various species and seems to be general

for mammalian ventricular muscle (Rougier 1969, Mascher and Peper 1969, Beeler and Reuter 1970a, McDonald and Trautwein 1978a).

In comparison to the fast sodium current, which is responsible for the fast upstroke of the AP the maximum value of the slow current is much less and the reversal potential is more positive. Beeler and Reuter (1970b) with voltage-clamp experiments on dog ventricle held the membrane potential at -40mV, therefore, inactivating completely the Na^+ current. In response to depolarization from -40mV to -25mV in the presence of 1.8mM Ca^{++} , they clearly showed an inward current, that required about 200 ms to inactivate, and was absent in the Ca^{++} free solution. The magnitude of the i_{Ca} has been shown to depend on the concentration of extracellular Ca^{++} (Beeler and Reuter 1970a), increasing with increasing extracellular Ca^{++} concentration and vice versa. Rougier et al (1969) showed that this slow inward current was insensitive to TTX and was not abolished by depolarization which inactivated the gating mechanism for sodium (see above); on the other hand they showed that cations like Mn^{++} could abolish this current.

this slow inward current is insensitive to TTX and is not abolished by depolarizations which inactivates the gating mechanism for sodium (see above): on the other hand they showed that cations like Mn^{++} can abolish this current.

The current has also been shown to be reduced by Ca^{++} blockers like Verapamil and D600 which also reduced the plateau phase of the cardiac action potential.

Rougier et al (1969) have also shown that the Ca^{++} channels can conduct some sodium ions in frog atrial preparations. They observed

some inward current in TTX Ringer, free of Ca^{++} . Therefore, besides Ca^{++} ions, the slow channel seems also to accept Na^+ ions, mainly after removal of Ca^{++} ions. The extent to which Ca^{++} and Na^+ contribute to the slow inward current differs among atrial, ventricular and purkinje preparations, and varies from one species to another (Carmeliet and Vereecke 1980). In ventricular muscle of dog and cat changing the Na^+ concentration does not greatly effect the slow inward current. In purkinje fibre and in ventricular fibres of the guinea pig, cow and the rat the contribution of Na^+ is important (Carmeliet and Vereecke 1980).

i) Activation of i_{Ca}

Steady state activation curves have been obtained for different muscle preparations (frog, cat, sheep, pig and cow). The steady state activation curve is S-shaped. It saturates at about 0mV membrane potential. The activation time, measured as the time to peak current is longest at approximately threshold. In purkinje fibres the experimental values range from 10-50ms. In mammalian cardiac muscle the activation times are 20-30ms near threshold, and 8-10ms near 0mV. (See Carmeliet and Vereecke 1980, for references).

ii) Inactivation of i_{Ca}

The inactivation of the i_{Ca} has been extensively studied in dog, (Beeler and Reuter 1970b), cow (Reuter and Scholz 1977) and guinea pig (Gettes and Reuter 1974). In dog the time constants steeply increase between -35mV and +40mV with extreme values of 50ms near

threshold and 400ms at potentials more positive than zero. A similar potential dependence was observed in pig and cow. In frog ventricle the inactivation process is faster than in mammalian preparations. At -30mV to -20mV the time constant is about 50ms, it decreases at more positive potentials to about 20ms at +20 mV (Rougier et al 1969).

Lee et al (1983 Edinburgh phys. soc. meeting) showed an evidence from single heart cells to suggest that I_{si} is too brief to maintain a long plateau. They have also identified a Cd-resistant inward current that is different from I_{si} . The current is carried by Ca, voltage-dependent and very slow. At -60mV the current requires more than 200 ms to reach a peak and shows negligible inactivation. At -20mV inactivation occurs with a half time of about 350ms.

Recovery of I_{si}

The recovery of I_{si} from inactivation has been studied in various cardiac preparations over a wide range of potentials. Gettes and Reuter (1974) studied the recovery of I_{si} by voltage clamp technique in pig ventricular trabeculae at different holding potentials at 37°C. The time course whereby the I_{si} regained the steady state value at each potential could be fitted by exponentials with time constants which increased from about 60msec, when the membrane potential during repolarization was clamped to between -70 to -75mV, to 250 msec when the membrane was clamped to -9mV.

In cat ventricular muscle Trautwein et al (1975) found an exponential time course for I_{si} recovery with time constant equal to 118 msec at -80mV, 144 msec at -60mV and 198 msec at -40mV, also at 37°C whereas Kohlhardt et al (1975) found values ranging from 200 ms to 350 ms between -30 mV and -10 mV. In agreement with the above results Reuter and Scholtz (1977), using voltage-clamped ventricular trabeculae found the time course, whereby I_{si} approached the steady state, was exponential and the time constants of these exponentials increased as the membrane potential during hyperpolarization became more positive, between -70 and -10 mV. But the relation between membrane potential and the time course of I_{si} recovery was steeper in the study of Reuter and Scholtz as compared to the other above studies of I_{si} recovery.

The above results suggest that the time course of I_{si} recovery in ventricular muscle increases, as the membrane potential becomes less negative. At potentials between about -80 mV and -30 to -40mV the change is small, but becomes increasingly larger at more positive potentials than -40 mV. In general therefore, the recovery of I_{si} becomes slower at less negative potentials especially at less negative potentials than about -40 to -30 mV.

2.1.4 Factors affecting I_{si} Kinetics

Beside membrane potential, temperature, intracellular and extracellular Ca can modify I_{si} kinetics.

i Temperature Modulation of I_{si}

In guinea pig papillary muscle, Gettes and Reuter (1974) found the time constant of I_{si} recovery (as indicated by the recovery of AP plateau duration) was increased from about 40 msec to about 75 msec at -75 mV when the temperature of the preparation was lowered from 37°C to 27°C. In the same preparation Q10 ranged between 1.5 - 2.3 within the potential range of -84 to -65 mV. In keeping with these results Kohlhardt et al (1975) analysed I_{si} kinetics in cats ventricular myocardium within a range between 32°C and 22°C. Cooling caused an increase of time to peak current and time constant of recovery. Temperature coefficients (Q10) of 2.7 and 2.3 respectively were obtained. In contrast the magnitude of I_{si} proved less sensitive to change in temperature. McDonald and Trautwein (1978a), using single sucrose gap technique on cat ventricular muscle, depolarized the preparation from -60mV to 0mV for appropriate durations and found that the deactivation rate of I_{si} increased as the temperature was raised from 24 to 31°C and then to 37°C. The estimated value of Q10 was about 3.

The above results, therefore, suggest that the rate of activation, inactivation and recovery of I_{si} are slower at low temperatures.

ii $[Ca]_o$ Modulation of I_{si}

It is an interesting observation that variations in external Ca concentration not only influence the magnitude of I_{si} in various ventricular preparations (Mascher and Peper 1969, Beeler and Reuter 1970a, Kohlhardt et al 1975, Reuter and Sholtz 1977), but simultaneously change the time course of I_{si} .

An increase in $[Ca]_o$ shortens the time to peak current and accelerates inactivation. In cat ventricular muscle, Kohlhardt (1975) observed increased rate of I_{si} inactivation when Ca_o was raised from 1.8 to 8.2 mM.

Beeler and Reuter (1970a) looked at the magnitude of I_{si} , at 0.2, 1.8 and 7.2 mM $[Ca]_o$, in dog ventricular trabeculae and found increased magnitude with increasing $[Ca]_o$. The current voltage relation in their study was shifted upward towards less I_{si} , when $[Ca]_o$ was lowered. Mascher and Peper (1969), Kohlhardt et al (1975) and Reuter and Scholtz (1977) found similar dependency of I_{si} on $[Ca]_o$ in sheep, cat and cow ventricular trabeculae respectively.

It was suggested that this increase in I_{si} was attributed to an increase in the inflow driving force for Ca.

iii $[Ca]_i$ Modulation of I_{si}

From section ii it can be predicted that if $[Ca]_i$ is elevated, I_{si} magnitude would be reduced. The experimental evidence is in keeping with the prediction. When long voltage clamp pulses were applied to sheep ventricular muscle, Bassingthwaite and Reuter (1972) were cited by Boyett and Jewell (1980) to have observed a reduction of the reversal potential of I_{si} which they attributed to intracellular accumulation of Ca^{++} . If the fast inward Na^+ current of the AP is abolished, slowly rising AP's which are mediated by I_{si} can be produced upon large enough excitation (Reuter 1973). The rate of rise and overshoot of these Ca^{++} mediated AP's have been used as indices of I_{si} .

Tritthart et al (1976) found the rate of rise, overshoot and duration of these AP's to be markedly reduced when the frequency of stimulation was increased. It was suggested that increasing Ca_i decreased the driving force for the inflow of Ca^{++} which in turn resulted in decreased I_{si} . When the rate was decreased the rate of rise, overshoot and duration returned slowly to normal. Simurda et al (1976) showed a progressive decrease of I_{si} in cat ventricular trabeculae and papillary muscle during repetitive stimulation by voltage-clamp depolarization in spite of several fold increase

in contractility. They suggested that the decrease in I_{si} was due to a decrease in Ca^{++} driving force as a consequence of intracellular accumulation of Ca^{++} which produced the observed increase in contractility. Also in some experiments of Beeler and Reuter (1970a) on dog ventricular muscle, a reduction was observed in I_{si} after the removal of Na^+ from the bathing solution which they attributed to the increased internal accumulation of Ca^{++} which in turn resulted in a decrease in the Ca^{++} inflow driving force.

In contradiction to the above results Isenberg (1977b) looked at the affect of intracellular injection of Ca^{++} on I_{si} in sheep purkinje fibres. The result was curious in that he obtained an increase in I_{si} , not a decrease.

He suggested two explanations:-

1. The injected Ca^{++} was rapidly removed from the cell via an extrusion process, which produced an increase in the extracellular $[Ca^{++}]$ and the transmembrane Ca^{++} concentration gradient.
2. Ca^{++} increases its own conductance.

The effect of increased $[Ca^{++}]_{o,i}$ on the kinetics of I_{si} is not clear. Kohlhardt et al (1975) found elevated Ca^{++}_o capable of speeding rate of activation, inactivation and recovery of I_{si} in cat ventricular trabeculae whereas Simurda et al (1976) observed slower activation and inactivation during repetitive voltage-clamp pulses also in cat ventricular trabeculae which they attributed to elevated Ca^{++}_i . In the study of Isenberg (1977b) he observed increased rate of activation and inactivation of I_{si} which is the opposite of Simurda's et al study for an increase of $[Ca^{++}]_i$.

2.1.5 Role of I_{si} in maintaining the AP plateau

Beeler and Reuter (1970a) studied the voltage dependence of I_{si} inactivation rate in dog ventricular muscle. At more negative potentials than -30 mV they found the rate of inactivation to be fast but, in the region of the plateau (positive to 0mV) the rate of decay was very slow.

The time constant at more positive potential than 0mV was about 500ms which is comparable with the ventricular action potential duration. The I_{si} current therefore decays slowly during the plateau of the ventricular action potential. In agreement with this, Gettes and Reuter (1974) found the time constants for the AP plateau to regain its steady state value correspond to the time constants for reactivation of the slow inward calcium current in pig ventricular trabeculae. In keeping with this they found similar voltage and temperature dependence of the recovery of the plateau duration and the recovery of I_{si} and concluded that the duration of the AP plateau is maintained by I_{si} . Reuter and Scholtz (1977) studied the activation and inactivation kinetics of I_{si} in cow ventricular trabeculae and related the findings to the plateau of the AP and have reached similar conclusion as in the above studies.

2.2 Repolarization of the AP (phase 2)

The most striking feature of cardiac AP's when compared to those of other excitable cells, such as skeletal muscle, is that the repolarization process is slow. In ventricular fibres, for example, the maximum rate of voltage change during the final phase of repolarization is lower than 0.5 v/sec which is about 0.2% of the maximum rate of depolarization which is in order of 200-300 V/s.

Repolarization occurs due to an increase of outward currents or a decrease of inward currents. The actual process will depend upon a balance between these two processes.

2.2.1 Delayed outward currents in mammalian ventricular muscle

When a Purkinje fibre is subjected to depolarizing voltage step from a holding potential of -30 mV to -20 mV and -10 mV an outward current that increases with the magnitude of the depolarization is seen. The current was analysed by Noble and Tsien (1969) as the sum of two currents which were

labelled I_{x1} , and a slower I_{x2} . The opening of these channels is both time and voltage dependent. The I_{x1} current which activates in the plateau range of -50 mV and +20 mV appears to be specific and largely carried by K^+ , and it plays a major role in the termination of the AP in Purkinje fibres.

In mammalian ventricular muscle, time dependent outward current/s (I_k) are seen during depolarizing steps but the importance of these currents seems to vary from one species to another. During short clamp steps corresponding in time to the plateau duration, time-dependent outward currents are small or absent in sheep ventricular muscle (Giebisch and Weidmann 1971) and in calf ventricular muscle (Mascher and Peper 1969). A time-dependent outward current is present in dog myocardium, but only during large depolarization (Beeler and Reuter 1970c). In guinea pig (Ochi 1970), in cat (McDonald and Trautwein 1978a, b) and in cow ventricular trabeculae (Boyett et al 1980), a time-dependent increase in outward current is a regular finding. The reversal potential of this current is -100 mV in the presence of 3 mM $[K]_o$. This value corresponds to the calculated equilibrium potential to K^+ current. Its voltage dependence was demonstrated by the shift in the reversal potential to -55 mV in 20 mM $[K^+]_o$ (McDonald and Trautwein 1978b). In cat ventricular fibres. McDonald and Trautwein (1978a, b) analysed the current as two outward currents labelled I_k being faster than the other I_x . At 0 mV potential the time constant of activation was found to be 370 msec for I_k and 3 sec for I_x . The voltage dependence of I_k time constant of deactivation was bell-shaped, being about 150 msec at -90 mV. A maximum value of 500 msec was obtained at about -15 mV; for positive membrane potential the time constant decreases again to a value of about 300 msec at +40 mV. Boyett et al (1980) found the time-dependent outward current in cow ventricular muscle to be similar to that in cat ventricular muscle as described above.

2.2.1.1. Factors affecting I_k

i Temperature

McDonald and Trautwein (1978a) used single sucrose gap techniques to control the membrane potential of cat ventricular fibres. In response to depolarizations from -60 to 0 mV, they found the deactivation rate of I_k increased as the temperature of the preparation was raised from 24 to 31°C and then to 37°C. The decay was exponential at each temperature and the estimated Q_{10} was about 3. Isenberg and Trautwein (1975) found gradually lowering the temperature from 37 to 18°C to reduced the outward currents in Purkinje fibres. The effect was best seen during the temperature fall from 30°C to 26°C. This effect of lowering the temperature was immediately reversed upon rewarming.

ii $[K^+]_o$

The fully activated current-voltage relationship is shifted to less negative potential at higher $[K^+]_o$ and the slope of the curve in the voltage range near the reversal potential is steeper at higher K^+_o (McDonald and Trautwein 1978b; Boyett et al 1980). Raising $[K^+]_o$ from 3 to 30 mM induced a cross-over between the current-voltage relation curves in both ventricular preparations, similar to that seen in Purkinje fibres (Noble 1981). This cross-over suggests that the K^+ current is larger at higher $[K^+]_o$ beyond the point of intersection. The conclusion reached in the above studies was that raising $[K^+]_o$ increased the membrane conductance to K and this resulted in increased K^+ outward current (i.e. inward-going rectification).

2.2.2. Background Currents

By definition background current is the remaining current when all currents carried by time dependent channels are accounted for. It can be obtained by subtracting the steady state values of the time dependent current from the steady state current-voltage relationship.

The background current-voltage relationship exhibits inward-going rectification (McDonald and Trautwein 1978b). Potassium, calcium and sodium can contribute to the total background current. For potentials within the range covered by the action potential, the K^+ current is outward, the Na^+ and Ca^{++} currents are inward.

Outward Background Current (Time-independent current) I_{kl}

An approximation of the K contribution to the outward background current in Purkinje fibres was made by determining the current-voltage relationship in Na-free media. The current voltage relationship displays inward-going rectification and a reversal potential at about -90 mV at $[K^+]_o$ of 2.7 mM.

That the inward going rectification is a characteristic of the K^+ channel is further suggested by the direct measurement of changes in K^+ concentrations, elicited by depolarizing clamps to different potentials, in the extracellular clefts of frog ventricular muscle (Cleeman and Morad 1976). The increase in the cleft K^+ concentration exhibited the same anomalous behaviour as the current.

2.2.2.1 Factors affecting I_{kl}

i Temperature

Isenberg and Trautwein (1975) observed a reduction in the late outward current, (i.e. a current flow 2 sec after a clamping pulse from -90 to -60 mV) as the temperature was decreased from 37 to 21°C. The current-voltage relationship was shifted

downward (i.e. in a negative direction). Since the current was in the anomalous rectification range Isenberg and Trautwein suggested this current to be (I_{kl}).

ii $[K^+]_o$

Raising $[K^+]_o$ had similar effects on I_{kl} as on I_k described above, i.e. the current-voltage relation cross-over thus indicating greater I_{kl} current at higher $[K^+]_o$ attributed to increased membrane conductance to K^+ (McDonald and Trautwein 1978b).

iii $[Ca^{++}]_o$

Kass and Tsien (1976b) looked at the effect of $[Ca^{++}]_o$ on the current-voltage relationships in calf Purkinje fibres at different $[K^+]_o$, and found that at high $[Ca^{++}]_o$ (7.2 mM) the relationship shifted in the outward direction throughout the potential range between -90 and -40 mV, but in the inward direction for potentials positive to -20 mV. They were also able to demonstrate that in steady state conditions the fraction of the current that is affected by Ca^{++} exhibits inward-going rectification and has a reversal potential at the expected E_k . Also the curves for 4 mM and 16 mM cross each other. Kass and Tsien concluded that each of these features is characteristic for the inwardly rectifying K^+ channel in Purkinje fibres and was identified as I_{kl} . They were able to demonstrate that the effect does not depend on an inward flow of Ca^{++} ions through the slow channel, but could not exclude an eventual rise in $[Ca^{++}]_i$. In

sheep Purkinje fibres, Isenberg (1977b) was able to show increased I_{kl} during micro-electrophoretic injection of Ca^{++} . Bassingthwaite et al (1976) by means of double sucrose gap technique on sheep and calf ventricular bundle, found that when internal Ca^{++} was increased either by increasing the frequency of voltage clamping to the plateau range potential, or by the addition of cyanide and a reduction of external Na^+ , the background outward current was enhanced. When this increase was due to repetitive depolarization, the effect

on I_{kl} was less marked after Ca^{++} current blockers such as D600 or verapamil. Bassingthwaite et al suggested that Ca^{++} at the inner side of the membrane sets the level of the background outward current. Urata and Goto (1982) also using double sucrose gap technique, looked at the effect of Ca^{++} -free and Na^{+} -free solutions on I_{kl} and other currents in frog atrial muscle. In Ca^{++} -free solution I_{kl} was decreased, but was enhanced in Na^{+} -free which they attributed to decreased and increased $[Ca^{++}]_i$ under these conditions respectively.

2.3 Determinants of Action Potential Duration

One of the major questions which investigators have sought to resolve is which ionic current is primarily responsible for the determination of the duration of the myocardial AP. The plateau phase of the AP is determined by the balance between the outward K currents and the inward I_{si} current which have been described above. Thus, the termination of the plateau of the AP could result from either the activation of the K outward currents or the inactivation of I_{si} .

Noble and Tsien (1969) held the membrane potential of a Purkinje fibre at -30 mV, which is beyond the threshold for activating outward current changes, the membrane was then subjected to depolarizing and hyperpolarizing current pulses of different magnitude. Depolarizations to -20 mV and -10 mV activated an outward current that increased with the magnitude of the depolarization. Hyperpolarizations to -50 mV and -70 mV switched off the current. These repolarizing interventions were found to increase the opening of the channels that carry K outward current thereby hastening the outward flow of K^{+} . The fast activated I_{x1} current was demonstrated by McAllister et al in 1975 to play a major role in the termination of the AP in Purkinje fibres (Noble 1981). In keeping with the role of I_{x1} , in calf Purkinje fibres Kass and Tsien (1976b) artificially elevated the plateau

level of the AP by applying a current and found accelerated repolarization and shorter APD at higher plateau level and concluded that these results reflect the importance of the repolarizing currents in bringing about early repolarization in Purkinje fibres.

The experimental evidence, therefore supports the notion that repolarization of the AP in myocardial Purkinje fibres is brought about by the activation of the K^+ outward currents.

In ventricular muscle the situation is different because similar outward current to I_{x1} is not a consistent finding and the evidence suggests that the inactivation of I_{si} seems to be more important. In a study by Giebisch and Weidmann (1971), bundles of sheep ventricular fibres were voltage-clamped by means of sucrose gap and an AP was triggered, but the membrane voltage was forced back to its resting level (-82 mV) at progressively later stages of the plateau. Clamping the membrane back to its resting potential during the early part of the AP resulted in a surge of inward current. The amplitude of the inward current decreased when the clamp was switched progressively later during the AP. When the membrane was clamped to inside strongly positive (+40 to +60 mV), there was no clear indication of a slow increase of outward current as a function of time. From these results Giebisch and Weidmann suggested that the normal repolarization of sheep ventricle depends on a time-dependent decrease of inward current, rather than on a time-dependent increase of outward K current.

In mammalian ventricular muscle, I_{si} inactivation becomes slower as the membrane potential becomes more positive (Beeler and Reuter 1970a, Trautwein et al 1975, see I_{si} inactivation above). It follows, therefore, that, if inactivation of I_{si} is of primary importance for the repolarization of myocardial AP, the decrease of the rate constant of inactivation upon depolarization, should help maintain the plateau of the AP. Moreover, a depolarizing

current pulse at the beginning of the plateau should prolong the APD. Bassingthwaite et al (1976) performed the procedure on sheep and calf ventricular bundles and indeed observed a prolongation of the APD by a constant current pulse applied at the same time as the upstroke of the AP at 38°C and in 0.36 mM $[Ca^{++}]_o$. In their study pulses as short as 50 msec induced prolongation. Similarly Reuter and Scholtz (1977) consistently found lengthening of the AP in response to depolarizing constant current pulses of 40 msec at the beginning of the AP in cow ventricular trabeculae. The greater the current applied, i.e. the higher the depolarization, the greater was the AP prolongation.

These results are consistent with the idea that the inactivation of I_{si} is more important in determining the repolarization phase of the AP, hence the APD, in ventricular muscle.

Gettes and Reuter (1974) found similarity between the time constant of recovery of the plateau duration and the time constant of I_{si} recovery in sheep and pig ventricular trabeculae which suggests that in ventricular myocardial fibres, the kinetics of I_{si} can account for the plateau duration in premature responses.

This observation provides an additional piece of evidence linking the plateau duration, hence the duration of the ventricular myocardial AP to I_{si} rather than to the slowly increasing K^+ outward currents.

2.3.1. Factors Affecting APD

i Temperature

Lowering the temperature is well known to prolong the APD. Coraboeuf and Weidmann (1954) lowered the temperature of the bathing solution of calf and sheep Purkinje fibres from 38°C to 10°C and found pronounced prolongation of the APD. Thyrum (1974), using isolated atrial strips from guinea

pigs, observed large prolongation when lowering the temperature from 31 to 28°C. In isolated epi- and endocardial strips from sheep ventricle, Cohen et al (1976) found prolongation of the AP in the order of 200 ms when they lowered the temperature from 38 to 28°C. Similarly Benndorf and Nilius found large progressive prolongation under temperature ramp condition in papillary muscle from rabbit's right ventricle. They found maximum APD at $23.5 \pm 0.5^\circ\text{C}$. In all the above studies a conventional microelectrode was used to record the AP.

Lab and Woolard (1978) used suction electrode to record the AP from the epicardium of the intact insitu pig heart and observed AP prolongation when the temperature of the epicardium at the site of recording was lowered with cooled drops (10°C) of saline. No attempt was made in any of the above studies in order to explain the APD prolongation at lower temperature. A likely explanation would be the effect of temperature on the kinetics of the currents that determine the APD - which have already been described above. It is possible, therefore, that a multiple effect on the I_{si} , I_{k} and I_{kl} and their kinetics in the right direction to be responsible for the prolongation, as for example a depression of the K outward currents, a decrease in the rate of I_{si} inactivation and an increase in I_{si} . In this context the results of Lederer and Eisner (1982) might be relevant. They studied the effect of inhibiting the Na^+ pump activity on I_{si} magnitude in sheep cardiac Purkinje fibres with a two-microelectrode voltage-clamp method. They found lowering the Na^+ pump activity, by reducing $[\text{K}^+]_o$ or Ouabain, led to time-dependent increase in I_{si} magnitude. Measurements of the Na^+ pump activity showed that this increase in I_{si} was due to intracellular elevation of $[\text{Na}^+]_i$ secondary to the pump inhibition and not to the instantaneous Na^+ pump turnover rate. Similar situations may exist under low temperature conditions, since the pump activity is expected to be lower (Glitch 1982).

ii $[K^+]_o$

Elevating $[K^+]_o$ has been demonstrated to shorten APD in various isolated and intact cardiac preparations. Weidmann (1956) injected high K^+ solution into the coronary artery of turtle heart during the plateau of the AP at 10°C . At such temperature the APD was 3-5 S. Increasing $[K^+]_o$ from 2.7 to 20 mM profoundly shortened the APD. Shortening of APD in response to $[K^+]_o$ elevation has also been seen in sheep Purkinje fibres (quoted in Weidmann 1956), Langendorff perfused rabbit heart (Surawicz et al 1959), frog ventricular strips (Kline and Morad 1978), cat trabeculae and papillary muscle (Boyett et al 1980, Hennekes et al 1981) and in dog Purkinje fibres (Kline and Kupersmith 1982, Lee and Dagostino 1982). In these studies conventional microelectrode was used to monitor the AP, but Tsutsumi et al used the interval between minimum QRS and maximum T derivatives of the electrogram in insitu pig hearts as a measure of local repolarization duration. In agreement with the other studies the effect of hyperkalemia (i.e. elevated $[K^+]_o$ in the blood) reduced the repolarization duration. Lab and Yardley (unpublished data) used suction electrode to record the AP in Langendorff rabbit heart. They found large shortening of APD at 70% repolarization when $[K^+]_o$ in the perfusing krebs was elevated from 4 to 6 mM

In order to explain this effect, Weidmann (1956) suggested K^+ induced increase in K^+ permeability to explain this shortening. McAllister and Noble 1966 (Noble 1981) found a large shift to the right in the current-voltage relationship along the voltage axis at high $[K^+]_o$. The curves also intersected. It was concluded, in agreement with Weidmann, that beyond the intersection point, the K outward current is always larger when $[K^+]_o$ is high. McDonald and Trautwein (1978) increased $[K^+]_o$ from 3 to 10, 20 and 30 mM and found increased conductance of the membrane to K^+ with increased outward K^+ repolarizing currents in cat ventricular muscle. In keeping with above results, Boyett et al (1980) studied the current-voltage relationship

in cow trabeculae at different $[K^+]_o$. They found the curves to cross-over and concluded that there was more outward current in the presence of high $[K^+]_o$ indicating an increase of conductance.

In disagreement with the above studies Kline and Morad (1978) using K^+ ion-selective microelectrode and conventional microelectrode to simultaneously monitor K^+ activity in the extracellular spaces and transmembrane potential respectively in frog ventricle. They found no increase in the permeability of the cardiac cells to K^+ during the plateau of the AP nor was there a relation between the rate of development or magnitude of the accumulated K^+ during the plateau and the accompanying APD. They concluded that increasing external K^+ has no effect on time-dependent and delayed K^+ current, despite the fact that APD shortened at higher $[K^+]_o$ in their preparation which they attributed to a shift in the voltage dependence of the inward rectifier current (i.e. an early K^+ current). The experimental evidence, therefore, suggests that the shortening of the AP at high $[K^+]_o$ may be attributed to an increase in the K^+ outward repolarizing current.

However, Weidmann (1956) put forward another possible mechanism which implied an increase in outward current due to active Na^+ extrusion produced by K^+ .

Increasing the activity of the Na^+-K^+ pump is an alternative explanation. Increasing $[K^+]_o$ has been shown to stimulate the Na^+-K^+ pump in frog ventricle, dog and sheep Purkinje fibres, rabbit ventricular muscle (Ellis 1977, Eisner and Lederer 1980a, Ruiz-Ceretti et al 1981, Martin and Morad 1982, Kline and Kupersmith 1982, Glitsch 1982 a). The pump is known to pump 3 Na^+ out for 2 K^+ in, which indicates that it is electrogenic generating an outward current carried by outward Na^+ (Glitsch 1982). Indeed a good deal of evidence showed the pump to be electrogenic and generates an outward current in Purkinje ,

atrial and ventricular muscle fibres (Gadsby & Cranefield 1979a,b, Gadsby 1980, Eisner & Lederer 1979b, 1980a, Glitsch 1982a,b).

Eisner et al (1979b, 1980a, 1981a), reduced $[K^+]_o$ to 0 mM in order to inhibit the $Na^+ - K^+$ pump in sheep purkinje fibres and on addition of an activator (e.g. Rb which is known to activate the pump) they observed a transient outward current which was abolished by 10 μ M strophanthidin. The amplitude of the current was dependent on the degree of $Na^+ - K^+$ pump activation. They concluded that the outward current was due to the activation of $Na^+ - K^+$ pump. Similar transient outward current was seen in dog Purkinje fibres when the pump was reactivated by the readmission of K^+ into the bathing medium, which was also inhibited by 2 μ M strophanthidin (Gadsby and Cranefield 1979a, b, Gadsby 1980).

A post-stimulus hyperpolarization of the resting membrane potential was found to be dependent on the $Na^+ - K^+$ pump activity in guinea pig atrial and ventricular muscle in sheep and frog ventricular muscle as well as dog purkinje fibres (Kunze 1977, Kline et al 1980, Martin and Morad 1982, Cohen et al 1982). Isenberg and Trautwein (1974, 1975) looked at the possible contribution of the $Na^+ - K^+$ pump current to the AP of sheep Purkinje fibres under steady state conditions by the voltage-clamp method. They described a voltage and time-independent outward current that was inhibited by cardiac glycosides. The current affected the plateau phase of the AP and was attributed to the activity of an electrogenic active Na^+ pumping. Gadsby and Cranefield (1979b) demonstrated AP shortening caused by stimulation of the $Na^+ - K^+$ pump in dog Purkinje fibre. The pump was first inhibited in K^+ free-medium and the AP was prolonged.

When K^+ was re-admitted to the bathing medium they observed a transient hyperpolarization of the membrane potential and transient shortening of the AP. They attributed these changes to a transient increase in outward $Na^+ - K^+$ pump current. Kline and Kupersmith (1982) concluded that the slow shortening in APD when increasing the rate of stimulation is related to the degree of the pump activation. Boyett and Fedida (1982) obtained direct evidence for electrogenic Na^+ -pump in cardiac Purkinje fibre and showed more outward current at higher rates of stimulation which was reduced by $0.5\mu M$ Ouabain indicating that the outward current was due to increased activation of the $Na^+ - K^+$ pump.

In conclusion, therefore, the shortening of the APD at high K^+ is probably produced by a combining effect of a shift in the current-voltage relationship and an increase of outward current produced by the increased activation of the Na^+ / K^+ pump.

iii $[Ca^{++}]$

Shortening of APD with increasing extracellular Ca^{++} has been demonstrated in dog ventricular and Purkinje fibres (Hoffman and Suckling 1956, Temte et al 1967, Siegal and Hoffman 1980, Colatsky and Hogan 1980), rabbit ventricle (Surawicz et al 1959, Johannsson and Wohlfart 1980, Lab and Yardley unpublished data), calf Purkinje fibres (Kass and Tsien 1976), calf and sheep ventricular muscle (Bassingthwaight et al 1976) and in frog atrial muscle (Urata and Goto 1982). In both studies of Hoffman and Suckling and Temte et al the shortening of AP was explained as being due mainly to shorter plateau duration. Kass and Tsien (1976) suggested two main factors responsible for the shortening. First, the high plateau level, which was attributed to increased inward driving force for Ca^{++} , thus larger I_{si} , accelerates changes in time and voltage dependent K^+ current which triggers early repolarization. Secondly, an increased activation of the background time-dependent K^+ outward current, I_{kl} . The first factor may apply to the Purkinje fibres only because elevating the plateau level of the AP, as discussed in section 2.3, prolongs APD in ventricular muscle, but the second factor may apply to both Purkinje fibres and ventricular muscle.

Bassingthwaight et al (1976) increased $[Ca^{++}]_i$ in sheep and calf ventricular bundles by various means and observed an increase in time-independent outward current; an effect which was enhanced with higher $[Ca^{++}]_o$. This effect was reduced by the Ca^{++} current blockers, like verapamil and D600. These results led Bassingthwaight et al to conclude that increasing Ca^{++} at the inner side of the membrane sets the level of the background outward current I_{kl} and thus the action potential duration. Isenberg (1977c,d) injected Ca^{++} directly into sheep cardiac Purkinje fibres in order to increase $[Ca^{++}]_i$ and observed a shortening of the action potential duration and an increase in K^+ conductance. Siegal and Hoffman (1980) using agents that can effect K^+ permeability

(e.g. aminoacridine which decreases K^+ permeability) showed increasing $[Ca^{++}]_i$ by elevating $[Ca^{++}]_o$ can modify the action potential in the directions described above by modifying the K^+ permeability. The extent of this effect determined the effect of $[Ca^{++}]_o$ on the action potential in dog cardiac Purkinje fibres. Caroni et al (1980) demonstrated increased permeability of highly purified sarcolemma vesicles to K^+ when $[Ca^{++}]_i$ was increased. However, Attwell et al (1981) reported that blocking Ca^{++} activated K^+ conductance by the dye 3, 3 diethylthiadicarbocyanine in guinea pig ventricular muscle has no significant effect on the rate dependent shortening of the AP.

iv - Rate of steady state of stimulation

(See chapter 3).

CHAPTER 3

Rate dependent changes in action potential

- 3.1 Incomplete recovery of the ionic currents
- 3.2 Ion concentration changes
 - 3.2.1 K^+ Accumulation
 - 3.2.2 Ca^{++} Accumulation
 - 3.2.3 Na^+ Accumulation
- 3.3 Consequences of ion concentration changes
- 3.4 The changes in the ventricular action potential and shape when the frequency is increased
 - 3.4.1 The repolarization of the action potential
 - 3.4.1.1 Premature action potential
 - 3.4.1.2 Steady state changes
- 3.5 The effects of prolonged pause and reduction in frequency
 - 3.5.1 possible mechanisms
 - 3.5.1.1 Intracellular accumulation of Ca^{++}
 - 3.5.1.2 Changes in extracellular K^+
 - 3.5.1.3 Incomplete recovery of ion conductances
- 3.6 Prolongation of the AP at high rates and at low rates

Chapter 3

Rate Dependent Changes in Action Potential

With changes in frequency important changes occur in the duration as well as the configuration of the action potential.

Two main causes of these rate dependent changes are:-

1. Incomplete recovery of the ionic currents, which are responsible for the production of the action potential, during the diastolic interval.
2. Changes in ion concentration intracellularly or extracellularly as a result of changes in the rate of stimulation.

3.1. Incomplete Recovery of the ionic Currents

As described in the previous chapter, several currents are activated during an action potential, including the fast Na^+ current, slow inward Ca^{++} current and time-dependent K^+ current. These currents, except the K^+ current, undergo inactivation during the action potential and time is required for these currents to recover from inactivation.

An action potential triggered before the complete recovery of these currents will result in less I_{Na} and I_{si} and more time-dependent K^+ current which will produce changes in duration and configuration of the early triggered action potential. These changes can be responsible for the changes of the first and probably second action potential after the rate increase but if the rate increase was continued, other mechanisms become more important.

3.2 Ion Concentration Changes

The ion fluxes across the cell membrane during an action potential may bring about significant changes of ions concentration on either side of the membrane.

3.2.1. K^+ Accumulation

Kline and Morad (1978) showed the diffusion from the extracellular clefts to the bulk of the medium is restricted in cardiac tissue. Therefore small movements of ions like K^+ across the cell membrane into the clefts would produce significant changes in the ion concentration because the clefts have a large surface area to volume ratio. Such accumulation of K^+ has been demonstrated using K-sensitive microelectrodes in frog ventricle (Kline and Morad 1978) and in rabbit atrium (Kunze 1977).

3.2.2. Ca^{++} Accumulation

It is probable that at high rates of stimulation more Ca^{++} enters the cells, carried by I_{si} , thus resulting in increased loading of the Ca^{++} stores which results in more Ca^{++} being released on depolarization and therefore greater contraction. Higher intracellular Ca^{++} concentration, as measured by aequorin (Ca^{++} -activated bioluminescent protein) has been observed at higher rates of stimulation in frog atrial muscle (Allen & Blinks 1978) and cat papillary muscle (Allen & Kurihara 1980). Winegrad and Sands (1970) reported intracellular accumulation of Ca^{++} at high rates of stimulation in frog ventricle too.

Recently Lado et al (1982) and Cohen et al (1982) provided further direct evidence for Ca_i^{++} accumulation at high rates of stimulation.

3.2.2. Na^+ Accumulation

Deitmer and Ellis (1980) using Na^+ selective microelectrodes have shown intracellular accumulation of Na^+ in purkinje fibre at high rates of stimulation. They measured an increase of 1-3mM. Boyett and Jewell (1980) suggested that because the cardiac cells have smaller diameters than purkinje fibres the rise might be greater than in purkinje fibres.

3.3. Consequences of ion Concentration Changes

The consequences of these ion concentration changes can be divided into four categories:-

a. Reduction of the driving forces across the sarcolemma. For example, intracellular accumulation of Na^+ and Ca^{++} can decrease the gradient across the cell membrane thus resulting in a reduced ion influx during an action potential. Extracellular accumulation of K^+ will reduce the efflux of K^+ .

b. Alteration of the resting potential.

The currents responsible for the action potential are voltage-dependent in their activation, inactivation and recovery from inactivation, therefore depolarization of the resting membrane potential, as a result of extracellular K^+ accumulation, will effect the magnitude of these currents during an action potential.

c. Alteration of conductances.

McDonald and Trantwein (1978b) showed that extracellular accumulation of K^+ increases the K^+ conductance which obviously resulted in an increase of K^+ current. Such extracellular accumulation has been reported to depress the Na^+ current (Kern et al 1978). Increased bathing Ca^{++} concentration has been shown to increase the rate of I_{si} inactivation (Kohlhardt et al 1975) and it affects the background K^+ current as well as the time-dependent K^+ current (I_x) in purkinje fibre (see Boyett and Jewell, 1980, for references). Also intracellular accumulation of Ca^{++} can increase the K^+ outward current in both purkinje and myocardium fibres as outlined in the previous chapter.

d. Alteration of ion pump activities

At higher rates of stimulation the intracellular accumulation of Na^+ and extracellular accumulation of K^+ can stimulate the Na^+-K^+ pump. Should the pump be electrogenic as described by Glitsch (1982) it will have a direct effect on the action potential as well as the resting potential (Trantwein and Isenberg 1974, 1975, Gadsby 1979). Since Na^+ is also involved in the $Na^+ - Ca^{++}$ exchange its intracellular accumulation can result in increased intracellular concentration of Ca^{++} (Eisner et al 1981a, Lado et al (1982), Lee and Dagostino (1982) Sheu and Fozzard 1982). Recent evidence also suggests that the $Na^+ - Ca^{++}$ exchange is electrogenic (Mullins 1982). If this is so its stimulation at high rates probably as a result of intracellular accumulation of Ca^{++} can also directly affect the action potential and probably the resting potential.

3.4 The changes in the ventricular action potential and shape when the rate of frequency is increased

3.4.1. The repolarization of the Action Potential

The repolarization phase of the action potential is determined by a balance between the slow inward and K^+ outward currents and it is not surprising that the repolarization is very sensitive to rate of stimulation.

3.4.1.1. Premature Action Potential

The membrane undergoes a period of recovery (or electrical restitution) after an action potential. Two types of electrical restitution have been demonstrated if a premature action potential is triggered after a control response.

a) In many preparations a premature action potential is characterised by a reduced plateau duration and amplitude, and total duration. When the interval between the control and premature responses is increased, these parameters return to their control values.

b) In some reports (Bass 1975, Noble 1978) the above three parameters did overshoot their control in a premature action potential. They observed supernormal premature action potential characterised by higher and more prolonged plateau. The total duration maybe greater or unchanged.

3.4.1.2. Steady State Changes

A good deal of evidence shows that the action potential recorded from isolated purkinje and ventricular fibres from various species are shorter at high rates of stimulation than at low rates (see Boyett and Jewell 1980 for references). Recently Elzinga et al (1981) showed that steady duration is also shorter at high rates of stimulation in intact insitu right ventricle of dog heart. Similar results have been obtained in intact pig heart insitu (lab and Yardley unpublished data), and in intact isolated ejecting cat heart (Elzinja et al 1980). In human ventricle the action potential duration as indicated by the QT interval has also been shown to be shorter at high rates of stimulation (Eisner et al 1982).

At high rates of stimulation there is an increase of the rate of repolarization during the plateau and at very high rates the plateau may be abolished and the action potential may become triangular in shape. This has been observed in isolated cat ventricular muscle (Boyett 1980), in isolated guinea pig ventricular muscle (Eisner et al 1982).

In Cat and guinea pig the shortening of the steady state action potential depends on external Ca^{++} concentration. It is accentuated in low external Ca^{++} and reduced or abolished in high external Ca^{++} , (see Boyett and Jewell 1980 for references).

If the rate of stimulation is continued beyond the first beat, two phases in the change of the action potential duration is observed: these are initial fast changes and slow progressive changes. The initial change can be lengthening or shortening as described above. The slow progressive change is always shortening of the action potential duration to a new steady state (see Boyett and Jewell 1980 for references).

3.5 The effects of prolonged pause and reduction in frequency

Generally, the changes occurring with a reduction of frequency are the reverse of those described for an increase in frequency. After a brief pause the first action potential may be longer, as in cat ventricular muscle (Boyett & Jewell 1978) and in dog purkinje fibre (Greenspan et al 1967) , or it may be shorter as in rabbit (Gibbs and Johnson 1961, Wohlfart 1980). If the decrease in rate is continued, progressive prolongation of action potential is observed.

The experimental evidence suggests that the above described rate dependent changes may be due to three possible factors.

1. The first factor is responsible for the shortening of premature action potential and it would account for the initial shortening when the rate of stimulation is increased (for one beat) and for the initial prolongation when the rate is decreased.
2. The second factor tends to shorten the action potential, at high rate of stimulation, beyond the first and second beats. The effect slowly develops after the rate changes and will also be responsible for the gradual prolongation of the action potential when the rate is decreased.
3. The third factor is responsible for the prolongation of the premature action potential when the rate is suddenly increased.

3.5.1. Possible Mechanisms

3.5.1.1. Intracellular accumulation of Ca^{++}

There is experimental evidence to suggest that the rate dependent changes in ventricular and purkinje fibre action potential duration are closely related to changes in internal Ca^{++} , as indicated by changes in peak tension (see Boyett and Jewell 1980 for references). The likely explanation is that the Ca^{++} released, during depolarization, into the sarcolemma determines both the level of tension and the action potential duration. The intracellular Ca^{++} may effect the action potential duration via changes in I_{Si} (Simurda et al 1976) or changes in K^+ conductance (Kass & Tsien 1976, Bassingthwaight 1976, Isenberg 1977) thus altering the outward repolarizing potassium current. It is also suggested that the slow shortening of the action potential after an increase in rate is mediated through intracellular accumulation of Ca^{++} .

3.5.1.2. Changes in Extracellular K^+

Increasing the bathing K^+ concentration is well known to shorten the action potential duration, although no change has been observed in some preparations, and a prolongation of the action potential has also been reported, (Carmeliet & Vereecke 1980): but the prolongation took place in chloride free solution. Kline & Morad (1976) observed large increase in the cleft K^+ concentration measured with K^+ sensitive microelectrode of frog ventricle during an action potential. Therefore the increase in cleft K^+ could be responsible for the rate dependent changes in action potential duration. The experimental evidence suggests that although this accumulation might be important in the initial phase of action potential shortening it is unlikely to make a major contribution to the slow changes after increase of rate for the following reasons:-

First - In some ventricular preparations (see Boyett and Jewell 1980 for references) doubling the bathing K^+ , although producing large depolarization, produced only a small shortening of the action potential. In guinea pig preparations, rate increases produced large shortening of the action potential (Attwell et al 1981).

Second - in the studies of Kline and Morad (1978) on frog ventricle, the time course of extracellular K^+ accumulation was different from that of action potential shortening when increasing the rate of stimulation.

Third - in a rabbit atrial muscle (Kinze 1977) and in sheep purkinje fibre the $[K^+]$ in the extracellular spaces declined when the rate increase was maintained, but AP shortening was maintained (Kline and Kupersmith 1982). Finally, Attwell et al (1981) reported that the action potential duration and amplitude recover much more slowly than the resting membrane potential (an indicator of $[K^+]$ in the extracellular clefts) in isolated guinea pig myocardium after an increase in rate. The time course of recovery was slower after trains which produced more shortening of the action potential, thus suggesting that K^+ accumulation is not the main cause of the changes in action potential duration. Therefore, accumulation of K^+ in the extracellular cleft probably does not play a major role in shortening of the action potential during the slow phase of changes after increasing the rate, but the fact that K^+ accumulation does occur as observed in frog ventricle and rabbit atrium suggest that it may play a role in the initial phase of changes.

3.5.1.3 Incomplete Recovery of ion Conductances

A. Premature Action Potential

The repolarization of the action potential is partly brought about by an activation of outward current in a purkinje fibre and inactivation of I_{si} in ventricular muscle (see chapter 2). If an action potential is triggered early, the K^+ outward current could still be in an activated state, or the slow current, I_{si} , is still inactivated: either could result in shortening of the early AP.

B. Steady State AP

Boyett and Jewell (1978), from their restitution curve study, suggested that incomplete recovery of the plateau currents are partly responsible for the rate dependent shortening of the steady state AP. They found the steady state duration at low rates of stimulation occurred at the plateau of the electrical restitution curve, but at high rates (200/min) the steady state AP fell on the shoulder of the electrical restitution curve in cat ventricular muscle. Similar results have been obtained by Elzinga et al (1981) in intact insitu dog heart. The plateau of the restitution curve was also shifted downwards at higher rates in both studies suggesting a second factor contributing to the shortening of the AP which could be due to accumulation of intracellular Ca^{++} and/or other mechanisms:

3.6 Prolongation of the AP at high rates and its shortening at low rates

Several hypotheses have been suggested to explain the prolongation of AP sometimes seen at high rates or its shortening at low rates, but with a limited evidence for each one. Two separate Ca^{++} compartments for the control of action potential and tension have been suggested (Bass 1975, Wohlfart 1979). The two compartments are assumed to be in equilibrium. One of them is on the inner side of the sarcolemma and the amount of Ca^{++} which is presumably bound to the membrane determines the K^+ conductance. If Ca^{++} is released into the sarcoplasm during an AP, it takes time to rebind to the membrane. If an AP is triggered early there will be less Ca^{++} bound and thus less K^+ outward current.

At higher steady state rates of stimulation, as a result of increased intracellular Ca^{++} , there will be more Ca^{++} bound to the membrane and more outward K^+ current as a result. This hypothesis can thus also explain the shortening of AP at high rates of stimulation, which could be due to accumulation of Ca^{++} on the inner surface of the membrane. The $\text{Na}^+ - \text{K}^+$ pump may play a role. Cohen et al (1975, 1976) found that cooling abolished the prolongation of the AP and Ouabain reduced it. They suggested that during an AP, K^+ accumulated in the extracellular clefts which would stimulate the $\text{Na}^+ - \text{K}^+$ pump resulting in the $[\text{K}^+]$ in the cleft being reduced below normal, thus producing a prolongation of the AP. Cooling or Ouabain administration can inhibit the pump thus reducing the prolongation. Finally, some evidence suggests that I_{Si} plays a role. It has been found that in double pulses, I_{Si} in the second prolonged AP is greater in cow purkinje fibre. Hirakoa and Sano (1977) found similar increase in I_{Si} of the second of the two closely spaced voltage clamp steps. Low external Ca^{++} concentration and verapamil were found to reduce and abolish the prolongation respectively (Iinuma and Kato 1979).

Regional Differences in Electrophysiology in intact heart

The earliest regional electrophysiological differences were suggested by Bayliss and Starling in 1883 (listed in Noble and Cohen 1978). These regional differences were manifest in APD differences between the base and apex in frog ventricle.

In the last few decades the experimental evidence suggests that the phenomena is general for mammalian heart.

Autenrieth et al (1975) studied the sequence of repolarization on the ventricular surface in the dog heart using suction electrode and found that AP's were generally shorter on the posterior than on the anterior surface of the ventricle, and larger at the apex than at the base. This difference between base and apex in dog heart has been confirmed in other recent studies by Kuo et al (1983) and Tsutsumi et al (1983) who also found the repolarization at the endocardium to be longer than at the epicardium.

In Autenrieth et al study, the average maximum dispersion of APD was 22 ± 9 msec. Dispersion of similar magnitude was observed in the isolated cat heart by Sarachek, Roberts and Leonard in 1972 as reported by Autenrieth et al.

In agreement with the above differences between base and apex in dog ventricle, Cohen et al (1976) found longer APD in ventricular strips from the epicardium at the apex as compared to endocardium at the base in sheep left ventricle. Similarly Lab and Yardley (unpublished data) observed longer APD at the apex of the intact insitu pig heart, but in the isolated rabbit the duration at the base of the left ventricle was longer than at the apex.

Regional differences in the electrophysiological behaviour in response to a physiological manoeuvre have also been seen in the above studies. In sheep ventricular strips from base and apex, stimulation of a rested muscle resulted in larger shortening of the APD to the steady state in the apical than in the basal strips. The response to paired pulse stimulation at various intervals was also different between the two strips (Cohen et al 1976). In the same study reducing the temperature had a larger influence on the apex than at the base which was reversible.

Lab and Yardley (1979) changed the rate of pacing of insitu pig heart to higher and lower rates, while recording AP's from base and apex of left ventricle simultaneously using suction electrode. They consistently found the rate at which APD approached a new steady state to either change was greater at the apex than at the base over the first few beats.

The restitution curves (Bass 1978, Boyett and Jewell 1978) from both areas in the above study crossed over (unpublished), and the plateau of curve from apex was higher than that at the base. Also in response to increasing rate of pacing the change in the slope of curve was greater at the apex. Lab and Yardley also carried out similar study on the isolated rabbit heart and found the curves from base and apex not to intersect but at steady state rate of 150 min^{-1} , the curve from apex was significantly shifted down. Lowering Ca^{++}_o from 1.8 to 0.5 mM shifted the curves of both areas upwards and increased their magnitudes between the range of 250 and 600 msec test pulse interval (TPI), while elevating K^+_o from 4 to 6 mM shifted both curves downward but only the magnitude of curve from base was increased. Increasing the steady state rate of pacing shifted the curves downward and reduced their magnitudes. The changes in response to the above manoeuvres were always greater at the base than the apex of the rabbit left ventricle except when increasing K^+_o which shifted the curve from apex to a greater extent.

In open-chested pig Kuo et al (1983) found the shortening of premature AP to be greater at the base than at the apex, that is opposite to that reported by Lab and Yardley (1979) for the pig. The difference between the two studies may be related to species differences or to different areas of recording from base and apex. In Kuo et al study a gradual decrease in the extent of shortening was observed from base to apex. Finally Tsutsumi et al (1983) found non-homogeneous reduction of ventricular repolarization duration in response to hyperKalemia in insitu dog heart.

The cause of these regional differences in APD and its behaviour to a physiological manoeuvre is a mystery. A good deal of evidence shows regional differences in mechanical contraction (chapter 5). Also regional biochemical differences have been reported, all these factors could contribute towards electrophysiological differences. In this context the following points may be of some importance.

1. Regional biochemical differences

Cohen et al (1976) suggested regional differences in the Na^+ pump activity to be responsible for the rate-dependent differences between apex and base APD in sheep left ventricle. Because there were no differences between base and apex AP after a quiescent period they concluded that the properties of the sarcolemmal membrane permeability to ions are not different. At constant stimulation the differences appeared. Their appearance was temperature and Ouabain sensitive which suggested the possible involvement of the Na^+ pump.

Peters and co-workers in Germany (personal communication) studied the density of Na^+/K^+ ATPase in atria and ventricles in Langendorff perfused guinea pig hearts. They used the amount of tritiated Ouabain bound as an indicative measure for the Na^+/K^+ ATPase density. They found the ventricles to bind much more Ouabain than the atria, and the time courses of binding were slightly frequency dependent, being faster at higher frequency in both tissues. Such difference may be the result of differences in wall stress and strain because of

differences in the developed pressure between them and/or different wall dynamics. The regional differences in wall stress and strain between left and right ventricle and between endo- and epicardium have been suggested to be responsible for the presence of different types of myosin and for the differences in Ca^{++} -ATPase activity too, which was observed in mammalian hearts (Gorze et al 1981, Mercadier et al 1981).

The differences in wall stress and strain could be brought about by regional differences in wall dynamics which have also been observed in intact normal left ventricle of mammalian hearts (see chapter 5) and the most likely reason for the differences in wall dynamics is the morphological structure of the myocardial fibres (Streeter 1979).

2. Possible regional differences in electrolyte concentration

Lepeschkin (1976a) reported in his review, regional differences in K^+/Na^+ ratio, and its relation to the T-wave, between epi- and endocardium of cat and human heart, also Ca^{++} was slightly higher in the endocardium. But in these studies a flame-photometer was used and the values were of the total ion concentration. Browning and Strauss (1981) found 30mM gradient in intracellular K^+ activity from Purkinje fibres (which has higher activity) to ventricular muscle in cat heart, meanwhile Sheu and Fozzard (1982) reported 1.6mM gradient in intracellular Na^+ activity from purkinje to ventricular muscle of sheep heart. They have also found a small gradient (about 5mM) in intracellular Ca^{++} activity in the same direction. The Purkinje fibres had higher activities of both ions.

These differences, which are bound to reflect differences in electrical activities, may suggest differences in enzyme activity which are responsible for maintaining constant ion concentrations and gradients across the sarcolemma.

3. Possible regional differences in K^+ fluctuation

Browning and Strauss (1981) showed the shorter AP to be accompanied by a faster rate as well as greater magnitude of K^+ efflux in Purkinje fibres from dog left ventricle as compared to Purkinje fibres from cat left ventricle. The fact that both fibres have very close intracellular K^+ activity could suggest the differences in APD to be due to the differences in rate and magnitude of K^+ efflux during activity which in turn be attributed to differences in cellular membrane permeability to K^+ . Furthermore, in the same study the ventricular action potential was very much shorter than the Purkinje fibres AP in cat heart and was accompanied by much greater and faster rate of K^+ efflux during the AP. The average resting membrane potential was identical in both fibres which could discount any possibility of differences in extracellular $[K^+]$. These findings are in keeping with the suggestion of possible differences in the cellular membrane permeability to K^+ . The above results are in line with the results of Kline and Kupersmith (1982), in that shorter AP is accompanied by faster rate of K^+ efflux. They also observed some locations to have large fluctuations in extracellular K^+ activity in the same preparation: dog Purkinje fibre. Increasing $[K^+]$ in the perfusing Krebs in my study was observed to produce regional differences in AP shortening (fig. 9.5 vs 9.6, 9.9 vs 9.10).

Similar results were obtained by Tsutsumi et al (1983) in intact in situ dog heart during hyperkalemia. Watanabe et al (1983) attributed the differences in action potentials of rat left ventricular myocytes to differences in the magnitude of a transient outward K^+ current. Finally, Baumgarten et al (1981) observed a non-uniform response of guinea pig papillary muscle to hypoxia. Some cells maintained near normal while others had high K^+ activity and the resting potential in both populations responded differently to elevated extracellular K^+ .

From the above experimental data on K^+ handling it is suggested that regional differences in K^+ transport and possible regional difference in its intracellular and extracellular activity may play an important part in producing regional differences in electrophysiology. Perhaps via its effect on the membrane conductance and on the I-V relationship or/and via its effect on ion transports like the Na^+ pump and the Na^+/Ca^{++} exchange. However, the picture seems to be more complicated than that and a combination of multiple factors may be behind the differences.

Chapter 5

Regional Differences in Ventricular Wall Motion

The presence of non-uniform and asymmetric left ventricular contraction was usually considered to be an indication of dysfunction of the left ventricle (Herman & Gorlin 1969). Models for quantitative analysis in those studies in terms of regional dynamics or wall stress and strain relationships typically assumed uniform symmetric contraction, but recent evidence suggests that this assumption is unjustified.

Ingels et al (1981) using radiopaque markers implanted in the mid-wall of the left ventricle of the human heart found regional differences in contraction. The systolic excursion and velocity of shortening of posterior sites were significantly greater than those of their counterparts in the anterior wall. Also along both posterior and anterior walls the extent of contraction and velocity of basal segments were also significantly greater than those of other segments in the same wall towards the apex.

In disagreement with this was the excursion at the mid-point of the aortic valve at the base of the left ventricle, which had the smallest excursion of all sites studied. The apical sites moved inward and towards the aortic valve, the mid-base apex sites moved almost directly inward and the basal sites moved inward and towards the apex. They pointed out that these results would suggest non-uniform contraction in the normal left ventricle and can be explained by the anatomical variations in myocardial fibre mass and orientation in the wall of the heart. Le Winter et al (1975) in a study on dog left ventricle using ultrasonic crystals implanted along the hoop axis in the midwall found the extent of shortening of the mid-wall fibre of the free wall to vary along the major axis, i.e. from the mid of the aortic valve to the apex, but the extent of shortening expressed as % of the end diastolic dimension was significantly greater at the apex than at the base or at the mid-ventricle, thus opposite to the findings of Ingels et al (1981). They explained the small shortening of the basal fibres to be due to the proximity of these fibres to the relatively inelastic fibrous portion of the cardiac skeletons comprising the mitral and aortic valve rings. These results could be in agreement with Ingels's results if the mid-point at the aortic valve in Ingels study is considered to be equivalent to the basal site in Le Winter's study. M-mode echocardiographic methods confirmed the results, of Le Winter, in normal hearts (Shapiro et al 1981). Fractional shortening increased from 31.7% at the mitral valve level to 36.5% at the region of the papillary muscle. .

Regional differences were observed in the extent of systolic posterior wall thickening (expressed as % increase in thickness) which increased from 55% at the base to 106% at the papillary muscle. Also the peak thinning rate at the base was almost half that at the tips of the papillary muscle. There was greater systolic inward movement of the epicardium at the base (sphincter-like inward movement) than at the papillary muscle level. The septum thinned and thickened more slowly than did the posterior wall and did not show major differences between levels. Shapiro et al (1981) as other investigators, explained these results as being due to the circumferential arrangement of myocardial fibres at the base and a more prominent longitudinal component towards the apex.

Using biplane coronary cineangiograms, Kong et al (1971) found the extent of shortening of the epicardium to be significantly greater at the apex than at the base and less near the septum on the left anterior descending artery than that on the free wall of the left ventricle in human heart. They concluded that the extent of shortening is greater on the left ventricular free wall and apex and least along the ventricular septum and at the base. In disagreement with these results, Le Winter et al (1975) found no significant regional differences in the extent or amplitude of epicardium movement and Marier et al (1979) reported no differences in the fractional shortening, but the amplitude of epicardial movement at the base was greater than at the apex. The failure to observe regional differences in epicardial contraction by Le Winter et al could have been due to the fact the ultrasonic crystals were implanted in the same direction as and along the major axis, therefore, any movement in the transverse direction would not have been detected.

The difference between the results of Marier et al (1979) and Kong et al (1971) could be due to different methods of study, the former used M-mode echocardiography whereas the latter used biplane coronary cineangiography.

Despite the differences between different studies, the results suggest that at the papillary muscle level reduction in transverse axis is due almost entirely to wall thickening while at the base, reduced wall thickening is compensated for by inward movement of the myocardium in a sphincter like manner.

Experimental results also suggest regional differences in the response to changing the mechanics of contraction from normal to isovolumic contraction which is produced by aortic constriction. Le Winter et al (1975), during transient periods of aortic constriction, found that the end diastolic length of the mid-wall increased significantly and the extent of shortening was maintained for the fibres at the apical and mid-ventricular levels. At the base the end diastolic length did not change but shortening decreased. At the epicardium the end diastolic length increased equally at all sites (Basal & Apical) but the extent of shortening decreased by about 30% at the apex and mid-ventricular wall and increased by 25% at the base. These results suggested that the major change in left ventricular geometry during aortic constriction is an increase in end-diastolic segment length at the mid ventricle and apical locations.

Olsen et al (1981) using chronically implanted pulse-transit dimension transducers in conscious dogs also found significant re-arrangements in ventricular geometry during the isovolumic contraction produced by inflating a cuff round the aorta, but the magnitude and the direction of these re-arrangements varied with the ventricular volume. During diastole of normal contraction the left ventricle was more spherical at large volumes, and more elliptical at small volumes, than during ejection. During isovolumic contraction the left ventricle became more elliptical at large volumes, and more spherical at small volumes, thus the re-arrangement in geometry observed during isovolumic contraction appeared to be transient stages from the diastolic to the ejection phase. Also during diastolic filling the dimensional changes of the left ventricle were asymmetrical with a disproportionate increase in the minor (transverse) axis diameter as compared to the major axis diameter (from aortic valve to apex).

CHAPTER 6

Methods

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Chapter 6

Methods

6.1 Experimental Preparations

6.1.1 Isolated Rabbit Heart

New Zealand white rabbits (2-3kg) were obtained from Charing Cross animal house. The animals were instantly killed by a blow to the back of the neck. The chest was very quickly opened and the heart was excised as high as possible to make sure of good length of the aorta for cannulation. The heart was very quickly washed in cold saline, cannulated and slowly perfused via a glass cannula through the aorta, i.e. cannula C_1 in figs. 6.1 and 6.2. Care was taken to exclude any air bubbles trapped between the end of the cannula and the aorta. The flow was increased to maximum and the aorta was tied tightly round the cannula. A second cannula, C_2 fig. 6.2 was introduced into the left ventricle through the left atrium and tied tightly.

6.1.2 Langendorff Perfusion (fig. 6.1)

A top reservoir contained 2 litres of continuously oxygenated (95% O_2 , 5% CO_2) Krebs of the following composition in mM/Litre
100 NaCl, 25 $NaHCO_3$, 1.5 $MgSO_4$, 1.5 NaH_2PO_4 , 4KCl, 20 CH_3COONa ,
1.8 $CaCl_2$, 10 glucose, and have pH of (7.2-7.4).

A cellulose filter and prefilter of 47mm diameter and 8 μ m pores (satorius SM 11301/30 and SM 13400/26 respectively) were incorporated between the top reservoir and the heated coil, in a 50mm filter holder (SM 16508). Thus the Krebs was filtered before entering the coil.

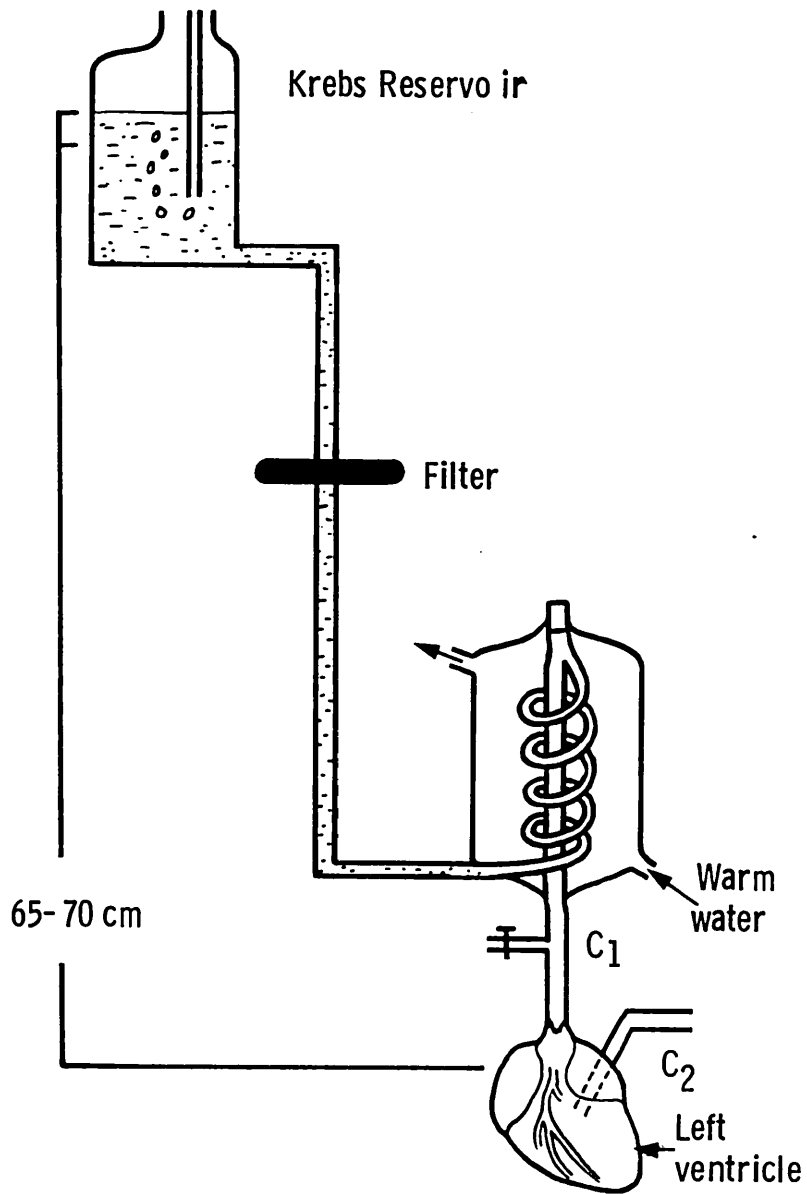


Fig. 6.1 The Langendorff perfusion apparatus.

C₁ A cannula in the aorta

C₂ A cannula in the left ventricle

The distance between the level of Krebs in the reservoir to the rabbit heart was 70 cm of H_2O . The Krebs was not allowed to drop more than about 5 cm, as a result of perfusion, by constant topping up. A suitably shaped cannula (C_1) with a side arm was fixed to the coil. The aorta of the heart was cannulated and tied tightly around the cannula. The Krebs flowing from the top reservoir, filtered by the in-line filter, was heated to $34 \pm 0.5^\circ C$ in the coil (Aimer) by the water jacket before perfusing the heart. A water heated chamber was placed immediately below the heart to maintain a warm environment. The perfusate dripped from the heart and was collected in a bowl below.

To change the perfusing Krebs, a double coil (Aimer) was used in place of the single coil. The two coils were built in a single glass chamber and were heated simultaneously, a tap at the bottom of the chamber enabled me to switch to the required solution. There was very little dead space. The heated water in the jacket was continuously circulated by a water pump circulator with a thermostatically controlled heater incorporated in it.

6.1.2.1 Quasi-Working Apparatus

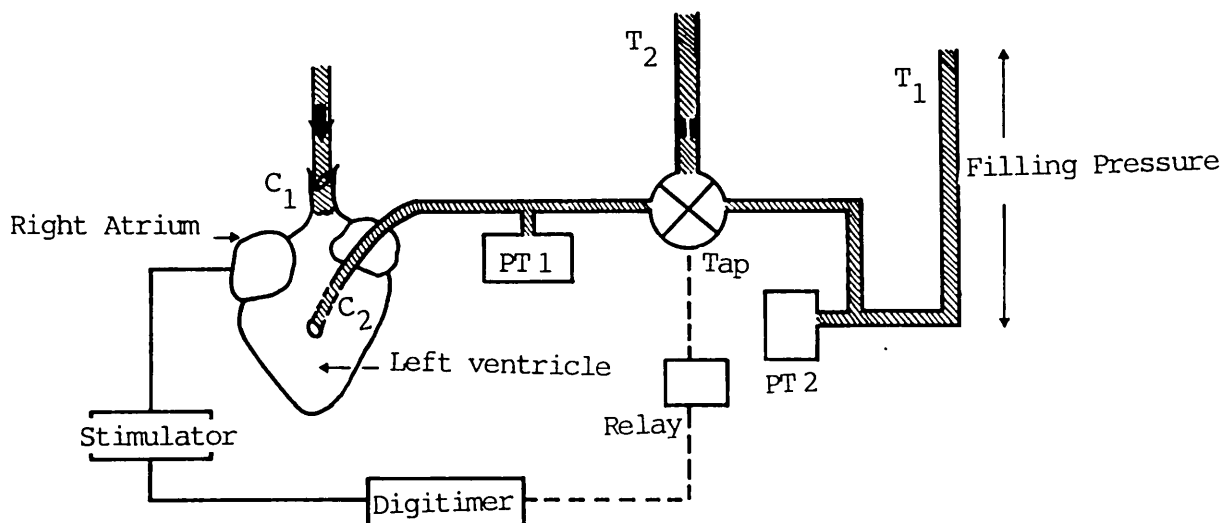


Fig. 6.2 The Quasi-working apparatus. (See text for details).

A. Description of Apparatus

The left ventricle of the rabbit heart was cannulated through the left atrium by a perforated cannula (C_2 figs. 6.1 and 6.2).

The cannula was connected to a solenoid tap of 2mm orifice diameter (Kuhnke Ltd) and, a pressure transducer, PT1, (Bell and Howell 4-422) was incorporated between the cannula and the solenoid tap to record the ventricular pressure.

The solenoid tap was connected to a graduated tube T_1 . The whole system i.e. from cannula 2 to the top of tube T_1 was filled with the perfusing medium. When the solenoid tap is opened to T_1 , the ventricle fills during diastole and empties back into T_1 during systole. Hence the level of fluid in the tube T_1 goes up and down with each contractile cycle. The length of the tube, which is 10cm in these experiments, determines the height of the column of Krebs and

thus the left ventricle filling pressure. Since the tube is graduated the volume change with each contraction could be read. In this way it is possible to determine the volume change on a beat to beat basis, and to record it with a sensitive pressure transducer PT2. Any excess of Krebs in the system, slowly developing from the thebesian drainage, spills over the top thus maintaining a constant filling pressure.

B. Changes in Mode of Contraction

The mode of contraction just described above produces free loaded contraction, i.e. fluid moving up and down the same tube, T1. Closure of the solenoid tap to T1 and any other direction produces isovolumic contraction.

The heart was stimulated through the right atrium by a stimulator (Devices stimulator type 2533). A digitimer, (Devices 3290) regulated the rate of stimulation and it also timed the operation of the relay to open or close the solenoid tap for free loaded and isovolumic contraction respectively. For maximum ventricular pressure under isovolumic contraction the relay is operated by the digitimer to close the solenoid tap at the end of diastole, i.e. when the level of saline in T1 has dropped to its minimum level.

6.1.2.2 Performance characteristics of the Apparatus in non-circulatory Mode

The results of figs. 6.3 and 6.4 were obtained when changing from free loaded to isovolumic contraction at different times of the cardiac cycle, i.e. different ventricular volumes.

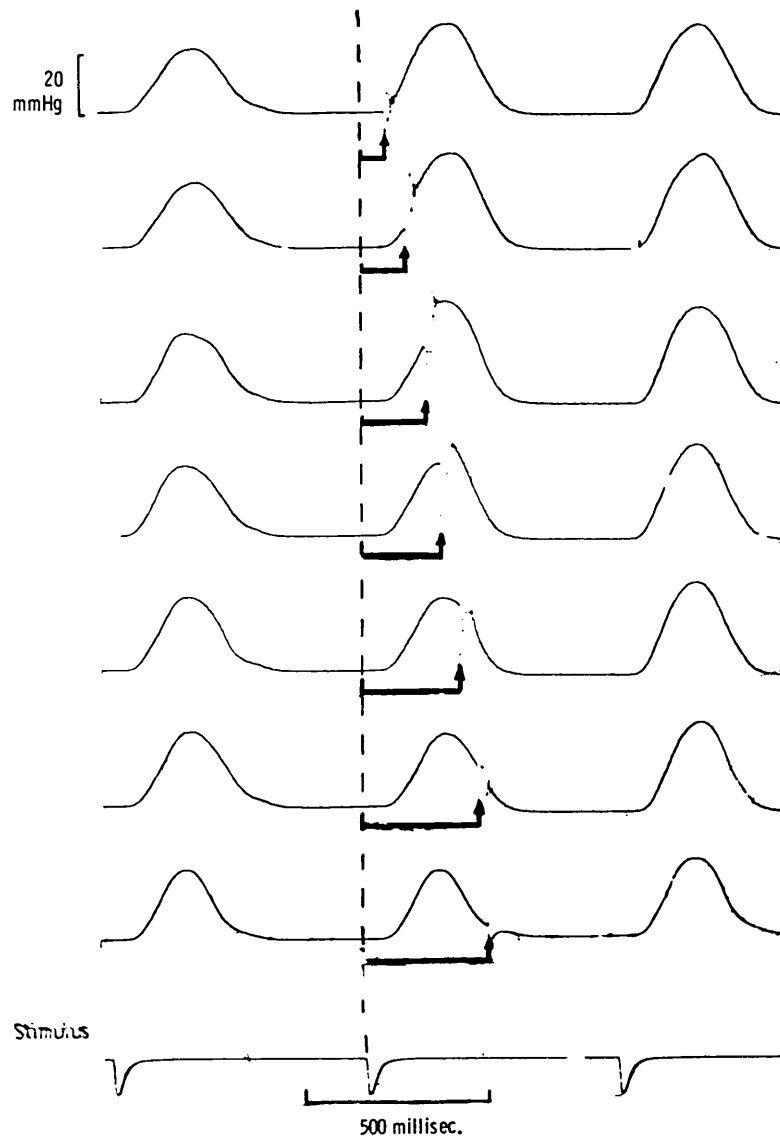


Fig. 6.3 The left ventricular pressure when changing from free loaded to isovolumic contraction at different times in the cardiac cycle.

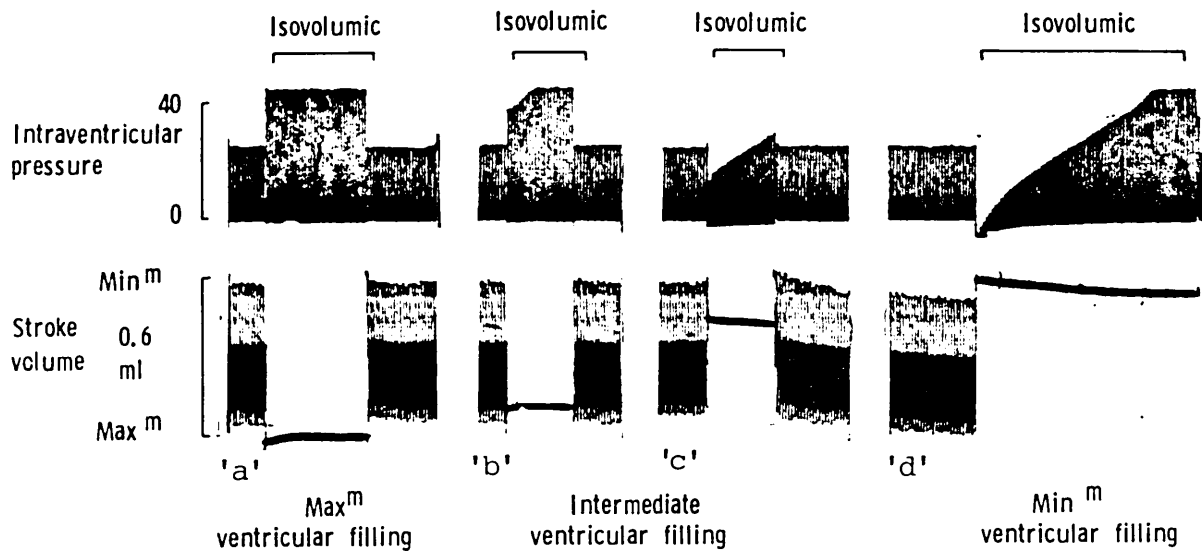


Fig. 6.4 Ventricular pressure (top trace) and the stroke volume, indicated by the recording of the sensitive pressure transducer, PT2 (bottom trace) when closing the solenoid tap at different times of the cardiac cycle.

When the solenoid tap is closed at maximum ventricular volume the level of saline in T1 is at its lowest level. The pressure due to the height of fluid in T1 can be recorded by incorporating a pressure transducer (PT2 in fig. 6.2) and such a recording is shown in fig. 6.4 (lower traces). The developed pressure is thus maximum (fig. 6.3 top and fig. 6.4 (a) top trace). When the tap is closed at the minimum ventricular volume, the level of saline in T1 is maximum and the developed isovolumic pressure is minimum (fig. 6.4 (d)). However, the pressure gradually rises to maximum due to ventricular filling by thebesian drainage. Fig. 6.4 (b & c) shows the ventricular pressure and volume in T1 at intermediate ventricular fillings.

6.1.2.3 Some Performance Characteristics under Recirculatory Conditions

For "re-circulation" a 3 way solenoid tap was used and an additional tube T2 connected to the solenoid tap (fig. 6.2). As described above, during diastole the solenoid tap is opened so that saline is allowed to flow from T1 to the ventricle. But now at the beginning of systole the solenoid tap is opened into T2. As the ventricle contracts Krebs is ejected into T2. At the end of systole the tap opens back to T1 and the ventricle fills from T1 again. The timing of opening and closing of the solenoid is regulated by the digitimer. A suitable arrangement as shown in fig. 6.5 allows the saline to flow from T1 back to T2.

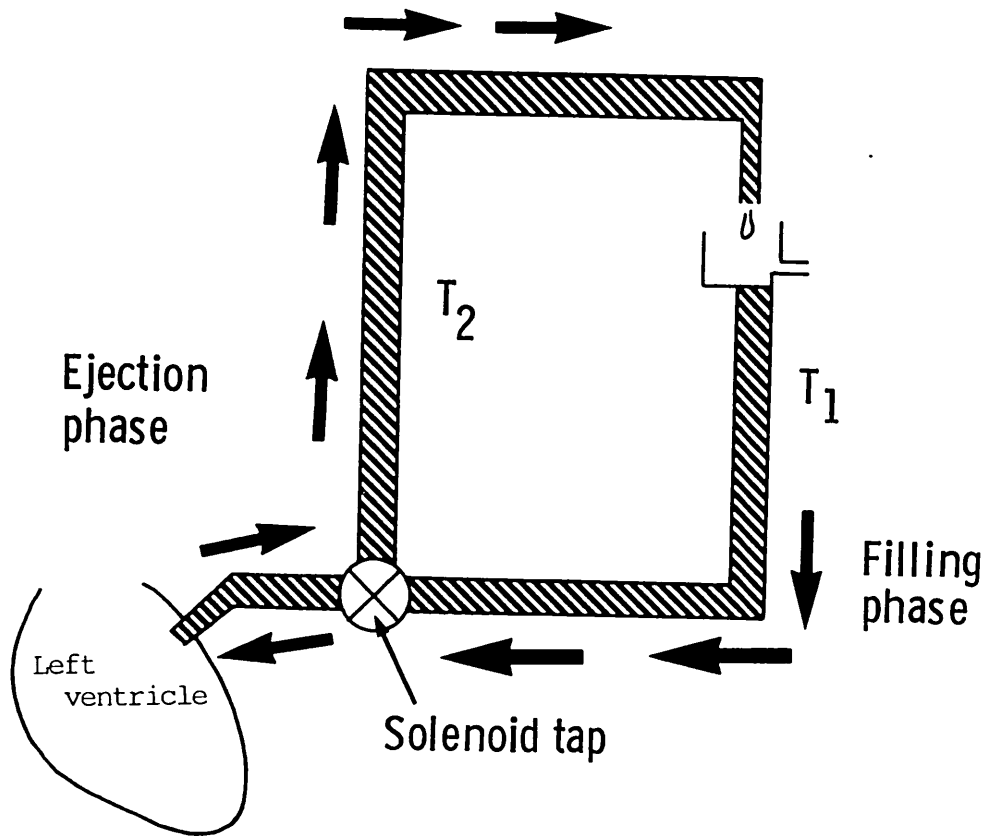


Fig. 6.5 The arrangement for re-circulation.

T_1 and T_2 are as in

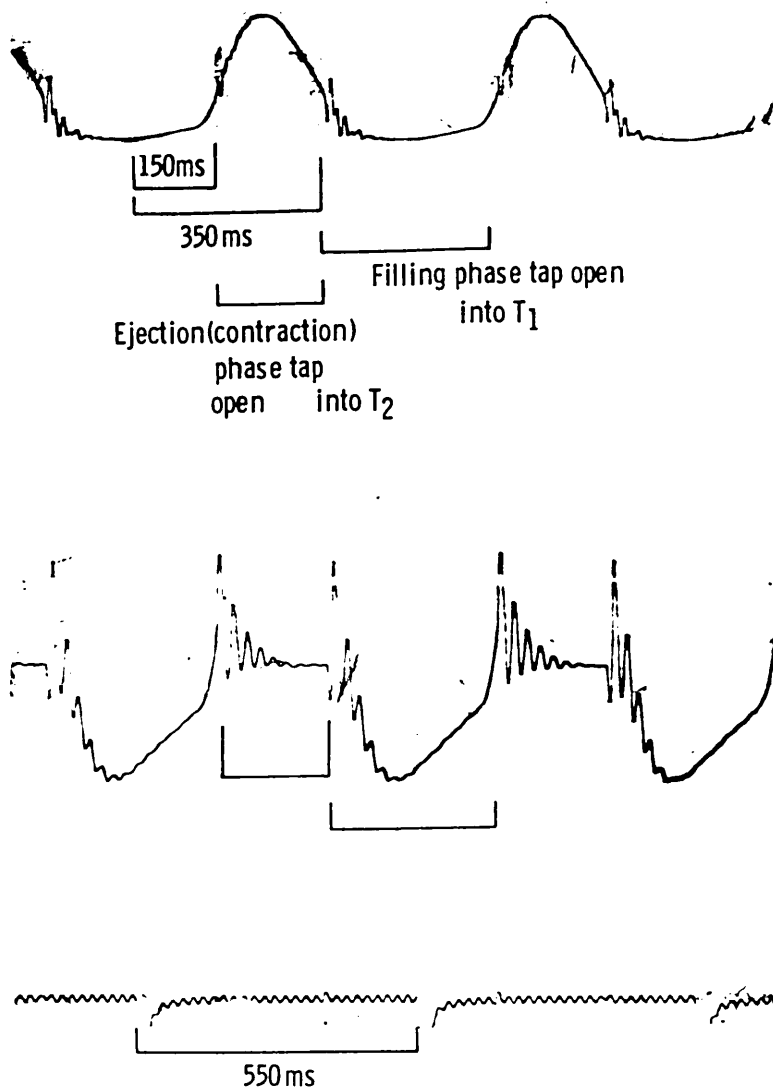


Fig. 6.6. Ventricular pressure (top trace) and the stroke volume, as indicated by the change in the column of Krebs in T1, recorded via pressure transducer PT2.

Fig. 6.6 shows records obtained under re-circulation conditions, with 10cm H₂O filling pressure or preload, from T1 and about 14cm H₂O afterload in T2. The noise on both records top trace and middle trace is recording artifact due to mechanical movements of the apparatus as a consequence of opening and closing of the solenoid tap. The top trace is ventricular pressure. The middle trace is the pressure recorded by the pressure transducer PT2 and reflects the volume change. The lowest trace in the stimulus at intervals of 550ms. The solenoid

tap was opened into T2 150ms after the stimulus signal, i.e. it was timed for the ventricle to open into T2 350ms after the stimulus near the end of the falling phase of ventricular pressure. Therefore, the duration of the ejection phase into T2 is 200ms i.e. (350-150). The duration of the filling phase from tube T1 is 350ms. This system produces a circulation of flow from T1 into T2. T2 can be arranged to empty into T1 from the top. During the ejection period the pressure developed by the ventricle recorded by PT1 is a function of the afterload i.e. height of volume of fluid in T2, the frictional resistance, and the preload as determined by the height of fluid in T1.

Appropriate arrangement of the pressure and volume can produce pressure-volume loops (fig. 6.7) analogous to those obtained in the working preparation. The pressure changes (ordinate) are recorded by PT1 and the volume changes (abscissa) are recorded by PT2. The area in the loop is related to the ejection work done by the ventricle, and maybe intergrated to find this value.

6.1.2.4 Advantages of the System

The apparatus imposed no major difficulties, on the contrary it had the advantage of simplicity of use and there is no other apparatus available to carry out mechanical studies on isolated intact heart. The quasi-working arrangement had the advantage of being simple and requiring a simple heating system as compared to the working apparatus (Taegtmeier et al 1980). A possible disadvantage of the arrangement I used was the fact that the volume of the fluid filling the ventricle during diastole could not be controlled for long test pulse intervals while obtaining the restitution curve. So that at long test intervals,

the ventricle was possibly over-filled and maybe stretched, but this situation was in fact quite close to the physiological condition in situ. The most serious difficulty I had to overcome with the working apparatus was a technical problem. Sometimes an air bubble got into the ventricle while cannulating it and this air collected just below the aortic valve. A postextrasystolic potentiation under isovolumic contraction can exceed the perfusing pressure and open the aortic valve. Any air trapped would go up the aorta and could enter the coronary arteries. To get rid of any trapped air bubbles, the inflow of Krebs was stopped simultaneously as when changing to isovolumic contraction. A side arm in the aortic cannula (C1 in fig. 6.1) was opened. Should there be any bubble, it was pushed out into the side arm. 10cm H₂O filling pressure was used to avoid stretching the ventricle, because there was no-one way valve in the system used. Should the filling pressure be too high, the ventricle would be over stretched under diastole. The increase in filling pressure would also increase O₂ demand up to a limit, after which the O₂ delivered might not meet the demand. Under this condition the ventricular pressure would decline gradually with time. Pilot experiments showed a maximum developed pressure at 10cm H₂O filling pressure with little increase at higher filling pressures. Weber et al (1980) observed aerobic limit in isolated blood perfused dog hearts, paced at 150/min, at 26cm H₂O filling pressure, beyond which the ventricular pressure decreased and alternans developed.

The main problem in Langendorff perfusion is oedema due to protein free perfusate. The effect of oedema on APD is not known. A second possible problem with Langendorff perfused preparation is the diffusion of O_2 into tissue. However, in coronary perfused Langendorff preparation, diffusion from O_2 saturated medium to the O_2 consuming sites of contractile structures is thought to be the same as under physiological condition, (Reichel 1976). It is worth remembering, however, that the coronary arteries are maximally vasodiluted with Langendorff perfusion. Coronary flow did not significantly alter when comparing free loaded with isovolumic pressure.

However, in comparison to the working system, the above quasi-working arrangement has the following advantages:-

- i) This system is simpler and needs less complicated heating. Only the perfusing medium is heated before perfusing the heart and the left ventricle acts as a heating chamber for the saline in the quasi-working system.
- ii) In the conventional working apparatus it can be a complicated affair to change the coronary perfusing medium because the ejecting fluid and perfusion fluid are part of the same system, but because the quasi-working arrangements and the perfusion of the heart through the aorta are two individual and separate arrangements, perfusion changeover is relatively simple.

iii) Mechanical studies can be carried out with minimal interference with the perfusing pressure or flow. For example, it is relatively easy to quickly change the modes of contraction from free to isovolumic and vice versa.

iv) It is easy to change the pre-and/or afterload in re-circulating mode by using longer or shorter tubes of T1 and/or T2 respectively.

v) It is possible and not expensive to build an electronic device to operate the opening and closing of the solenoid tap which in itself is operated by the ventricular pressure so that at the beginning of the rising phase the solenoid tap opens into T2 and at the end of the falling phase of pressure the solenoid opens into T1.

A one way opening pressure valve in the direction of T2 will allow only the flow from the ventricle into T2 and prevent any retrograde flow into the ventricle. This one way valve mimics the aortic valve. The ventricle now acts as a simple force pump.

In our hands some 50 preparations were studied using the above assembly for an average of 6 hours (range 5-8 hours) each with little change in the ventricular pressure under free loaded or isovolumic contraction at the end of the experiment from that at the beginning of the experiment.

6.1.2.5 Limitation of the preparation

The isolated heart preparation in this study, as all krebs perfused preparations, suffers from the draw back of being perfused with a solution free of red blood cells (i.e. 0% Hematocrit).

Isolated heart preparations under free hematocrit perfusion have been found to have higher coronary flow, decreased external work and reduced myocardial O_2 consumption compared to in intact animal. For example, in a working isolated rat heart perfused with pig red cells suspension, the coronary flow was halved, external work doubled and myocardial oxygen consumption more than doubled when the hematocrit was increased from 0% to 30% (Duvelleroy et al 1976). These results suggest a better performance can be obtained with red blood cell suspension than with free red cell solution.

Duvelleroy et al (1977) found the coronary sinus O_2 partial pressure in isolated rat working heart to be higher with a red cell free perfusate which suggested that the coronary flow is partially shunted under such conditions. These results led Duvelleroy et al (1977) to conclude that oxygenation of the myocardium is inadequate with an electrolyte perfusion which could suggest that the isolated rabbit heart preparation used in this study may have been hypoxic. However, changing from free loaded contraction to isovolumic contraction had no effect on the coronary flow as measured with a measuring cylinder. It must be borne in mind that in comparison to insitu heart the Langendorff preparation may be hypoxic. However, restitution curves results from intact insitu pig heart are similar to those results from the isolated rabbit heart which suggest that the two preparations are comparable.

6.1.2.6 Stability of the Experimental Preparation

For some unknown reason, the preparation was very stable for as long as my experiments went on. The ventricular pressure under free loaded contraction at the end of all experiments was almost identical to that at the beginning. Under isovolumic contraction the ventricular pressure remained constant over the whole of any restitution curve, discounting the possibility of any hypoxia as would be shown by a drop in ventricular pressure. In other isolated preparations the ventricular pressure declined quickly over the first two hours of perfusion. This might be due to the obstruction of the thebesian drainage which is considered as part of the coronary flow. This flow was measured in some of my experiments and found to be in the order of 1-2ml/min. If this flow is stopped a slowly developing hypoxia could develop.

One possible explanation for the long stability of the preparation that may be worth investigating is related to oedema formation. Protein free perfusion facilitates oedema in Langendorff preparation and it is possible that intramyocardial pumping action produced by the quasi-working heart (Khatib and Lab 1982b) removes oedema fluid.

6.1.3. Intact Pig Heart Insitu

6.1.3.1 Anaesthesia and Respiration

Large, white, landrace cross pigs 18-22kg were premedicated with

intramuscular 'Suicalm' (Azaperone), 8 mg/kg (Janssen pharmaceutical). Anaesthesia was induced 20 mins later with brietal (Methodexitone Sodium 10 mg/kg Eli Lilly & Co. Ltd.) through a size 18 medicut which had been inserted into an ear vein. Once the animal was anaesthetized deeply enough (no eye reflex) it was turned onto its back. Strips of bandage were used to open the jaws, and the larynx was sprayed with Xylocaine to decrease the mechanical sensitivity of the vocal cords. With the aid of a specialised laryngoscope the epiglottis was held back and a 7mm endotracheal tube was inserted into the trachea, with the aid of an introducer. The cuff around the tube was inflated enough to keep it in place, and the jaws were then tied together with the tap. The laryngoscope was of a special construction (Penlon Abingdon Oxon). The pig has a larynx set at an angle to the trachea, and in order to hold back the epiglottis a longer blade is necessary.

Anaesthesia was maintained through the experiment with 1% Halothane vapourised and carried in a mixture 1:1 of oxygen and nitrous oxide. The gas mixture was passed through a 'Fluotec' vapourised from a Boyle's Machine, (British Oxygen Co. Ltd.). This mixture of anaesthetic gasses was delivered by a Manley positive pressure ventilator on which the tidal volume could be set (Blease Medical Equipment Ltd): the appropriate minute volume having previously been determined by setting the flow meters on the Boyle's machine to deliver the required volume.

The pig was placed on its back, prior to attachment to the anaesthetic machine, on a thermostatically controlled heated operating table. The diathermy earthing plate was placed under the animal's back.

6.1.3.2 Surgery

A carotid artery was exposed in the neck using a diathermy point for the overlying skin and a clamp was placed round it. A heparinized saline filled polythene cannula was inserted through a small hole in the artery and tied in place once the clamp was removed. This cannula was connected by a plastic three way tap to a pressure transducer (Statham), for the continuous monitoring of the arterial pressure. In the other artery a catheter tip pressure transducer (Gaeltec) was inserted all the way down to the left ventricle for recording of the ventricular pressure. The front of the animal's chest was removed by wide incisions made either side of the sternum using a cutting diathermy and bone cutters for the ribs. All the small blood vessels were sealed with diathermy but larger vessels such as the internal mammary veins and arteries were tied. The exposed cut ends of the ribs were sealed by the application of a thin layer of bone wax. A pericardial cradle was fashioned to support the heart. The edges of the anterior surface of the pericardium were sutured to the free walls of the chest. The surface of the heart was always thereafter constantly kept moistened with saline, or the exposed surface of the heart covered with moist swabs between recordings. A physiological saline drip was set up via a cannula in one of the jugular veins. This was done to replace any blood loss sustained during surgery and to maintain the body fluid level throughout the experiment. A constant check was maintained of the animal's temperature throughout the experiment. The pH of the blood was also occasionally monitored and the gases appropriately altered. In practice the latter was never necessary.

6.1.3.3 Changes in Mode of Contraction

To produce an isovolumically contracting left ventricle the aorta just above the heart was freed of all surrounding tissue and a strong polyvinyl tubing, 25cm long, was passed around the aorta and both ends were secured in a clamping device (Jewell 1969). The aorta was trapped between the polyvinyl tubing going round it and a perspex piston. By applying pressure from the other end of the device the piston moves down clamping the aorta underneath it. The aorta can be clamped as long as the pressure is on, and when the pressure is released a spring recoils pushing the piston back and thus releases the clamp.

6.2 Recordings

6.2.1 Monophasic Action Potential (AP)

6.2.1.1 Construction of Suction Electrode (Fig. 6.8)

Three holes were drilled in a block of perspex to mount 4mm sockets and a plastic cannula, to which a blunted 19 gauge needle was fixed by its luer fitting. A length of polythene tubing (20cm long, 1.4mm diameter) was attached to the blunt end of the needle. A silver wire was passed through the wall of the tubing, and pulled level with the open free end (i.e. recording end). The other end of the silver wire was soldered to an electrical wire that was attached to one end of the socket on the perspex block. The hole was then sealed with silicone rubber and a small piece of shrinkage tube put around the polythene tubing above the hole and then shrunk. To ensure no leakage and to protect the soldered joint, the ends of the shrinkage tubing were also sealed with silicone rubber.

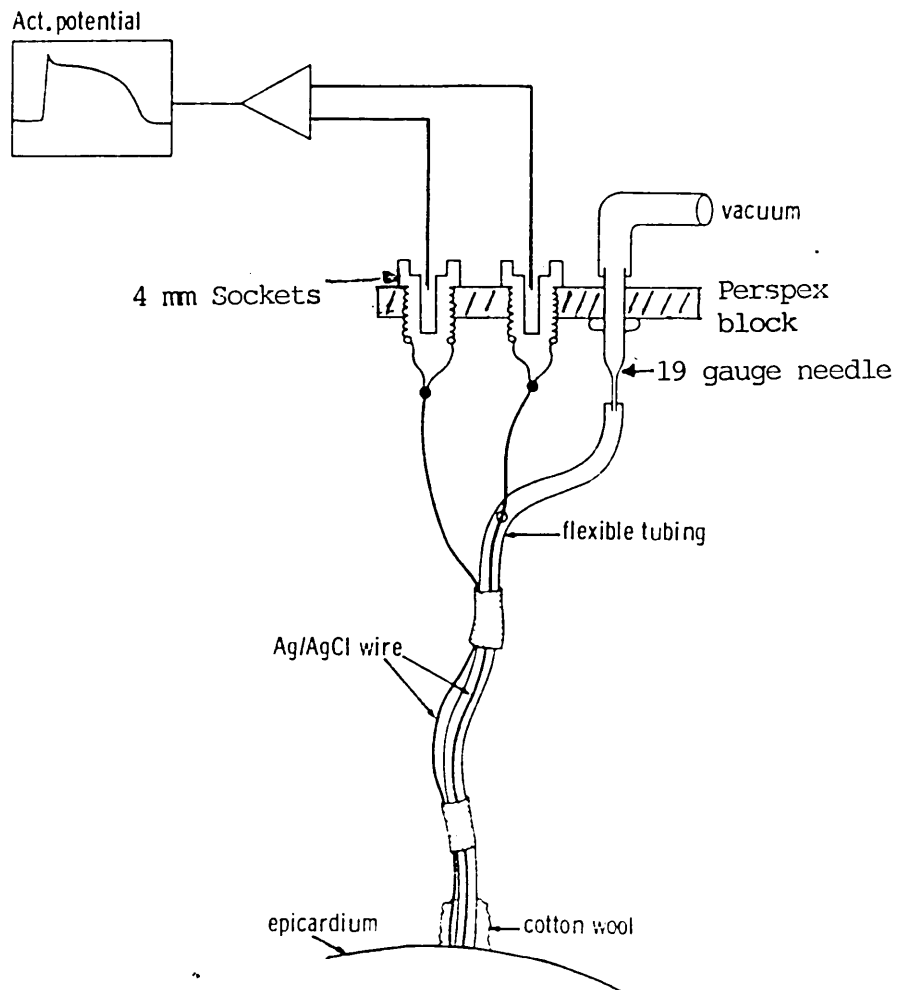


Fig. 6.8 The structure of the suction electrode.

A small piece of cotton wool was then fixed around the outside of the recording end of the tubing, and another piece of silver wire was wrapped and securely fixed around the cotton wool. The free end of the silver wire was soldered to a second electrical wire which was attached to the perspex block in the second socket.

The silver/silver chloride (Ag/AgCl) wire on the inside of the tubing recorded an injury potential which was fed into one input of a differential amplifier (Devices 3461). The other input was from the Ag/AgCl wire around the cotton wool. This arrangement allowed the potential differences between the cells beneath the centre of the tube and the immediately surrounding normal myocardium to be recorded and provide a monophasic action potential.

6.2.1.2 Action Potential Recording

Monophasic action potentials were recorded from different regions of the left ventricle with suction electrodes, applied to the epicardium by vacuum. Care was taken to avoid including the more obvious epicardial blood vessels.

The signals were recorded as the differences between the voltage of the inner electrode (Ag/AgCl) and the immediately surrounding cotton wick electrode with a Devices DC differential amplifier 3461. When an electrode is placed on the myocardium, the AP has large amplitude (50-80mV) but rapidly (20-30 seconds) decreases to a new steady state amplitude (30-40mV). The AP amplitude continued to decrease but now very slowly. The protocols were executed when the AP had reached the steady state (after 30 seconds of application) and this procedure was repeated every time that any one of the electrodes was replaced.

6.2.1.3. Action Potential Duration (APD)

This was measured in the same way in both pig and rabbit as the distance between the upstroke of the action potential and the repolarization phase at the point of 70% repolarization using a magnifying glass. The duration in divisions was multiplied by 10, since the paper speed was 100 div/per second to convert it into milliseconds. The paper speed was often checked by test pulses from the stimulator, and, in any case, the recorder itself has a timer at one second intervals. However, it was easy to check the paper speed from the regular pacing rate prior to all attempts in changing the rate.

6.2.1.4 Criticism of the Suction Electrode

An ideal suction electrode would be one which provides completely intracellular recordings. Our suction electrode is certainly not ideal but still reliable for the duration (Hoffmann et al 1959, Churney et al 1964) and has the advantage of being easy to use in studies involving the whole intact heart as compared to the glass microelectrode. The principle of its recording is that myocardial cells are drawn, by suction into the tip of tube. This creates a mass of damaged cells, which are partially depolarized and with none excitable membranes. The inner electrode which is in contact with these cells, records the potential between them and the external electrode in contact with the immediately adjacent normal myocardium. The potential is reduced by the short circuiting effect of the extracellular fluid.

Chloriding of the Electrode

It was essential, for good chloriding and eventually for accurate recording to make sure that the silver wires were degreased with acetone before use. For chloriding, the recording end of the electrode was immersed for 15 minutes in a solution comprising; 60ml distilled water, 60ml concentrated HCl and 3gm of Fe Cl₃. This prevented the wire from acting as a battery (metal in electrolyte) which could generate its own DC voltage. This process formed a thin layer of silver chloride deposit on the pure silver wire. Whenever the electrodes were used, they were chlorided for 10-15 mins and thoroughly washed under tap water and then soaked in distilled water for 30 minutes. This was to make absolutely sure that the cotton wool was free of acid which could damage the normal myocardium under the cotton wool, which was vital for the recording. The process of re-chloriding meant accumulation of an increasing thickness of silver chloride layer which might be non-uniform in thickness. Gedds et al (1969) examined the effect of the chloride deposits on the impedance of the silver electrode. They showed that the impedance was different for different layers of chloride. However, they doubted whether the thickness made a significant difference in recording.

6.2.1.5 Problems in Recording of Action Potential

A. Suction Problems

To maintain a stable AP with small changes in amplitude, it was important to have the correct suction pressure for that particular diameter of tubing used in the construction of the electrode. This was found to

be about 400mm Hg for the electrode used for this project. Suction below this value resulted in an AP of a small amplitude. Too much suction pressure, above 500mm Hg, produced a very rapid decrease in the AP amplitude, which could be due to changes in myocardial tissues under the wall of the tubing thus reducing the potential difference between the intact and the injured myocardium.

B. Action Potential Amplitude Problems

Although the suction electrode is a reliable index of the shape and duration of the AP (Hoffmann et al 1959, Churney et al 1964) the amplitude is only 40-50% of that expected from a microelectrode, even immediately after applying the suction electrode to the myocardium. The amplitude of the AP decreased very rapidly, immediately after application, for about 15-20 seconds after which the amplitude continued to decrease with time, but very slowly 30 mins or so. This could be due to the healing of the damaged myocardium under the tubing of the suction electrode. A typical AP amplitude at the start of a restitution curve (chapter 8) was 30-40 mV and after the 20 minutes of obtaining a single restitution curve it decreased to about 10-20 mV. It rarely went down below 10mV in all the experiments. If it did so, the electrode was repositioned. All the AP recordings for any particular area was obtained from a circular area of no more than 3mm in diameter. There was a small and insignificant differences between action potential's recorded from the same area under a particular mode of contraction as a consequence of replacements. If there was a big difference in APD on replacement, say in the order of 10ms, either the electrode was replaced again or the whole restitution curve was

repeated if the replacement took place at the middle of the protocol under free loaded contraction. However, the steady state AP was 'reasonably' steady over the whole of any restitution curve in most of the experiments. A standard deviation of 1-4ms was observed in about 80% of the experiments. This could well be the result of measurement errors because of the decrease in AP amplitude. Sometimes the signal was increased by changing the gain of the amplifier to reduce the error in measurements of the duration, fig.6.9

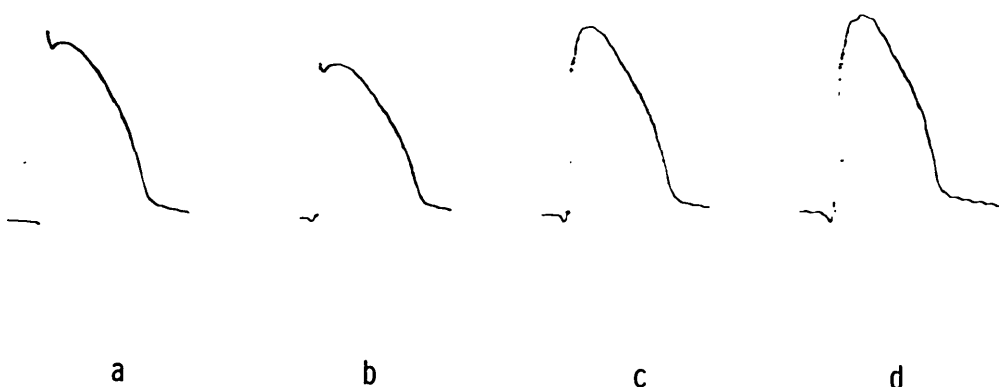
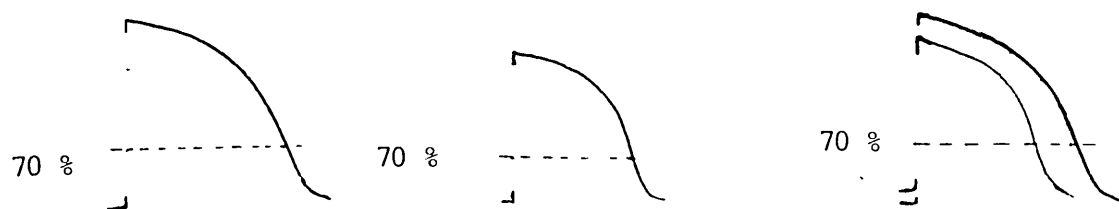


Fig. 6.9 Steady state AP's at the beginning 'a', intermediate 'b, c' and at the end of a restitution curve 'd'.

Provided everything went well and no replacement was required, as was often the case, it took approximately 15-20 minutes to construct a single restitution curve repeating each TPI twice. More time was needed when there was a replacement, but in no experiment did it take more than 30 minutes to obtain a restitution curve. An important

disadvantage of the suction electrode was that long recordings from the same place were needed, resulting in the need for replacements, and this leaves a damaged area. Such frequent replacements could result in a large area of the myocardium being damaged because every time it is replaced it probably shifted a small distance.

Figure 6.9 shows four steady state AP's at the beginning 'a', intermediate 'b', 'c' and at the end of a restitution curve 'd'. The average steady state APD as measured by a computer (Research Machines 380Z), for the experiment in the figure, was 156.5 ± 1.9 ms ($n = 20$) and my measurements gave 158 ± 2.3 ms, ($n = 20$) (The same AP's were measured in both cases). Occasionally records are shown in which an analogue output of action potential duration was provided by an amplifier designed for this purpose (Lab and Child 1979). Where action potentials were superimposed for the figures, this was done at the 70% repolarization level as indicated (below).



C. Measurement Problems

As a result of the presence of occasional after-depolarizations it was difficult to take the base line at the end of the repolarization phase as 100%, therefore the amplitude of the AP was measured from the base line of the fast upstroke to the plateau. The continuous change of the heart rate while studying the relation between APD and segment shortening in the intact insitu pig heart was a problem because I did not pace. For valid comparison, the APD was divided by the preceeding steady state interval when recording from a particular area. The ratio of APD/preceeding steady state interval was then divided by the largest ratio of the same preparation.

6.2.2. Epicardial Segment Length

6.2.2..1 The Tripod Construction

A hexagonal perspex block 6mm diameter and 3mm high was hollowed from the top, leaving a thin walled hexagonal perspex box. A hole 1mm was drilled on one of the faces and a piece of polythene tubing (0.5mm ID, 1mm OD) was fixed in. This tube was used to connect the tripod to a vacuum pump (see section on electrode construction). On the other side of the perspex box, three 1mm holes were drilled at the points of an equilateral triangle, the central point of which was the centre of the perspex box. Three lengths of soft polythene tubing (0.5mm ID, 1mm OD) were glued in the three holes. These three tubes formed the legs of the tripod.

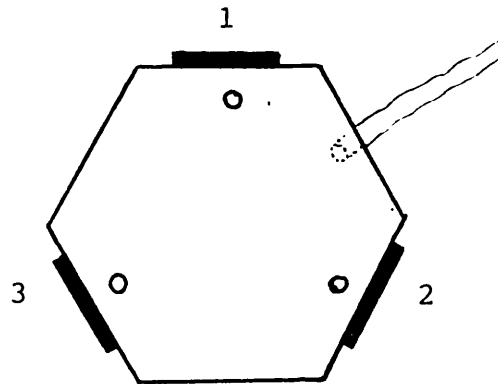


Fig. 6.10 The tripod surface with the phosphobronze shims 1, 2 and 3 at alternate sides.

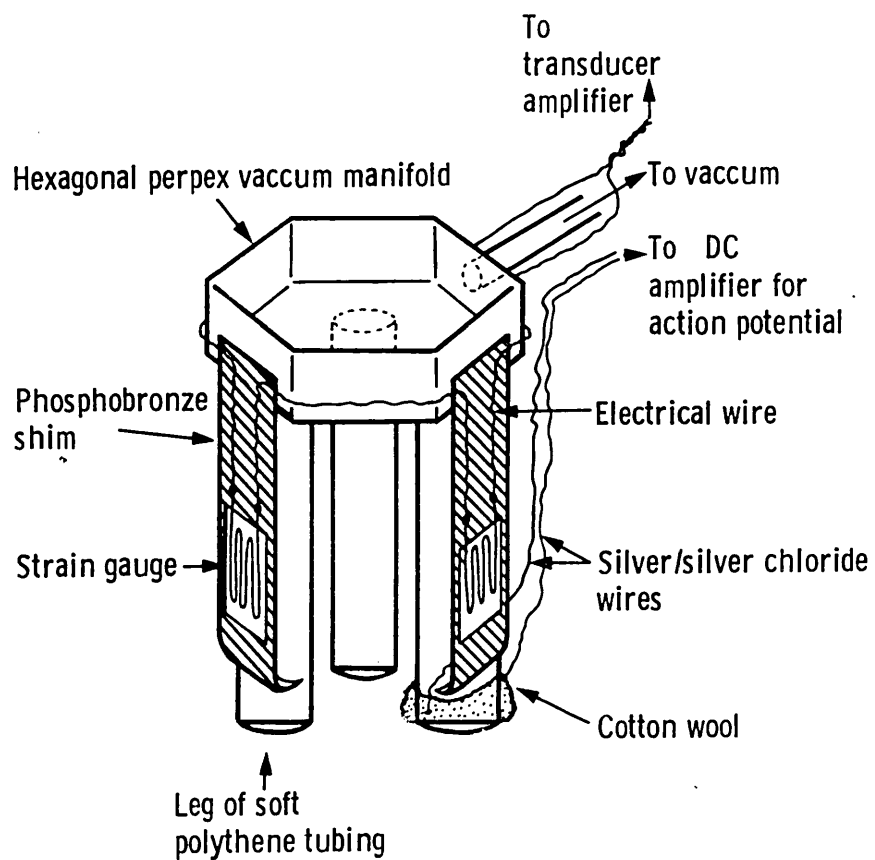


Fig. 6.11 The tripod Structure.

The holes were drilled in such a way that each one was at the central point of three alternate faces. Three strain gauges (type EA-09-031DE-120 or EA-09-062AP-120 Welwyn Strain Measurements Ltd) were glued on in the middle of three 7mm long, 2.5mm wide phosphobronze shims. The phosphobronze shims were then glued (with a 3mm overlap) on the alternate sides of the perspex box, each one facing a leg of the tripod (fig. 6.10). The shims were bent inwards approximately 1mm from the free end and glued firmly to the polythene tubing legs 1mm above the end of the legs. The three strain gauges were connected together in series by thin electrical wires and then to a bridge amplifier. Where action potential and epicardial shortening were to be recorded simultaneously from the same area of the intact ventricle of the pig, one of the tripod legs was modified into a suction electrode by passing a wire through the wall of a leg, and pulled through to the recording end. A small piece of cotton wool was wrapped around the bottom of the leg and a silver wire was wrapped around it (as shown in the diagram fig. 6.11). The areas where the polythene tubing joined the perspex box were covered with silicone rubber to ensure a good seal and prevent leakage. The resistance of the 3 gauges were checked to be 120ohms (this is what it should be) to ensure no strain on the gauges at rest.

This arrangement leaves legs of 7mm to bend in response to movement in the only possible direction, 120° to each other, and to thereby deform the strain gauge.

6.2.2.2 Ejection Shortening of Epicardial Segment and its Measurements

The tripod, applied to the area under study by vacuum, was connected to a wheatstone bridge device and the bridge was balanced until the output was zero. This procedure cancelled any output from the strain gauges due to any gauge deformation at rest. When a leg is bent in response to a movement the balance of the bridge is disturbed and the electrical output was recorded by a Devices DC amplifier. The linearity of response in the three legs to the same deformation, was determined by placing the tripod in the horizontal position and hanging weights of 1, 2 and 5gm on each leg. The tripod was turned until maximum output was obtained for each leg at a particular weight. The output of all three legs were similar at any weight as shown in fig. 6.12. The outputs are normally electrically summed. The outputs were also mathematically summed up to construct a calibration graph as shown in fig. 6.13.

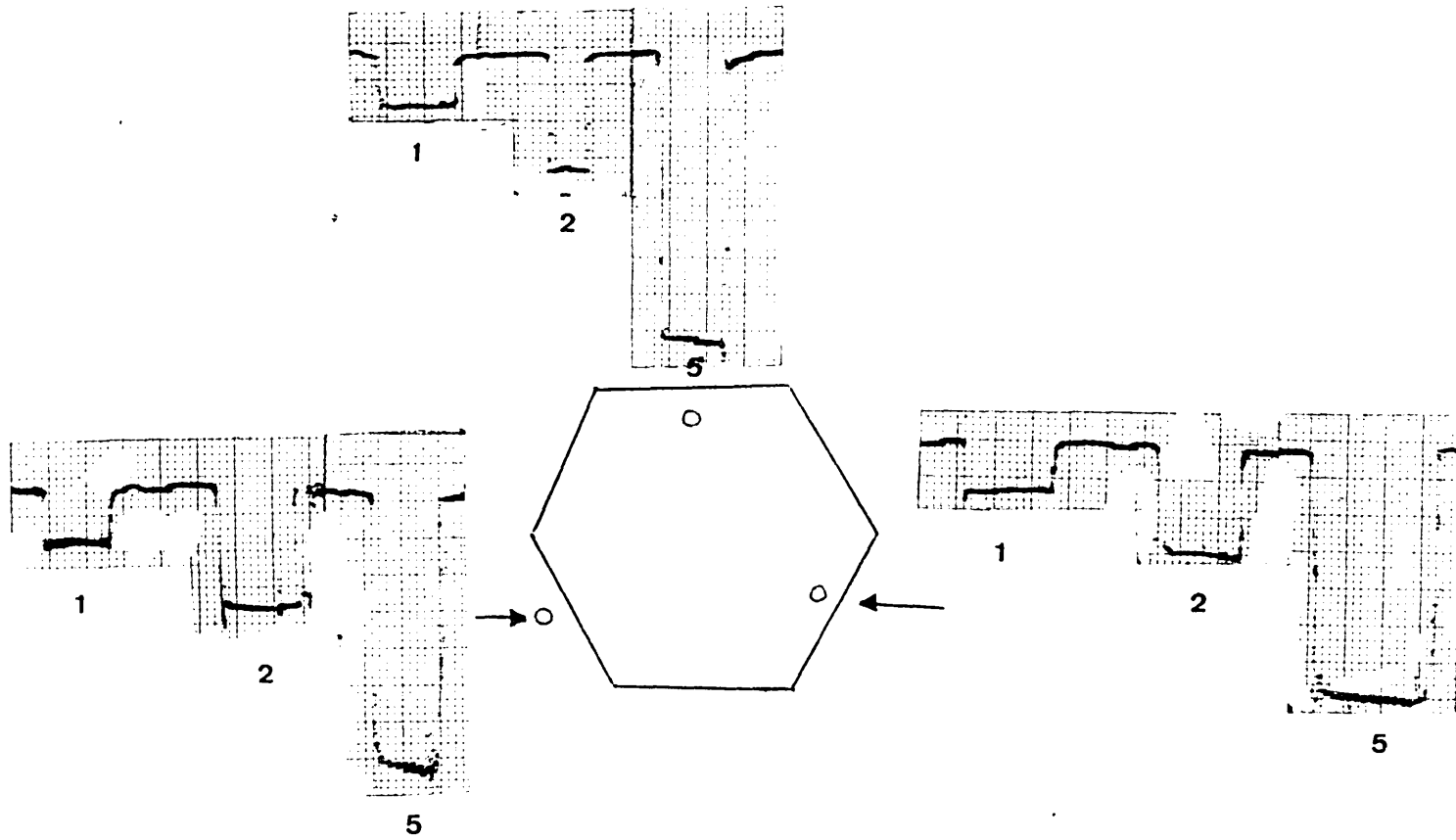
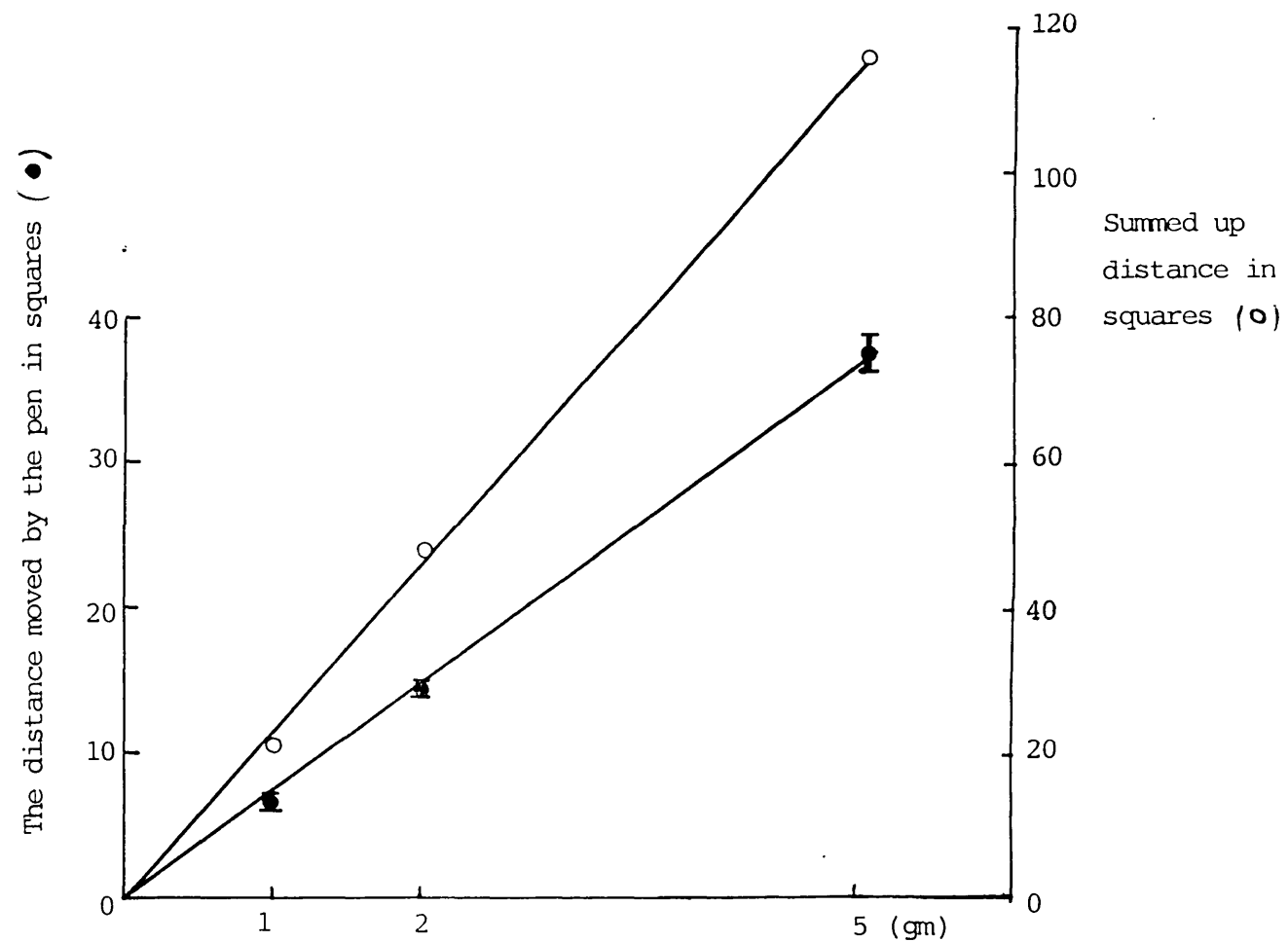


fig. 6.12 The output of each leg of the tripod at 1, 2 and 5 gm.weight.



(○) The summed up outputs of the three legs for each weight.
 (●) The average output(mean $\pm$ SD) of the three legs for each weight.

Fig. 6.13 Calibration curves for the tripod.

Measurements Epicardial Segment Shortening

It was difficult to study the mechanical changes in the rabbit heart because of the size of the tripod needed to carry out such a study with any accuracy. In the intact pig the AP and the ejection segment shortening were recorded simultaneously with the modified tripod from random areas at a fast paper speed. The AP was measured as described above and ejection shortening of that area was measured as the distance between the base line and the point corresponding to the 70% repolarization of the accompanying AP. This point was usually at the maximum ejection shortening fig. 6.14 and it is the point at which the mechanics could have a maximum affect on the AP (Kaufmann et al 1971).

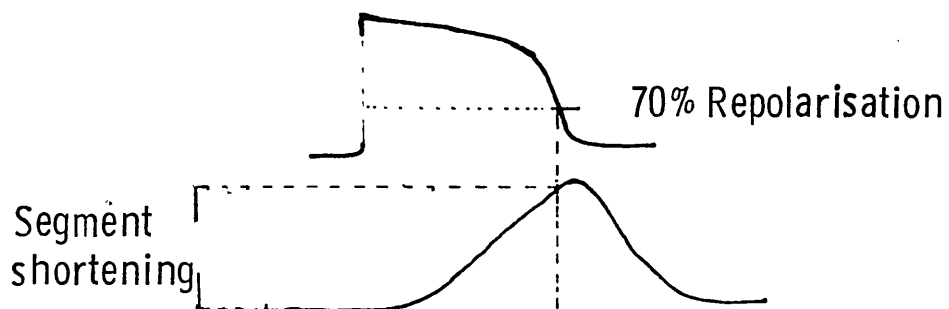


Fig. 6.14 The point at which the segment shortening (lower trace) corresponding to 70% repolarization of the AP.

Where the results are expressed as percent of steady state, whether action potential or segment shortening, the 3 or 4 complexes before the rate change were measured and the average was taken as 100%. For the restitution curves, the average of the preceding complexes before each attempt were used to calculate the percentage of the test AP for that attempt.

6.2.2.3 Problems with the Tripodal Device

The legs of the tripod were oriented at 120° to each other and can only move in one possible direction, i.e. along their axes.

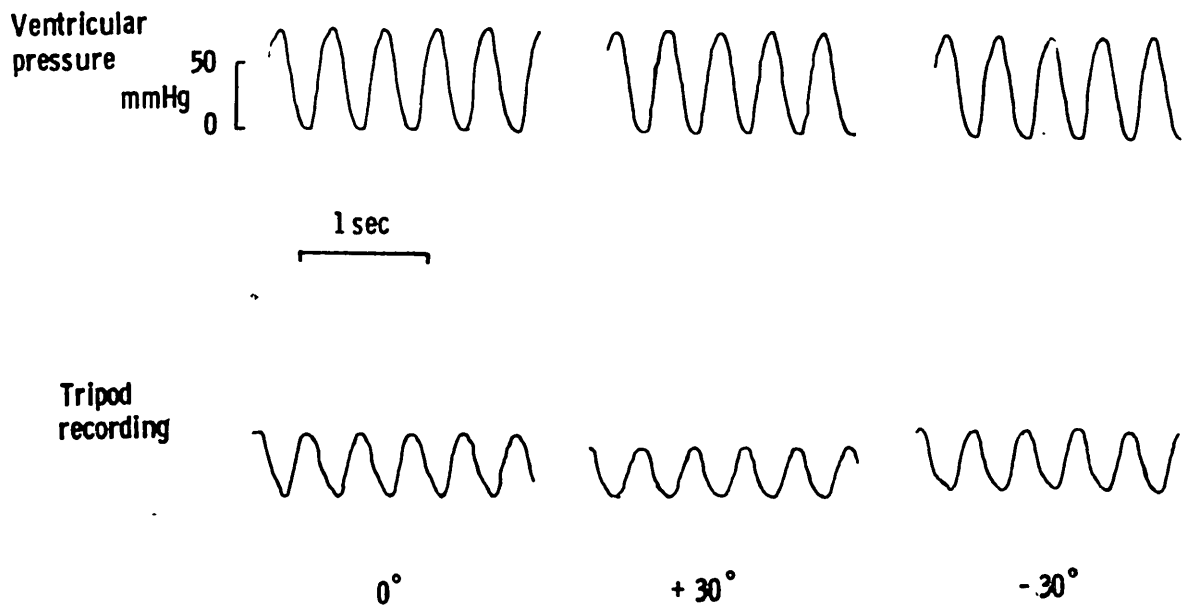


Fig. 6.15 The segment shortening recorded when rotating the tripod 0° , $+30^\circ$ and -30° .

It was essential, for good representative recording, to obtain three identical lengths responding equally to the same change. This was tested by hanging weights as described above. The results of this procedure are in fig. 6.12. In doing so I assumed that the response of the legs to outward movement is identical to the response to inward movement, i.e. away from the centre of the tripod and towards the centre of the tripod respectively. The second problem was the movement of the tripod in relation to the myocardium, if the movement is not symmetrical. To test this the tripod was rotated $+30^\circ$ and -30° , while recording from an area in the intact pig heart. It was expected to observe a difference in the order of 10-15% (Lab and Woolard 1978). The results of rotation are shown in fig. 6.15. There was a small difference on rotation but in agreement with the expected. The major problem in expressing the segment shortening results, was the change in heart rate experienced whilst recording from different areas of the epicardium during an experiment, because the hearts were not paced. It would be possible to predict that with a slow heart rate, the segment shortening would be greater in a specific area. To standardise the results, for valid comparison, the segment shortening of all areas at 70% repolarization (fig. 6.14) was divided by the immediately preceding steady state interval. To normalise the results the above ratios of segment shortening to the preceding interval of all areas were divided by the longest ratio of that particular experiment. The AP received the same treatment (as mentioned above). At all times, care was taken to avoid any leg of the tripod being applied to an obvious blood vessel.

6.2.3. Ventricular Pressure

The recording of all the parameters was done through the appropriate amplifier on Devices Mx4 or Mx2 for the isolated rabbit and on M19 for the pig experiments, and played out on a heated stylus chart recorder at a speed of 100 divisions per second. Where these recordings have been done simultaneously this is stated in the protocols section.

In the isolated rabbit heart experiments only, pressure transducer PT_1 was used and it was calibrated at the beginning of each experiment with 20, 40 and 50 mmHg (three columns of water 27, 55 and 68cm high respectively). The level of the transducer was adjusted to the level of the heart to eliminate hydrostatic pressure differences, and the zero level was set on the recorder before calibrating by opening to atmospheric pressure. The ventricular pressure was continuously monitored throughout all the experiments. In the intact insitu pig experiments all pressure transducers were calibrated only with 50 mmHg. All the recorded parameters from the pig experiments were displayed on Tektronix 5103N storage oscilloscope and on magnetic tape (Racal Thermionic tape recorder).

Measurements of Ventricular Pressure

The developed ventricular pressure in both the rabbit and the pig experiments was measured as the distance between the minimum diastolic pressure and the maximum systolic pressure. To convert it into a mm Hg, it was multiplied by the appropriate factor obtained from the calibration signals in each experiment.

6.2.4 The Electrocardiogram (ECG) Recording

In the isolated rabbit heart bipolar epicardial ECG was recorded by two cotton wick electrodes (pieces of cotton wool and AG/AgCl wires) placed halfway down the left ventricle and about 2-3cm apart. The inputs from both electrodes were fed into a Devices AC high gain amplifier type 3542. It was ensured that the cotton wool was wet all the time. In the pig experiments a conventional limb lead ecg or epicardial (V-lead) ecg was recorded. No protocol was adopted for ecg recording. It was recorded simultaneously with the other parameters while obtaining the restitution curve in a random selection of experiments. The Q-T interval was measured as the distance between the start of the QRS and the middle of the T-wave.

6.3 Experimental Protocols

6.3.1 The Isolated Rabbit Heart

After cannulating the aorta and the left ventricle, two pacing silver electrodes were hooked onto the right atrium about 1-2 mm apart and connected to a Devices stimulator 2533. The frequency of stimulation was controlled by a Devices digitimer 3290. In all the experiments the hearts were paced at a steady state (ss) of 150 beats/min i.e. 400 ms interstimulus pulse interval, except at low temperature (24°C), where the pacing rate was 100 beats/min. A heart rate of 150/min was chosen because the intrinsic rate was often just slower than this rate. This was also found to be the case in some earlier work done in this laboratory.

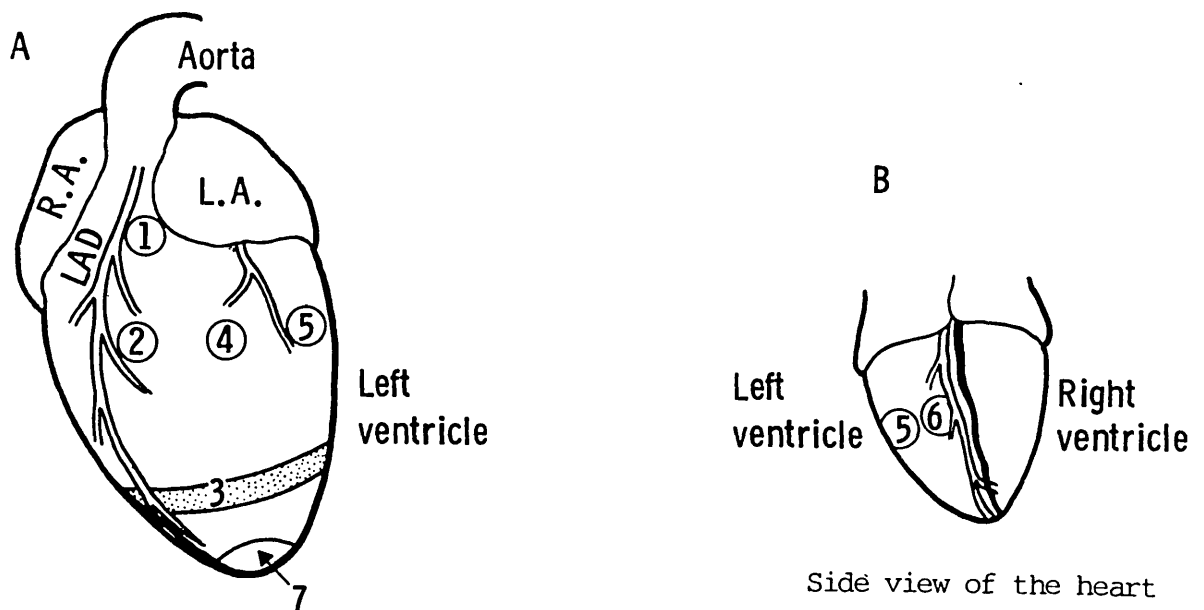


Fig. 6.16 The regions investigated in the left ventricle of the isolated rabbit heart.

L.A. and R.A. are left and right atria respectively.
LAD left anterior descending artery.

All the preparations were left for 15-20 mins to stabilise after starting perfusion and pacing. The rate of pacing was changed by the digitimer at the switch of a button. To change the conditions of contraction from free loaded to isovolumic, the digitimer was operated to close the solenoid tap (see apparatus section 6.1.2.1) at the maximum diastolic volume. The heart was left for about 5 mins to reach steady state before proceeding with the protocol.

All the areas investigated are shown in fig. 6.16. Usually two areas were investigated simultaneously, and at least two attempts of changing the rate were carried out under each mechanical condition of contraction. The steady state was allowed to be restored between attempts.

6.3.2. The intact pig experiments

As above, two silver electrodes were hooked onto the right atrium 2mm apart. The electrodes were connected to the stimulator.

The hearts were paced at rates ranging from 100 to 150 beat/min depending on the intrinsic rate. The rate was increased to between 150 to 200 beat/min in one step while the paper was running at 100 div/min.

This was repeated twice under each of the two conditions of contraction.

Under isovolumic contraction the rate change was done at the maximum developed ventricular pressure which increased gradually when putting the clamp on. The APD's of the first beat recorded from base and apex (figs. 6.17) was measured at 70% repolarization and expressed as a % of the steady state duration which is taken as the average duration of the three or four AP's immediately prior to the change in rate.

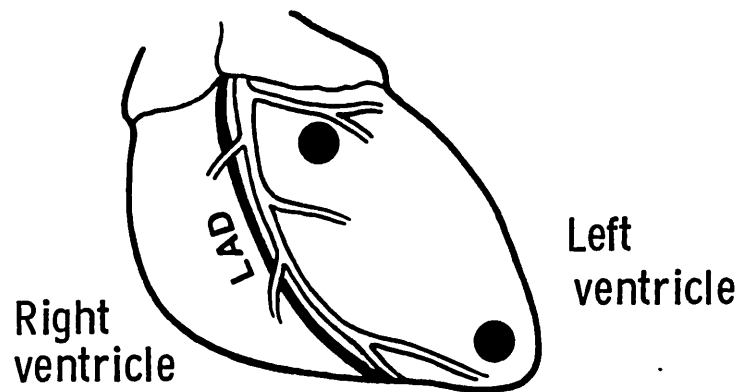


Fig. 6.17 The areas investigated on the left ventricle of the pig heart.

After the surgery the animal was left for 30 mins before starting the recording. For isovolumic contraction the clamp was put on by applying pressure from a 50 ml syringe for 10-15 beats. The clamp was released after the rate change and the steady state was restored before trying a second attempt.

To study the relation between the regional APD and regional ejection shortening, the tripod, with one leg adapted as a suction, was placed randomly on different areas.

Because of the nature of the data, it was not possible to apply a student T-test to test the significance of the results. In such a situation a Wilcoxon-matched signed ranks test was used to test the significance of the differences.



6.3.3. The Electrical Restitution Curve

Electrical restitution curves (Boyett & Jewell 1978) were obtained under both conditions of contraction for areas 1 & 6 (fig. 6.16) in the isolated rabbit and from the base and apex in the intact pig heart. At a pre-determined time (Test interval, TI, in fig. 6.18) a test pulse was interposed to produce an extrasystole after a regular beat. The AP's from area 1 & 6 in the isolated rabbit and from the base and apex in the intact pig heart and the ventricular pressure in both preparations were simultaneously recorded at fast paper speed. The transient changes in APD and ventricular pressure were allowed to return to the control steady state before the next stimulus was interpolated. The time interval between the last controlled response and the first test response was varied in each experiment between 250-600ms in the isolated rabbit heart and between 320-750ms in the insitu pig heart. The test APD duration was plotted against the variable time interval to construct a restitution curve.

For each test interval at least two attempts were made and the average durations of the test action potentials were used to plot the curve of any experiment. The same procedure was followed for the intact pig experiments and the action potentials from base and apex were recorded simultaneously. For restitution curves under isovolumic contraction in the pig, the aorta was clamped and an attempt was carried out at the maximum ventricular pressure and the clamp was released immediately after the change in rate. The transient changes were allowed to subside before the next attempt.

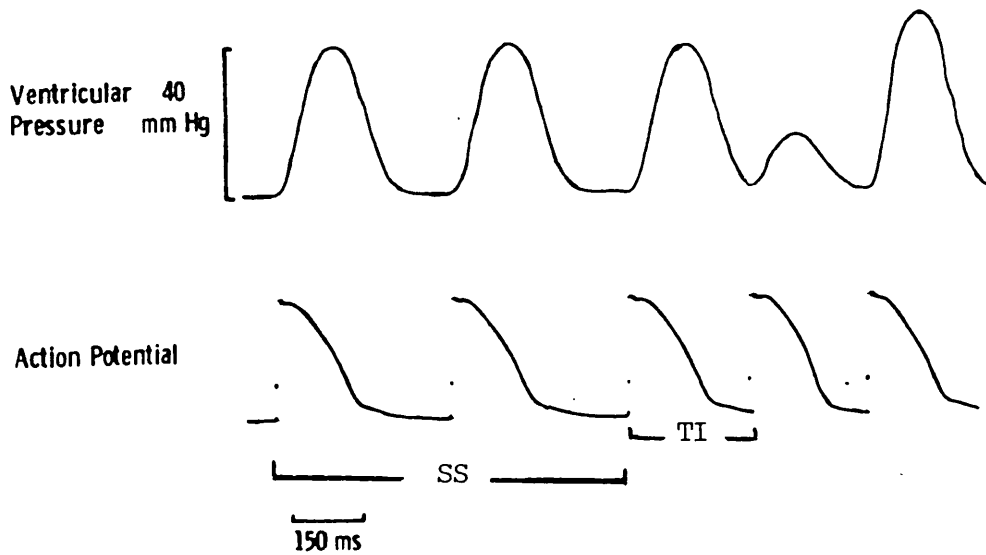


Fig. 6.18 The protocol for the electrical restitution curve construction. SS = Steady state. TI = Test Interval.

6.3.4. Calculation of Recovery Index ' τ '.

The time constant of the restitution curve has been used by Boyett & Jewell (1978) to indicate the rate of membrane recovery. In the present study it was not possible to calculate a single time constant and it seems that the curves have more than one time constant. Since the aim was to compare the steepness of the restitution curves, free loaded contraction vs isovolumic contraction, the curves were considered to be monoexponentials as assumed by Boyett and Jewell (1978) and the following equation was used to calculate a recovery index,

$$T^* = \tau_k / e^{-(x/\tau)}$$

$$\text{where } T^* = \frac{T_2 - T_1}{T_3 - T_2}$$

$T_1, 2, \text{ \& } 3$ can be read direct from the restitution curve and the values were chosen so that K is 2,3 or 4 (see below).

for $K = 2$

$$\tau = x / \log_e T^*$$

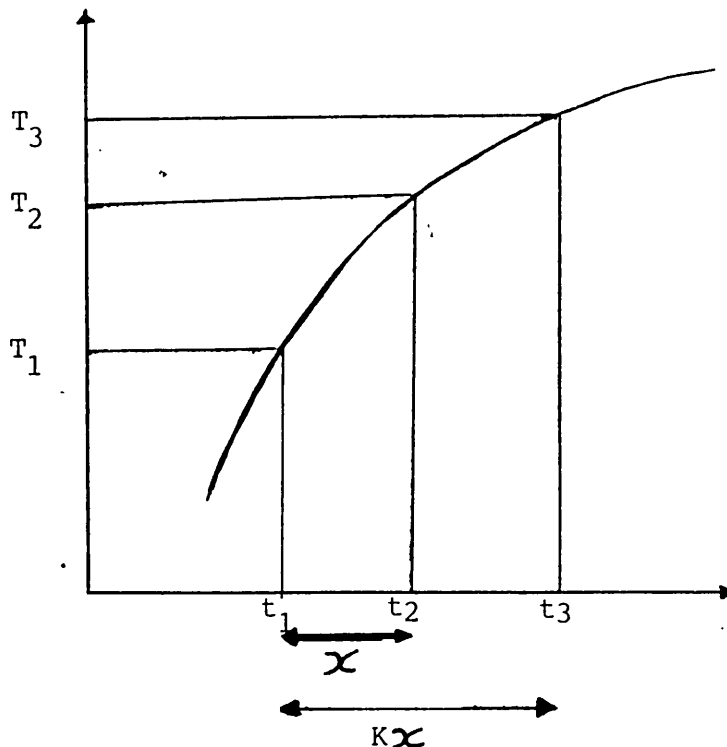
x and T^* can be obtained directly from the restitution curve.

For $K = 3$ and 4

For T^* , which can be calculated from the restitution curves, x/τ can be read off from graph 1.

For checking, x/τ was read off from graph 2 as a function of $\log_e T^*$.

For valid comparison of free loaded contraction vs isovolumic contraction the same value of K and $T_1, 2 \text{ \& } 3$ were chosen, for the free loaded contraction curve and for isovolumic contraction curve for any particular area under any perfusion conditions.



CHAPTER 7

REGIONAL DIFFERENCES IN ACTION POTENTIAL BEHAVIOUR UNDER DIFFERENT MECHANICS OF CONTRACTION

- 7.1 Changes in action potential and resting potential produced by changing the mechanics of contraction in the isolated rabbit heart.
- 7.2 Changes in action potential duration and resting potential produced by increasing the rate isolated rabbit heart.
- 7.3 Differences in action potential shortening under different mechanical conditions of contraction in intact insitu pig heart.
- 7.4 The relation between regional action potential duration and epicardial segment shortening.

Chapter 7

Regional Differences in Action Potential Behaviour under Different Mechanics of Contraction

7.1 Changes in action potential and resting potential produced by changing the mechanics of contraction in the isolated rabbit heart

When changing the condition of contraction from free loaded to isovolumic contraction, a small but consistent depolarization was observed. An example of such a depolarization is shown in fig. 7.1. This depolarization was observed in 12 preparations out of the first group of 22 experiments.

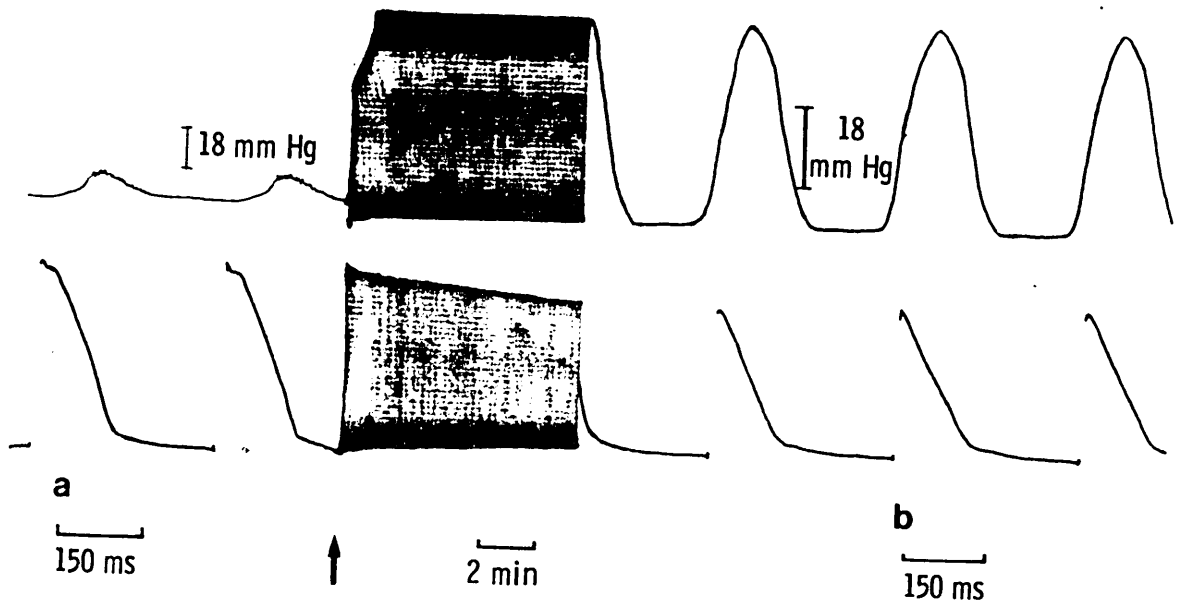
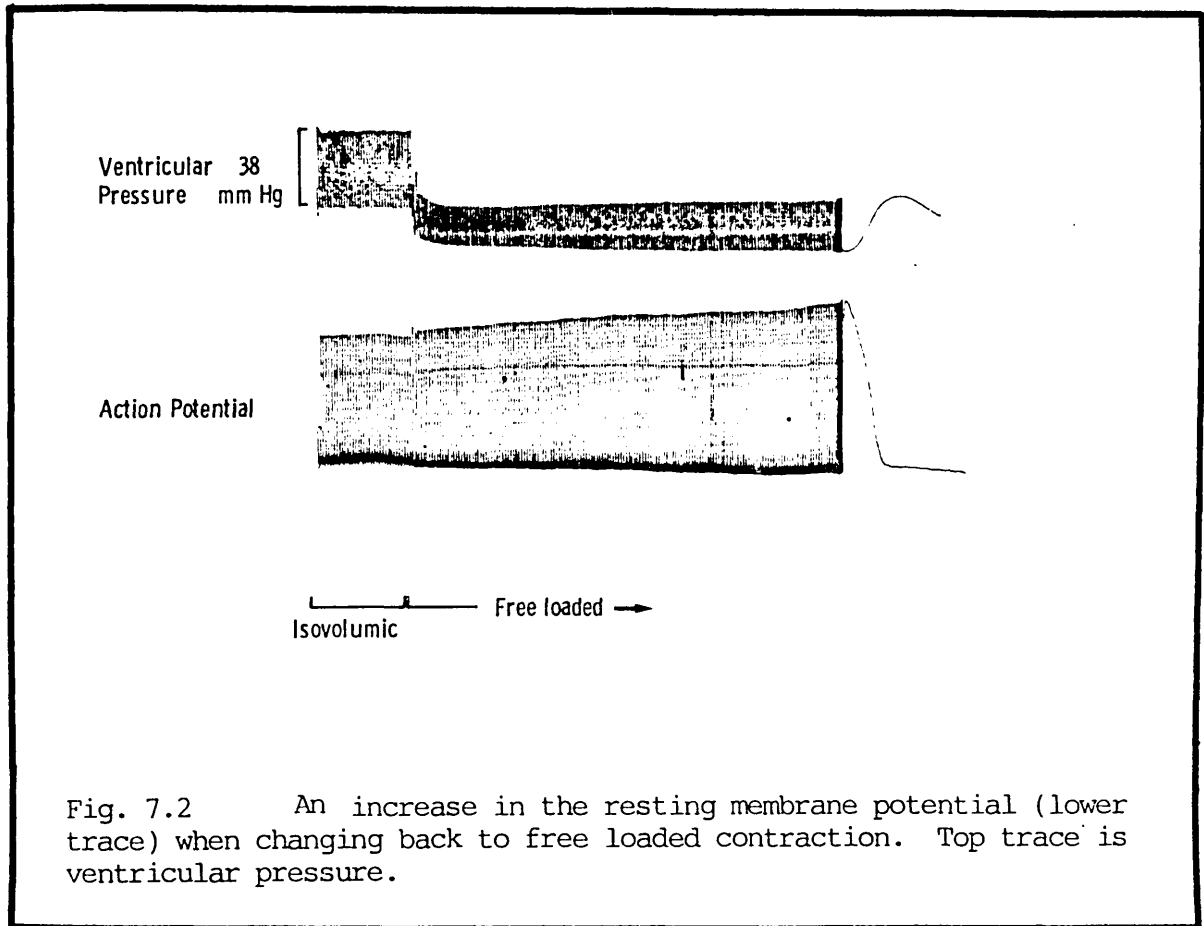


Fig. 7.1 The depolarization of the resting membrane potential as indicated in the AP recording (bottom trace) when changing from free loaded to isovolumic contraction at the arrow. Upper trace is the ventricle pressure.

The depolarization, seemed to be reversible and fig. 7.2 shows a small increase in membrane potential when the mechanics of contraction was changed back to free loading contraction. The two records (i.e. figs. 7.1 & 7.2) were obtained from different preparations.



In fig. 7.3A the action potential duration was measured continuously, with the AP meter, at 70% repolarization (Lab and Child 1979). The three parameters shown in the figure were recorded simultaneously. The figure shows a depolarization in the baseline potential (bottom trace) and an APD shortening on changing the mechanical condition of contraction from free loaded to isovolumic.

The shortening in APD was recorded continuously by the meter (top trace) and is also shown by superimposing an action potential just before the change and after about 2 mins of isovolumic contraction, (fig. 7.3B).

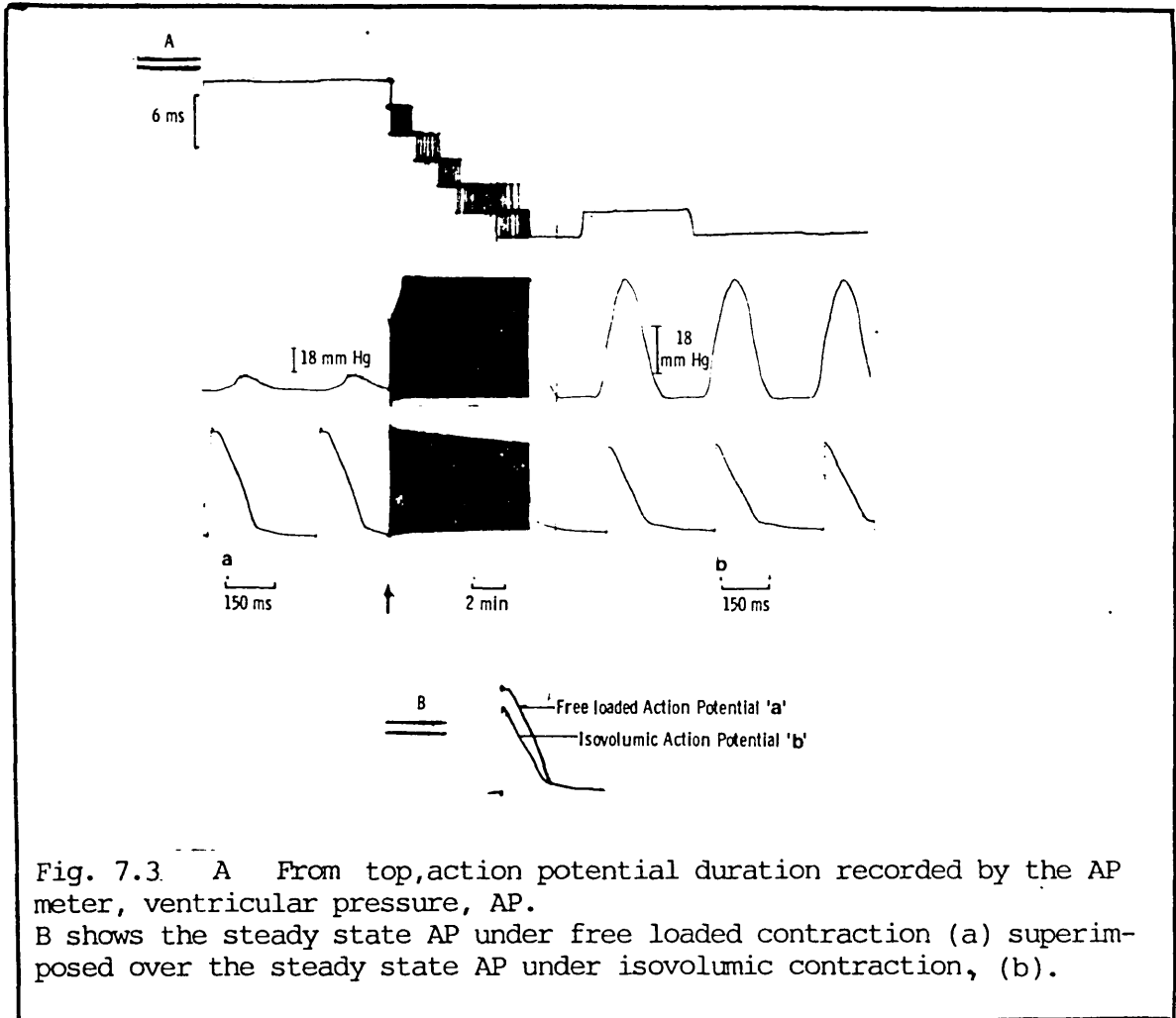


Fig. 7.3 A From top, action potential duration recorded by the AP meter, ventricular pressure, AP. B shows the steady state AP under free loaded contraction (a) superimposed over the steady state AP under isovolumic contraction, (b).

Fig. 7.4A shows that the changes in APD shown in fig. 7.2 are reversible on changing from isovolumic to free loaded contraction. A fast play out of the steady state action potential under isovolumic and free loaded contraction are shown in fig. 7.4B and superimposition of the two is shown in fig. 7.4C.

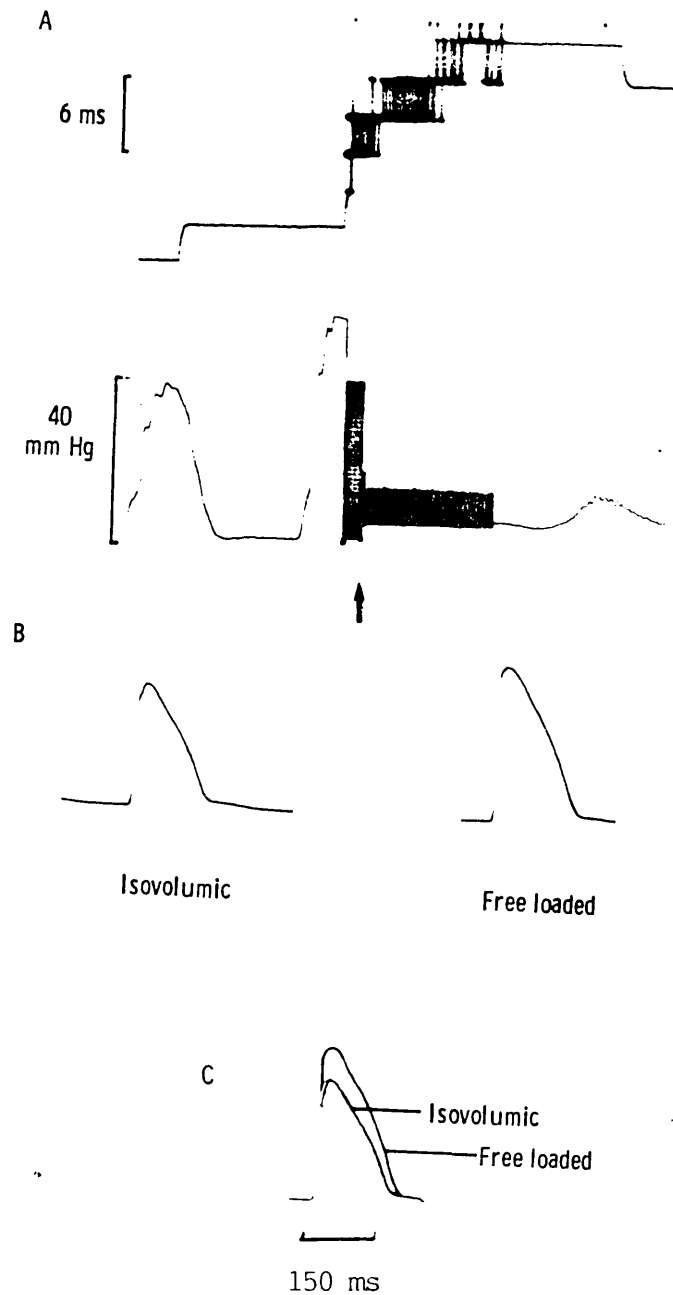


Fig. 7.4 A prolongation of APD recorded with AP meter (top trace) and ventricular pressure (bottom trace) when changing from isovolumic to free loaded contraction at the arrow.

B The steady states AP under the two mechanical conditions.

C The two steady state AP superimposed.

7.2 Changes in action potential duration and resting potential produced by increasing the rate of isolated rabbit heart: Regional differences in Action Potential behaviour under different mechanical conditions

It is well known that increasing the heart rate shortens the APD, decreases the resting potential i.e. depolarization of the resting membrane potential and increases force development. Such (or similar) changes are shown in fig. 7.5A. The top trace is APD recorded by the AP meter, the centre is the ventricular pressure under isovolumic contraction and the bottom trace is the AP. The depolarization in the action potential produced by increasing the rate from 150/min to 240/min in one step is clear and can be reversed by changing the rate back to 150/min. Figure 7.5B shows a faster play out of the AP of another attempt of increasing the rate, in the same preparation, showing clearly the depolarization and the appearance of alternans (alternating amplitude and duration of AP and ventricular pressure). This has been observed in isolated ventricular muscle (Spear and Moore 1971, Boyett and Jewell 1978).

To study the behaviour of the AP under both conditions of contraction when increasing the rate, it was recorded at fast paper speed and the rate was increased in one step from 150 to 240/min. An example of such change under isovolumic contraction is in fig. 7.6. The rate was increased for 10-15 beats and the action potential duration of the first 10 beats were measured.

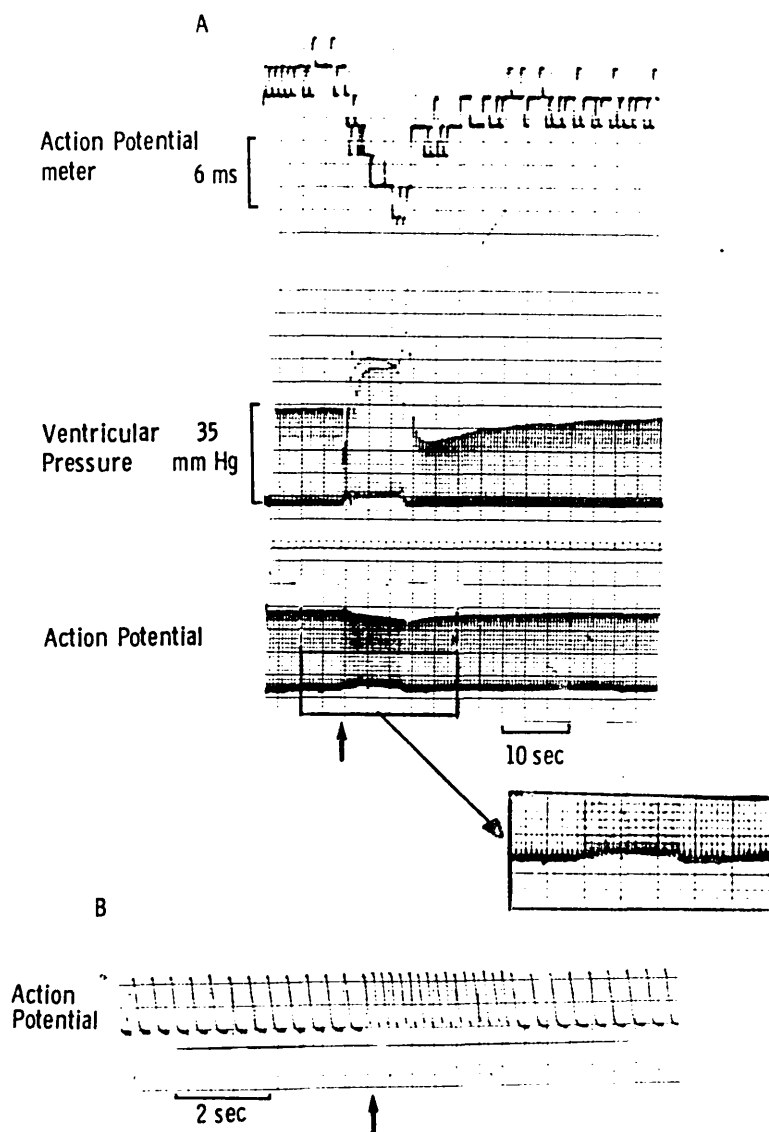


Fig. 7.5 A APD (top), ventricular pressure (middle) and AP (bottom trace), before, during and after changing the rate from 150 to 240/min.

B shows the AP at faster paper speed in response to a similar rate change as that in A. Note the alternans.

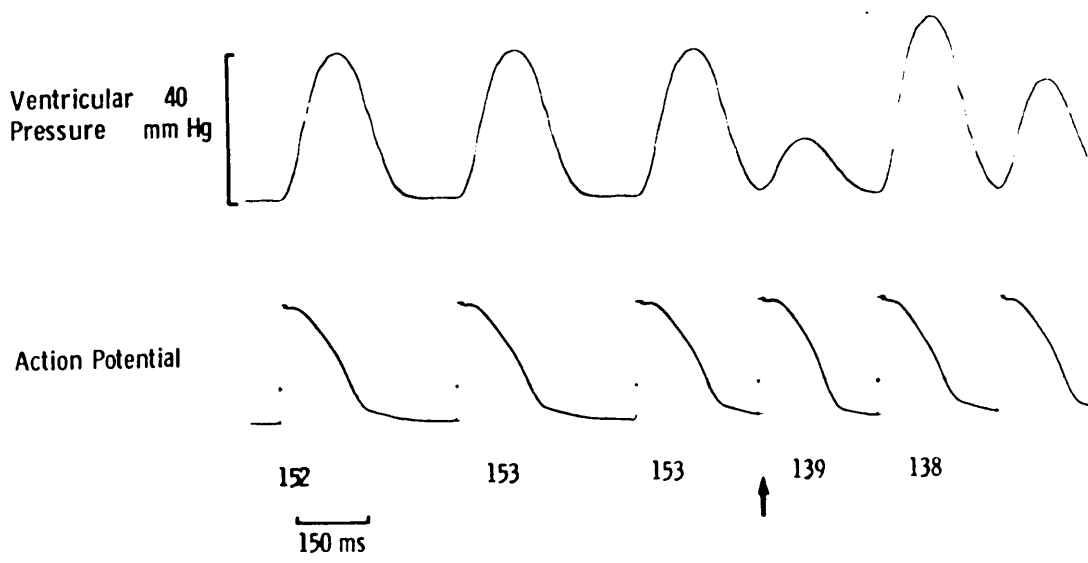


Fig 7.6 Ventricular pressure (top trace) and AP (bottom trace).
 AT the arrow the rate was changed to 240/min. The figures at
 the bottom are the durations of the corresponding AP'S in ms.

The absolute values (mean $\pm$ SE) from 5 preparations totalling 14 attempts under each mechanical condition are shown in fig 7.7. To present the results as % of the steady state, as in fig 7.8, the durations of the 10 beats were expressed as a % of the preceding steady state i.e just prior to the rate change.

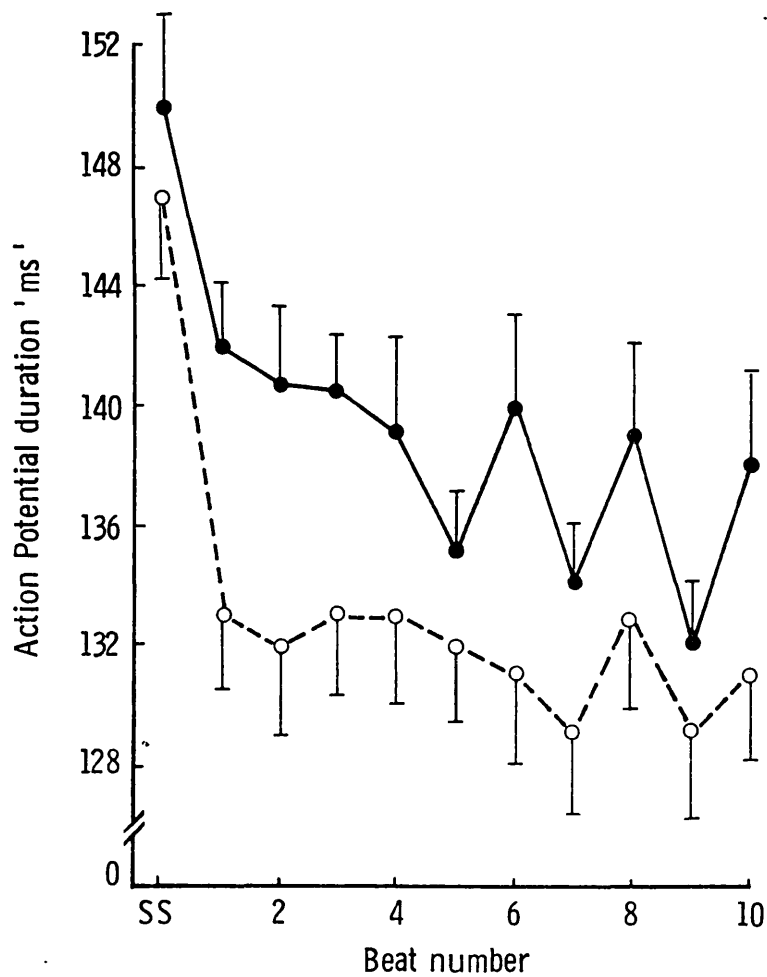
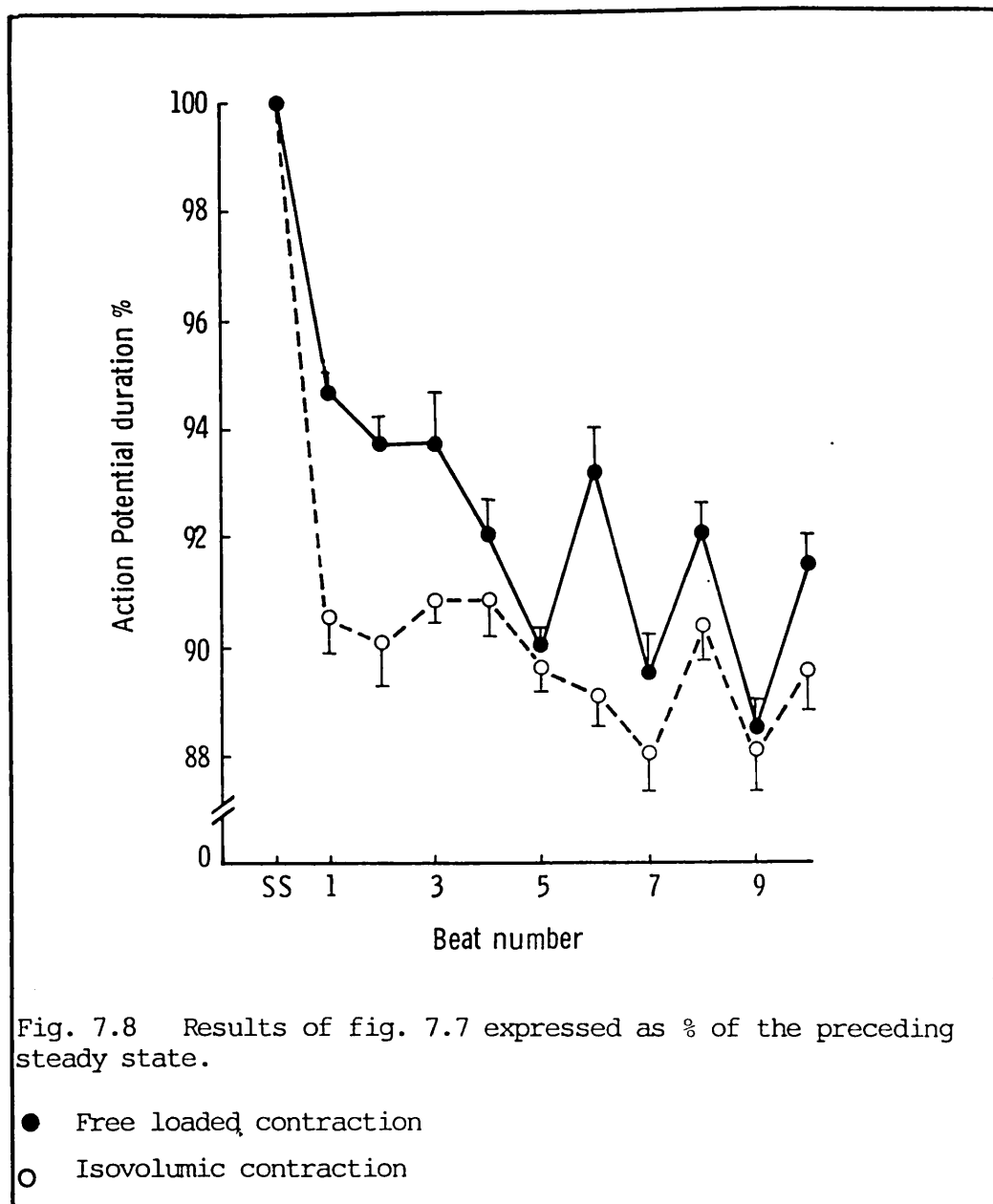


Fig 7.7 APD (mean $\pm$ SE) of the first 10 beats after changing the rate from 150 to 240/min. SS = steady state.

- Free loaded contraction
- Isovolumic contraction



The above results showed that the A_p shortened more under isovolumic than under free loaded contraction, and because most of the change took place in the first two beats, only the first beat was measured in a similar study of six more areas (fig. 7.9) mapping the left ventricle downward from base to apex and across from left to right.

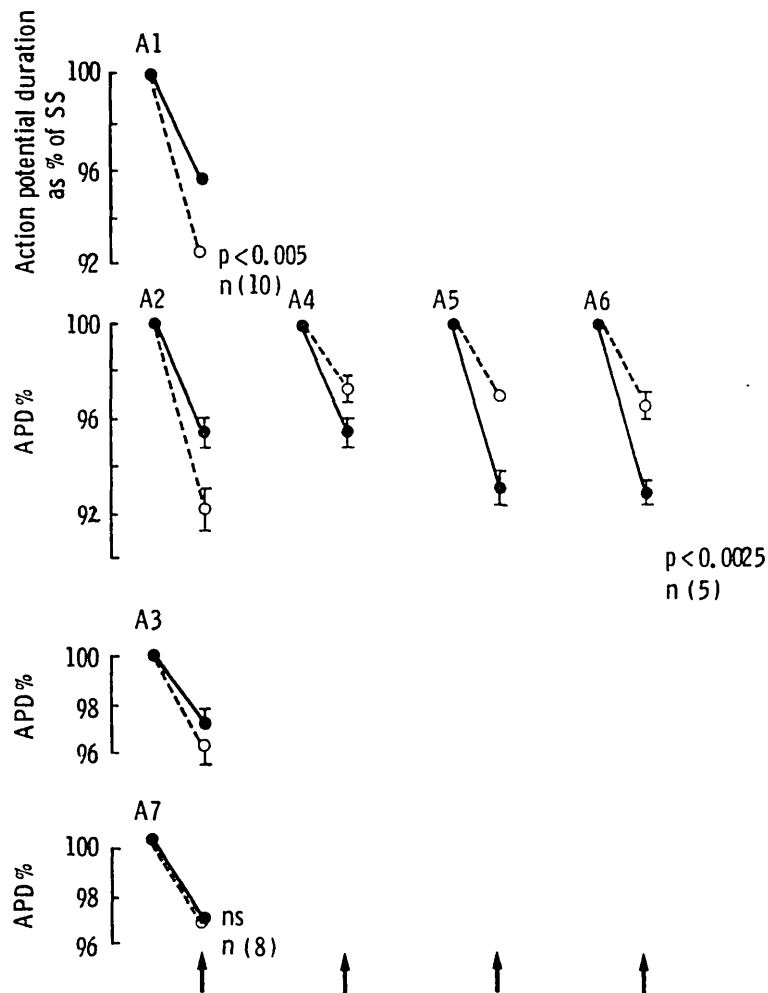


Fig. 7.9 APD of the first beat expressed as % of the preceding steady state (mean $\pm$ SE) of all areas. The points with no range indicate that the SE was within the circle.

Close circle is free loaded contraction and open circle is isovolumic.

(n) is the number of preparations

ns is not significant

A1-A7 are Areas 1- Areas 7

Area Number	<u>Free LOADED</u>			<u>ISOVOLUMIC</u>		
	Steady State APD 'ms'	1st beat APD 'ms'	1st beat APD as % of steady state	Steady state APD 'ms'	1st beat APD 'ms'	1st beat APD as % of steady state
A1	142 $\pm$ 15 (10)	136 $\pm$ 12 (10)	95 $\pm$ 2 (10)	137 $\pm$ 17 (10)	127.2 $\pm$ 14 (10)	92 $\pm$ 2 (10)
A2	143 $\pm$ 9 (3)	136 $\pm$ 2 (3)	95 $\pm$ 1 (3)	145 $\pm$ 11 (3)	134 $\pm$ 12 (3)	92 $\pm$ 2 (3)
A3	138 $\pm$ 3 (4)	134 $\pm$ 6 (4)	97 $\pm$ 3 (4)	130 $\pm$ 6 (5)	176 $\pm$ 8 (5)	96 $\pm$ 3 (5)
A4	131 $\pm$ 6 (4)	125 $\pm$ 2 (4)	95 $\pm$ 2 (4)	124 $\pm$ 7 (4)	121 $\pm$ 8 (4)	97 $\pm$ 2 (4)
A5	129 $\pm$ 5 (3)	122 $\pm$ 5 (3)	94 $\pm$ 1 (3)	121 $\pm$ 6 (3)	117 $\pm$ 5 (3)	96 $\pm$ 1 (3)
A6	144 $\pm$ 5 (5)	134 $\pm$ 7 (5)	92 $\pm$ 1 (5)	128 $\pm$ 7 (5)	123 $\pm$ 9 (5)	96 $\pm$ 2 (5)
A7	134 $\pm$ 12 (8)	130 $\pm$ 12 (8)	97 $\pm$ 2 (8)	129 $\pm$ 19 (8)	125 $\pm$ 19 (8)	97 $\pm$ 1 (8)

TABLE 7.1 Action potential durations (ms) of all areas (Mean $\pm$ SD) under free loaded and isovolumic contraction.

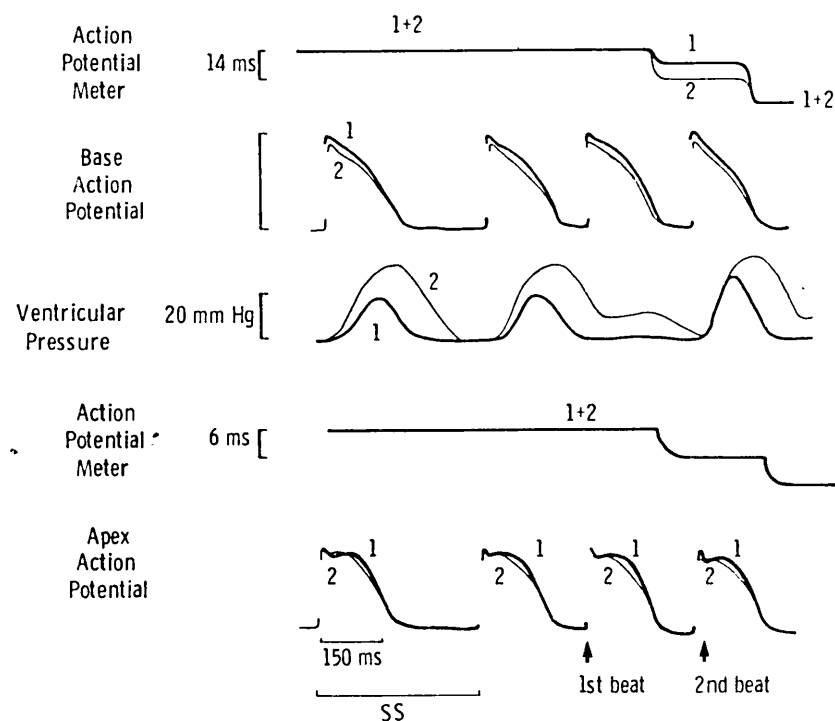
() number of experiments comprising 9-26 attempts.

Fig. 7.9 summarises the results of all areas where the APD of the first beat was expressed as % of the steady state duration before the rate change.

Table 7.1 contains the results of all areas in absolute values and the first beat as % of the steady state.

Area 1 (Base):-

The action potential of this area seemed to shorten more under isovolumic than free loaded contraction. Superimposed steady state, first and second beat action potentials under free loaded and isovolumic contraction are shown in fig 7.10 (second trace down), in which the rate was changed from 150 to 240/min. In six out of the ten experiments the steady state AP was significantly shorter under isovolumic than free loaded contraction. In two experiments there was no difference and in the other two the steady state AP was slightly but insignificantly longer under isovolumic contraction.



1-Free Loaded contraction, 2-Isovolumic, SS-Steady State

Fig. 7.10 Superimposed; Action potential meter recordings (top trace) which were recorded simultaneously with the action potentials from the base (second trace). Ventricular pressure (third trace) and action potential meter recordings for the apex action potentials which are shown in the last trace. The thick lines are the recordings under free loaded contraction and the thin lines are isovolumic contraction. The AP duration meter shows the recording of immediately preceding APD.

Three further experiments showed no difference in the amount of shortening between the two mechanical conditions on increasing the rate, but in two of the three, the steady state under isovolumic contraction was shorter than under free loaded contraction. One experiment only showed less shortening under isovolumic contraction i.e. opposite to the other experiments. This could have been due to the fact that the steady state AP under isovolumic contraction was very much shorter than free loaded steady state in this experiment. Paired student T-test applied to the % results showed the difference in AP shortening between free loaded and isovolumic contraction to be significant ($p < 0.005$).

Area 2

This area seemed to behave like area 1. Three experiments showed more shortening under isovolumic than under free loaded contraction on increasing the rate, in a fourth experiment there was no difference. The steady state duration was greatly shorter under isovolumic contraction in two experiments and longer in the other two. Paired student T-test applied to the % results showed the difference in AP shortening between free loaded and isovolumic contraction to be not significant, probably because of the large SE and the small number of experiments.

Area 3

There was no significant difference in action potential shortening between free and isovolumic contraction but in three out of the four experiments, the steady state under isovolumic contraction was shorter than in free loaded contraction and no difference in the fourth.

Area 4

The free loaded action potential seemed to shorten more in this area but a T-test showed this was to be insignificant. This behaviour is the opposite of area 1 and 2. The steady state duration was shorter under isovolumic contraction in three experiments but no difference in a fourth.

Area 5

The action potential duration in three experiments shortened significantly more under free loaded contraction when the rate was increased from 150 to 240/min. This behaviour was curiously opposite to that found in area 1, but the steady state AP was significantly shorter under isovolumic contraction. The action potential behaviour was opposite in a fourth experiment which showed no difference in the steady states.

Area 6

The behaviour of the action potential in response to increase in heart rate was also curiously opposite to that of 1 and 2 in that it shortened more under free loaded than it did under isovolumic contraction. The steady state AP was shorter under isovolumic contraction, but later experiments showed that this was not consistent. Paired T-test showed the difference in AP shortening between free loaded and isovolumic contraction to be significant ($P < 0.005$).

Area 7 (The Apex)

There was no significant difference in the behaviour of the apical action potential on increasing the rate fig. 7.10, and only in three out of the eight experiments, the steady state duration was shorter under isovolumic contraction than under free loaded contraction.

Fig. 7.10 (bottom trace) shows superimposed steady state, first beat and second beat action potentials recorded from the apex when the rate was increased from 150 to 240/min. The second trace from the bottom shows superimposed action potential meter recordings in the same preparation recorded simultaneously with the AP. There was no difference in the meter recordings or the APD at 70% repolarization between free loaded and isovolumic contraction. The results from all areas (fig. 7.9) expressed as % of the preceding steady state seem to suggest a gradual change from base to apex and from left to right. In four more experiments it was not possible to pace at 150/min because the intrinsic rate was high. These preparations were paced at between 200-170/min. Similar changes were observed in all areas at such rates and the differences between free and isovolumic contraction were as those obtained at steady state of 150/min.

Summary

Isovolumic contraction shortens the duration of AP and the resting membrane potential seems to partially depolarize. When the rate of beating is suddenly increased, the AP shortens more under isovolumic than under free loaded contraction in some areas, with no differences seen at the apex: but with greater shortening under free loaded contraction in some other areas. The results suggest a possible gradual change across the mid-ventricle from left to right and from base to apex.

7.3 Differences in Action Potential Shortening under different Mechanical Conditions of Contraction in intact insitu pig heart

The above difference in action potential shortening between free loaded and isovolumic contraction in the left ventricle of the isolated rabbit heart could be due to hypoxia developing under isovolumic condition. The preparation was isolated and the amount of O_2 in the perfusing medium was limited. Hypoxia or ischaemia is known to produce shortening of the action potential duration (Fozzard 1980). Although in my experiments there was no change in coronary flow on changing to isovolumic contraction, the ventricular pressure was higher. So it was possible that slow hypoxia developed under isovolumic contraction because of increased O_2 demand in the face of increased wall tension (Laplace and compression of capillaries). Therefore it was decided to carry out a similar study on the intact insitu heart of the open chested pig. The isovolumic condition was produced by clamping the aorta with a special clamp (Jewell 1969). The rate was increased for 2-3 beats at maximum developing ventricular pressure and AP's were recorded from base to apex. Figures 7.11 and 7.12 show the kind of records obtained when changing the rate from 100 to 140/min under normal and isovolumic contraction respectively.

Fig. 7.13 shows the action potential and the aortic pressure when changing from normal to isovolumic contraction. The aortic pressure dropped significantly as expected.

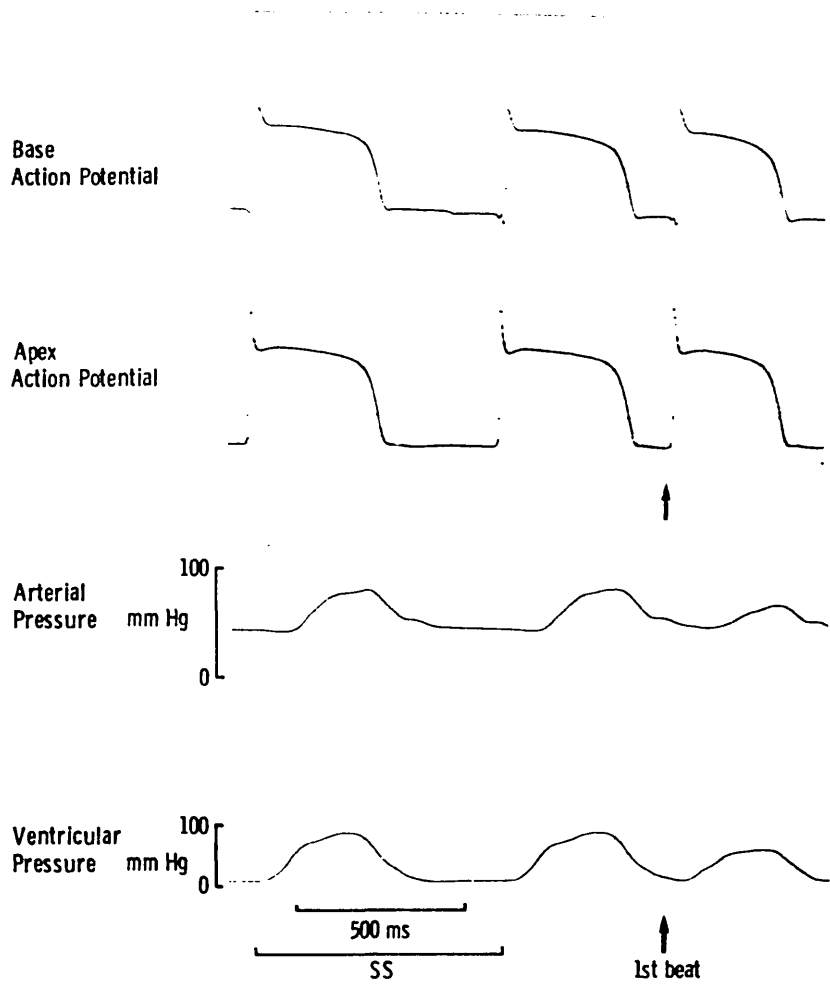


Fig. 7.11 Base (top) and apex (second trace) action potentials under normal contraction. The rate was changed from 100 to 140/min at the arrow.

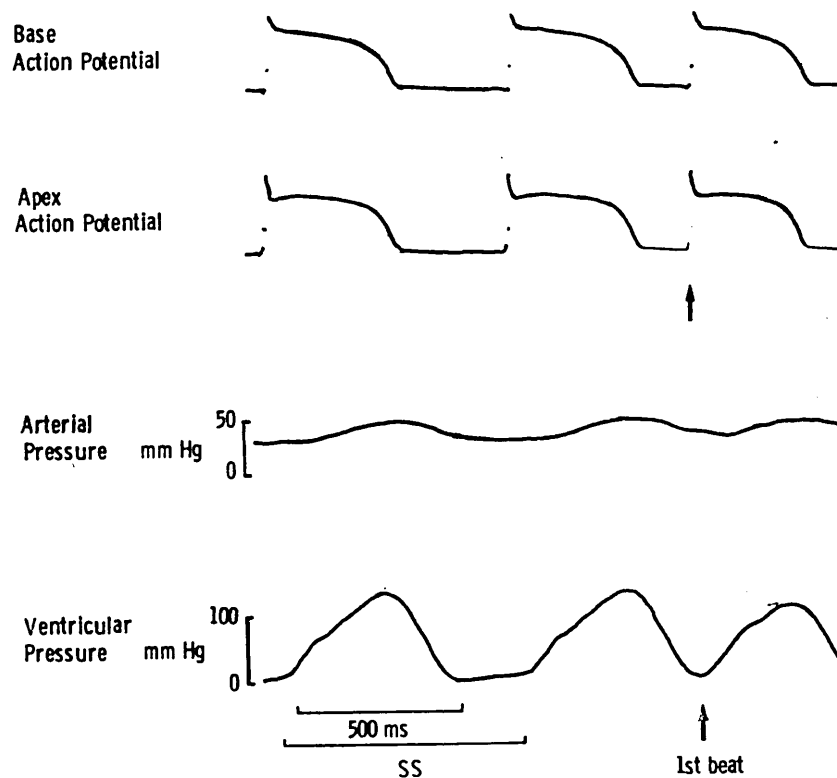


Fig. 7.12 Base and apex action potentials (top two traces respectively) under isovolumic contraction. The rate was changed from 100 to 140/min at the arrow.

Figure 7.13 shows a slow play out of action potential recorded from the base and the aortic pressure. It shows a depolarization which was reversible on releasing the clamp. Similar depolarization was observed in the apex. In some preparations large enough depolarization triggered an extrasystole (Lab 1982).

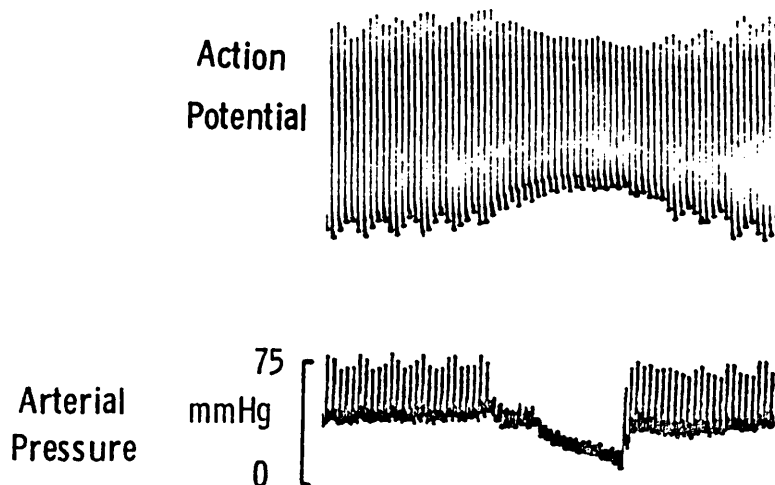


Fig.7.13 The resting membrane depolarization as indicated by the upward shift of the base line of the action potential (top trace) when changing to isovolumic contraction in the insitu pig heart and its repolarization on releasing the aortic clamp, thus returning to normal contraction.

The changes in APD on increasing the rate from between 100-150 to 140-240/min obtained from 8 animals are presented in table 7.2 for the base and in table 7.3 for the Apex.

Base

In 7 experiments out of 8 the AP was shortened more under normal than under isovolumic contraction, the exception being one experiment (number 3 in table 7.3) in which there was no significant difference. This behaviour of base in the pig is the opposite of that of the left ventricle in the isolated rabbit heart.

Figure 7.14A shows an example from the base when the rate was increased in one step from 110 to 145/min under normal (left hand panel) and under isovolumic (right hand panel) contraction. The AP of the first beat after the rate change is superimposed over the steady state AP (SS) at 70% repolarization. It also shows greater difference between the steady state and the first beat AP under normal compared to isovolumic contraction. It was not possible to apply simple paired T-test on the results, even when expressed as % of the steady state because, first, the rate changes were not uniform in all experiments and second, the steady state pulse intervals varied from one experiment to another depending on the intrinsic rate. Therefore, I applied Wilcoxon-matched pairs signed ranks test which showed a significant difference in action potential shortening between normal contraction and when the clamp was on ($p < .02$).

BASE	NORMAL CONTRACTION			ISOVOLUMIC CONTRACTION		
	Animal Number	Steady State APD 'ms'	1st beat APD 'ms'	1st beat APD as % of steady state	Steady state APD 'ms'	1st beat APD 'ms'
1	338 <u>+5</u> (4)	272 <u>+4</u> (4)	80 <u>+ 1</u> (4)	338 <u>+7</u> (3)	277 <u>+7</u> (3)	82 <u>+ 1</u> (3)
2	240	199	83	230	197	85.
3	165 <u>+11</u> (2)	159 <u>+ 1</u> (2)	96. (2)	165 <u>+ 1</u> (2)	157 <u>+ 1</u> (2)	95 (2)
4	296	248	83	290 <u>+56</u> (2)	250 <u>+84</u> (2)	86 <u>+1.2</u> (2)
5	272 <u>+2</u> (2)	246 <u>+8</u> (2)	90 (2)	233 <u>+14</u> (2)	220 <u>+5</u> (2)	94 <u>+1</u> (2)
6	283 <u>+1</u> (2)	229 <u>+3</u> (2)	81 <u>+1</u> (2)	281 <u>+4</u> (2)	246. <u>+14</u> (2)	87 <u>+3</u> (2)
7	316	298	94	317	307	96
8	245 <u>+4</u> (3)	221 <u>+13</u> (3)	90 <u>+3</u> (3)	251 <u>+1</u> (3)	241 <u>+2</u> (3)	96 <u>+ 1</u> (3)

TABLE 7.2 The steady state and the first beat APD from the base of the left ventricle of insitu pig heart,
under normal and isovolumic contraction. The first beat is also expressed as % of steady state APD.

MEAN $\pm$ SD

() number of attempts of rate changes.

APEX		<u>NORMAL CONTRACTION</u>			<u>ISOVOLUMIC CONTRACTION</u>		
		Steady State APD 'ms'	1st beat APD 'ms'	1st beat APD as % of steady state	Steady state APD 'ms'	1st beat APD 'ms'	1st beat APD as % of steady state
<u>Group A</u>							
1		372 $\pm$ 2 (4)	285 $\pm$ 3 (4)	764 $\pm$ 1 (4)	380 $\pm$ 0 (2)	282 $\pm$ 0 (2)	74 $\pm$ 0 (2)
2		238	192	81	247	191	77
3		180 $\pm$ 0 (2)	168 (2)	93. $\pm$ 0 (2)	180	163	90.
<u>Group B</u>							
4		312	260	83	297 $\pm$ 1 (2)	256 $\pm$ 1 (2)	86. $\pm$ 1 (2)
5		276 $\pm$ 0 (2)	242 $\pm$ 8 (2)	87 $\pm$ 3 (2)	266 $\pm$ 0 (2)	241 $\pm$ 6 (2)	94 $\pm$ 1 (2)
6		261 $\pm$ 8 (2)	221 $\pm$ 3 (3)	84 $\pm$ 2 (3)	249 $\pm$ 16 (3)	222 $\pm$ 14 (3)	85 $\pm$ 3 (3)
7		307	294	95	264	259	98
8		248 $\pm$ 2 (4)	228 $\pm$ 10 (4)	92 $\pm$ 3 (4)	243 $\pm$ 1 (3)	225 $\pm$ 14 (3)	92. $\pm$ 5 (3)

TABLE 7.3 The steady state and the first beat APD from the apex of the left ventricle of insitu pig heart, under normal and isovolumic contraction. The first beat is also expressed as % of steady state APD.
 MEAN $\pm$ SD () number of attempts of rate changes.

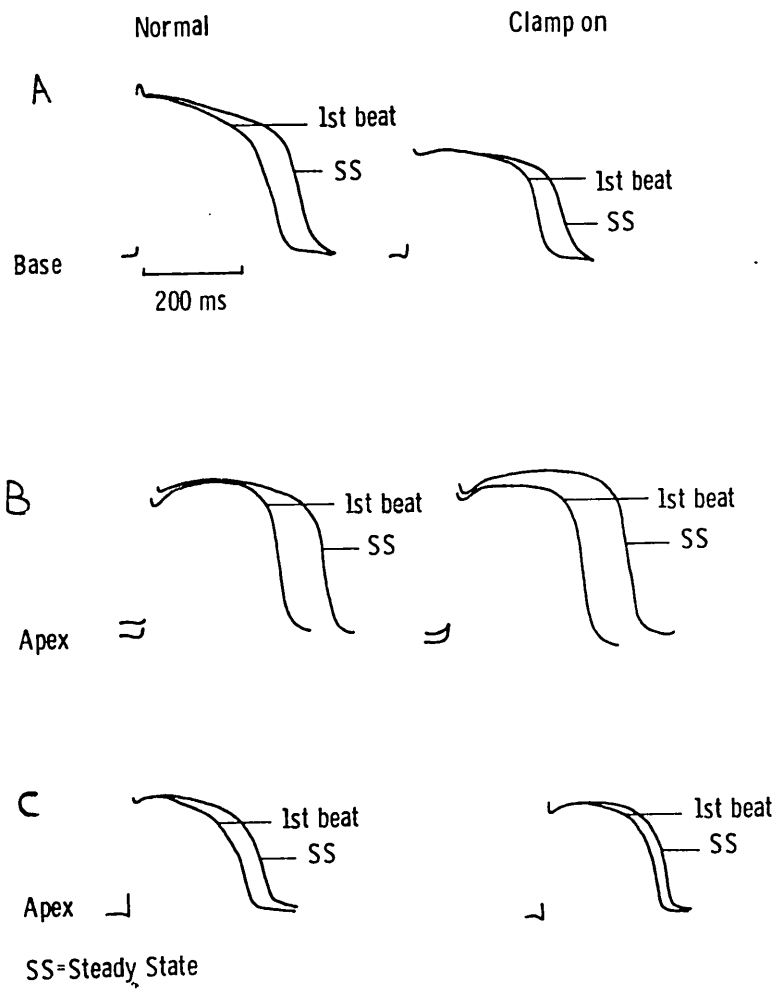
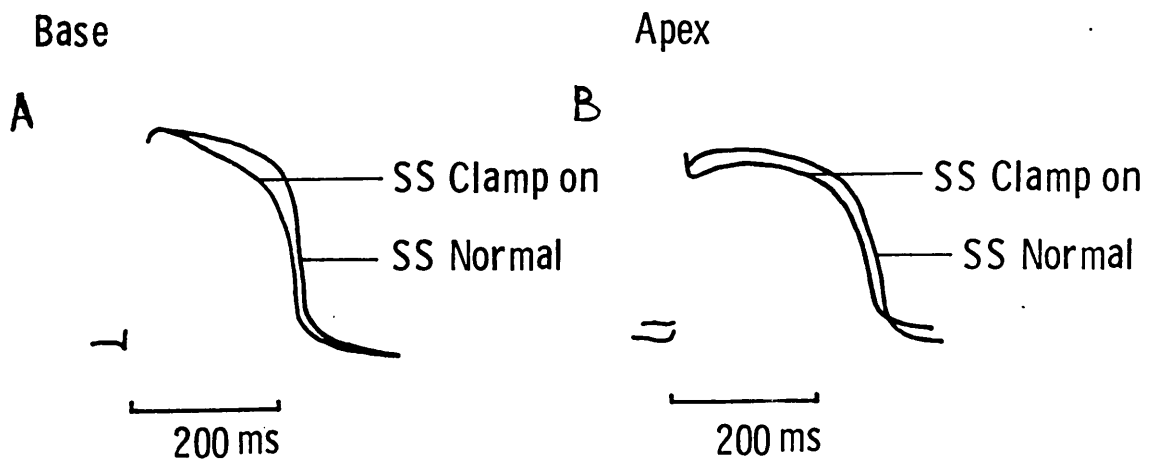


Fig. 7.14 See text for description

It was not possible to compare the steady state action potential under normal contraction with that under isovolumic contraction because in most of the experiments the suction electrode was often replaced. However, the action potential seemed to shorten under isovolumic contraction and an example of this is shown in fig. 7.15A. In this figure the action potentials under normal and just after changing to isovolumic contraction are superimposed at 70% repolarization level.



SS=Steady State

Fig. 7.15 Steady state AP under normal contraction and just after clamping the aorta from the base (A) and from the apex (B).

This shortening of the action potential on clamping the aorta has been shown by Lab (1982) and also in later experiments, when I was constructing restitution curves, (Chapter 8). The time course of AP shortening in the base is shown in fig. 7.16 (bottom graph).

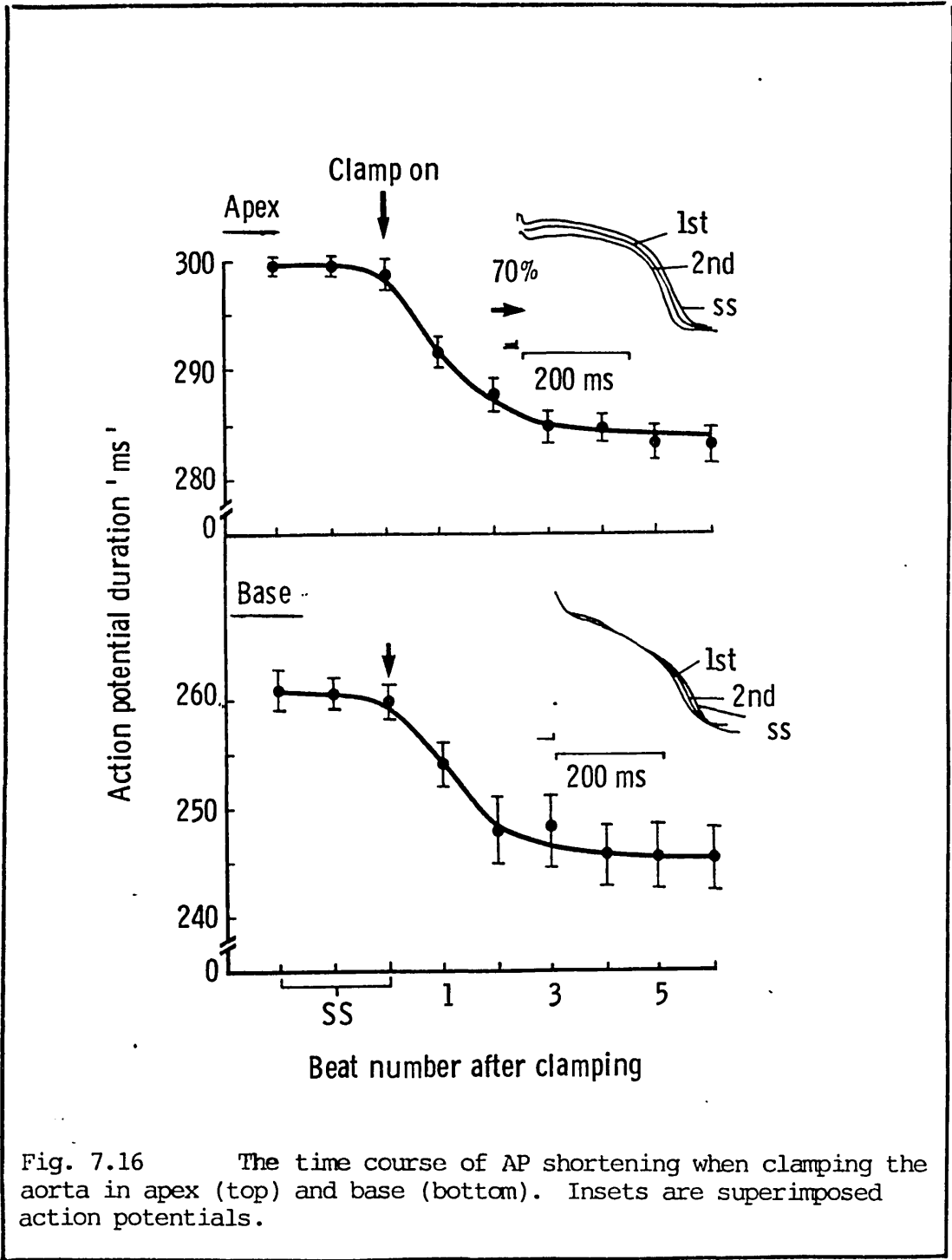


Fig. 7.16 The time course of AP shortening when clamping the aorta in apex (top) and base (bottom). Insets are superimposed action potentials.

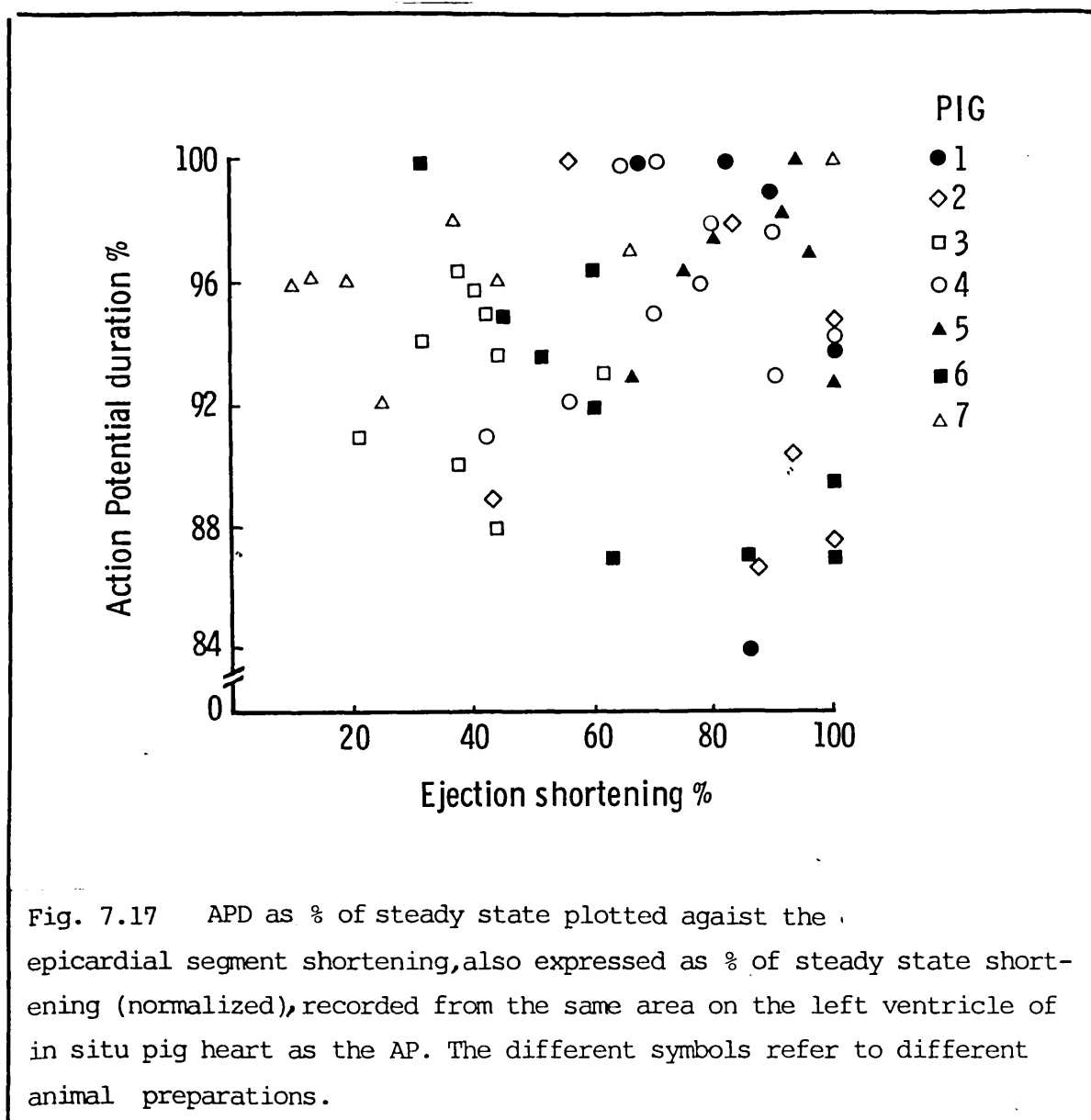
Apex

The results from the apex were not consistent. In three experiments (group A table 7.3) the AP shortened more under isovolumic contraction, than free loaded contraction when the rate was suddenly increased, an example was shown in fig. 7.14B. In another three experiments (group B table 7.3) the AP shortened under isovolumic contraction less than under normal contraction, an example was shown in fig. 14C. In two more experiments there was no difference between the two mechanical conditions. Thus there was a similarity between the base and group B experiments of the apex in that the AP shortened more under normal contraction, on increasing the rate of stimulation (pacing), than under isovolumic contraction. In the two experiments where the apex AP shortened more under isovolumic than under normal contraction, the steady state APD was longer under isovolumic than under normal contraction. This difference might have been due to replacement rather than as a result of clamping the aorta. A possible explanation for these differences in behaviour of the apex is that we were recording from different areas at the apex, i.e. from areas just adjacent to the tip, and from the tip itself.

7.4 The relation between regional action potential duration and epicardial segment shortening

The question that can be asked is that if APD is shorter under isovolumic than free loaded contraction and there are regional differences in the extent of epicardial muscle shortening, could there be a correlation between APD and local epicardial shortening in the intact insitu heart?

To investigate this a tripod was adapted (see Methods chapter) to record the epicardial segment shortening and APD from the same place. The adapted tripod was placed on a number of areas between (5-11) areas in each animal. The areas were selected randomly in each animal, so that the areas investigated in one animal were not necessarily investigated in another, but a good number of areas in common were investigated in all animals. If the results are considered as a whole, a correlation co-efficient of 0.11 is obtained, suggesting no correlation between ejection shortening and APD.



Mechanically Induced Depolarization of the resting
membrane potential

Changing the mode of contraction from free loaded to isovolumic frequently produced an upward shift in the baseline of the AP which could reflect depolarization of the resting membrane potential in the isolated rabbit heart (fig. 7.1). A similar base-line shift was observed in the pig left ventricle when clamping the aorta (fig. 7.13). It can be argued that this shift in base-line could have been due to electrical artifacts produced by mechanical distortion or damage of the tissue under the tubing of the suction electrode as a result of epicardial stretch or by conduction abnormalities (Dominguez and Fozzard 1979, Markhasian and Shklyer 1982). But this explanation is not favoured for the following reasons:-

1. Similar depolarization has been observed in various isolated ventricular and atrial preparations when the muscle was stretched, using glass microelectrodes for recording. Penefesky and Hoffmann (1963) demonstrated a stretch induced depolarization in cat, squirrel, chicken, hamster and terrapin ventricular and atrial muscles. Also Lab (1969) found a base-line shift recorded with suction electrodes when clamping the aorta of intact frog ventricle and, Covell et al (1981) occluded the pulmonary artery in dog and observed a similar change in the resting potential in the right ventricle which was accompanied by some stretch of the epicardial segment as recorded with ultrasonic crystals.

Boland and Troquet (1980) found depolarization of the resting potential in both ventricles (in the right more than in the left) when clamping the pulmonary artery in an open chested rat preparation recorded with floating glass microelectrode. Finally Dudel and Trautwein have found depolarization with large stretches in cat papillary muscle and explained it as being due to muscle damage, (Cited in Lab 1982).

Gordon and Ridway (1976) observed membrane depolarization in a single barnacle muscle fibre at longer muscle lengths than at shorter lengths. They also observed an effect of muscle length on Ca^{++} release which they attributed to changes in membrane properties. The fact that the base-line shift was reversible in this study (figs. 7.2 and 7.13), and in Lab (1969), Covell et al (1981) and in Boland and Troquet (1980) studies discounts the possibility of muscle damage being responsible.

2. If the base-line shift was due to some mechanical distortion as a result of myocardial stretch, the shift would be expected to reach steady state simultaneously with the mechanical stretch. Increase in ventricular pressure and stretch reach the new steady state simultaneously. (Fig. 7.18). The depolarization of the resting potential would therefore be expected to reach steady state simultaneously with the ventricular pressure, but the results in fig. 7.1 show the base line shift continued to rise well after the ventricular pressure had reached steady state. Similar arguments apply to the base line shift in the pig heart.

3. Some observations, obtained during the course of the present study suggest that the suction electrode can qualitatively pick changes in

the resting membrane potential, as indicated by a shift in the base-line of the AP. Similar to those recorded with a glass microelectrode.

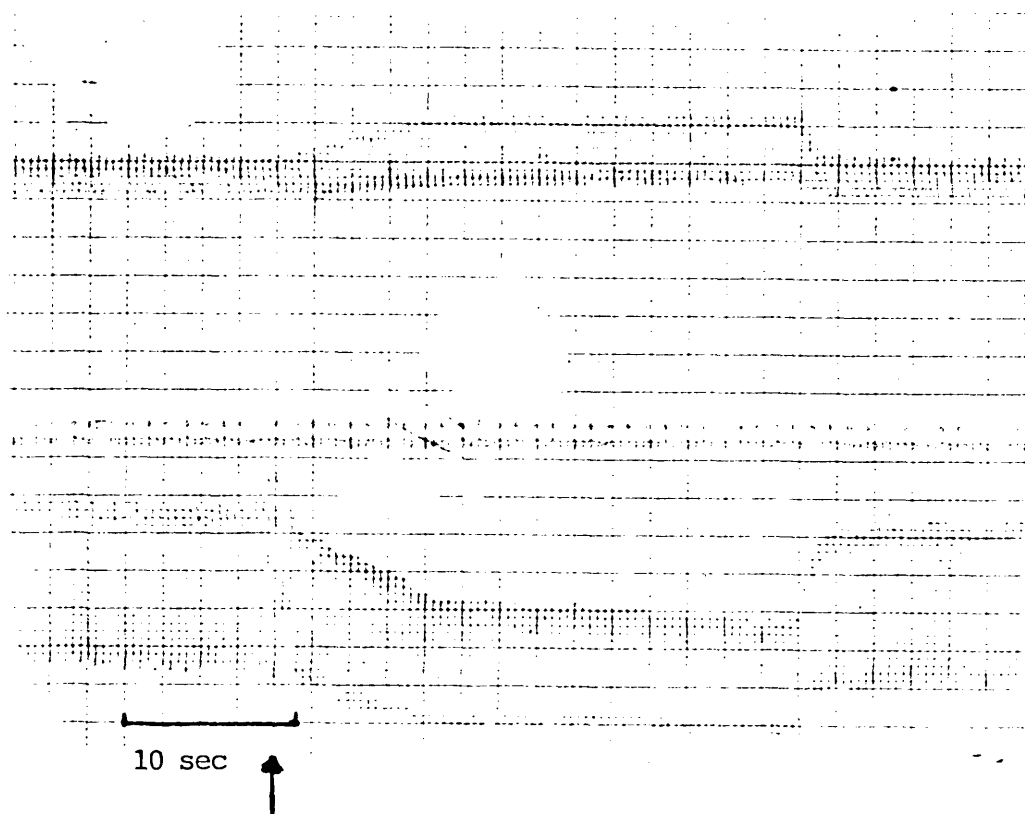


Fig.7.18 Ventricular pressure (top trace) and segment shortening (bottom) when changing from free loaded to isovolumic contraction at the arrow in the isolated rabbit heart.

For example, increasing the rate of stimulation is well known to produce initial depolarization of the resting potential in ventricular, atrial and purkinje fibres (Hoffmann and Suckling 1954, Edman and Johansson 1976, Kunze 1977, Kline and Morad 1978, Attwell et al 1981, Cohen et al 1982, Cohen and Kline 1982). In the present study increasing the rate of stimulation produced a clear base-line shift which was reversible as shown in fig. 7.5 A(bottom trace) and B.

Increasing and decreasing $[K^+]$ in the perfusing medium, $[K^+]_o$ have been shown by the means of glass microelectrode to produce depolarization and hyperpolarization respectively (Weidmann 1956, Lee and Fozzard 1975, Ellis 1977, Baumgarten et al 1981). Figure 9.1 (Chapter 9) and fig 7.19 show an upward shift and a downward shift of the AP base-line recorded with the suction electrode from both areas simultaneously in the isolated rabbit heart, when $[K^+]_o$ was changed from 4mM to 8mM and from 4mM to 0mM respectively.

Ouabain (0.1-10mM) has been shown to depolarize the membrane resting potential in many isolated cardiac preparations, as recorded with glass microelectrode (Ellis 1977, Gadsby and Cranefield 1979, Sheu and Fozzard 1982, Lee and Dagestino 1982, Bridge and Bassingthwaite 1983). Similar possible depolarization recorded with the suction electrode was seen when the isolated rabbit heart was perfused with Krebs containing 0.1mM Ouabain as shown in fig. 7.20. This possible depolarization was reversible when changing back to Ouabain free Krebs (fig. 7.21).

Finally, the amplitude of the AP was reversibly reduced under isovolumic contraction, in rabbit and pig (fig. 7.2 and 7.13), similar to that expected from a partially depolarized membrane.

However, a conventional microelectrode was not used in this study to confirm whether the shift represent a true membrane depolarization or just changes to a leakage resistance between the two electrodes attributed to muscle changes.

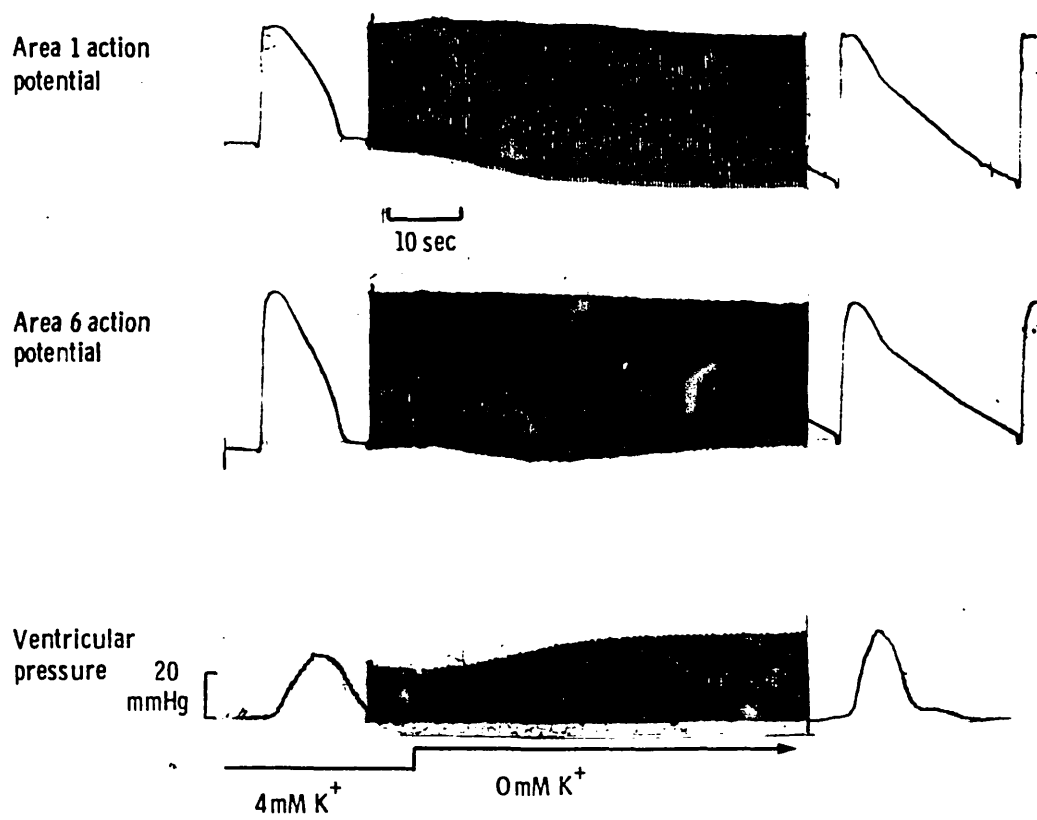


Fig. 7.19 Action potential from area 1 (top trace), area 6 (middle trace) and ventricular pressure (bottom trace) when changing the perfusing Krebs from 4mM to 0mM K⁺.

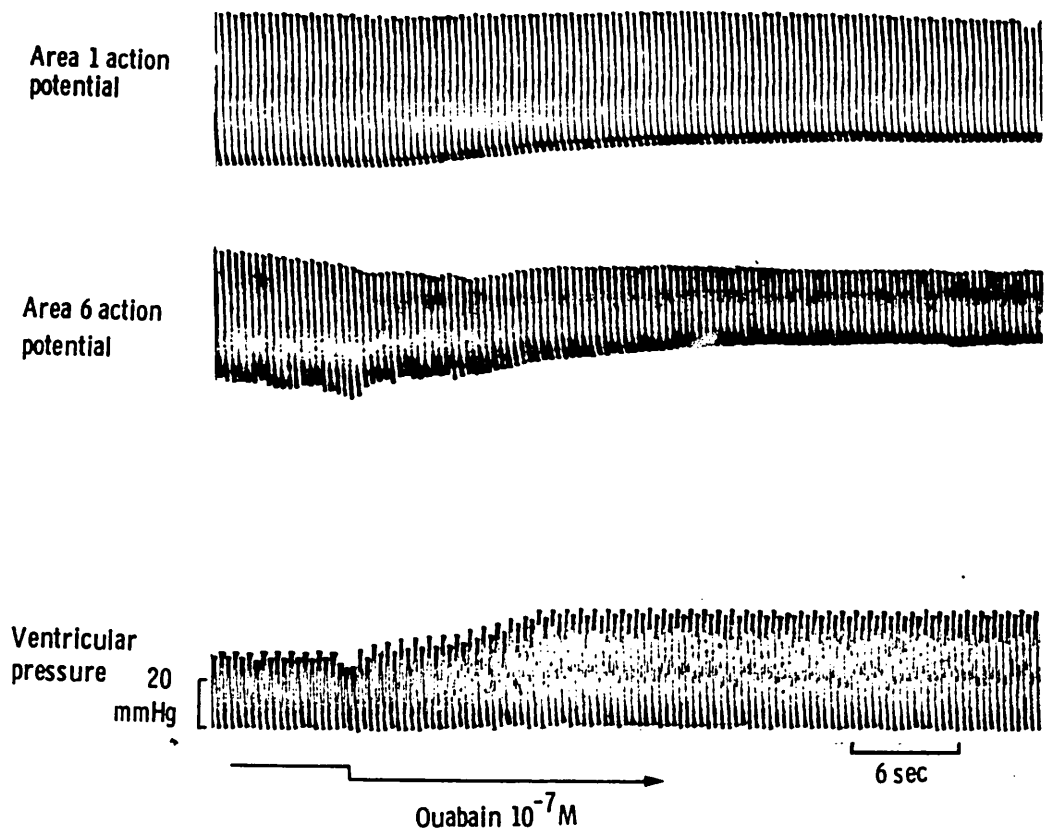


Fig. 7.20 Action potential from area 1 (top trace), area 6 and ventricular pressure (bottom trace) when changing to Ouabain containing Krebs.

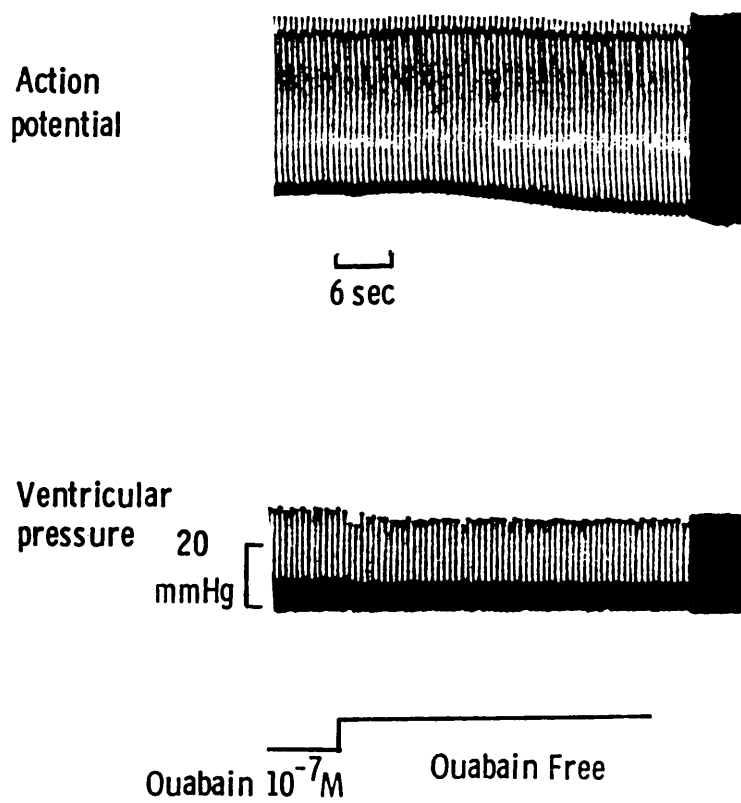


Fig. 7.21 Action potential (top trace) and ventricular pressure (bottom trace) when changing back to Ouabain free Krebs.

CHAPTER 8

Electrical restitution action potential

8.1 Introduction

8.2 Results

8.2.1 Isolated rabbit heart

8.2.1.1 Area 1 restitution curves

8.2.1.2 Area 6 restitution curves

8.2.2 Intact in situ pig heart

8.2.2.1 Base electrical restitution curves

8.2.2.2 Apex electrical restitution curves

Chapter 8

Electrical Restitution of Action Potential

8.1 Introduction

It was difficult to explain the different behaviour of the action potentials (AP's) under the two mechanical contractions when the rate was increased (chapter 7.2, 7.3) but the fact that the steady state AP's under isovolumic contraction were shorter than under free loaded contraction in most areas suggested either, some ion accumulation, as for example increasing intracellular Ca^{++} could shorten the AP as discussed by Boyett & Jewell (1978) and/or accumulation of K^+ in the extracellular spaces (Kline and Morad 1978) or changes in electrical membrane recovery. The electrical restitution curve is very useful tool in studying and making some interpretations about the recovery behaviour of membrane conductance, particularly in the intact heart in situ. For instance, it has been shown that the time course of the electrical restitution is affected by temperature, steady state beating rate and the ionic composition of the perfusing medium (Bass 1975, Boyett and Jewell 1978). Because the mechanical condition of contraction viz free loaded or isovolumic, affects APD (fig. 7.3 and 7.16) one might expect the mechanics of contraction to affect the electrical restitution curve. Therefore, it was decided to study the electrical restitution curves of areas 1 and 6 in the isolated rabbit heart, because of their opposite behaviour following an increase in rate (fig. 7.9) and also of the base and apex of the insitu pig heart under the two mechanical conditions.

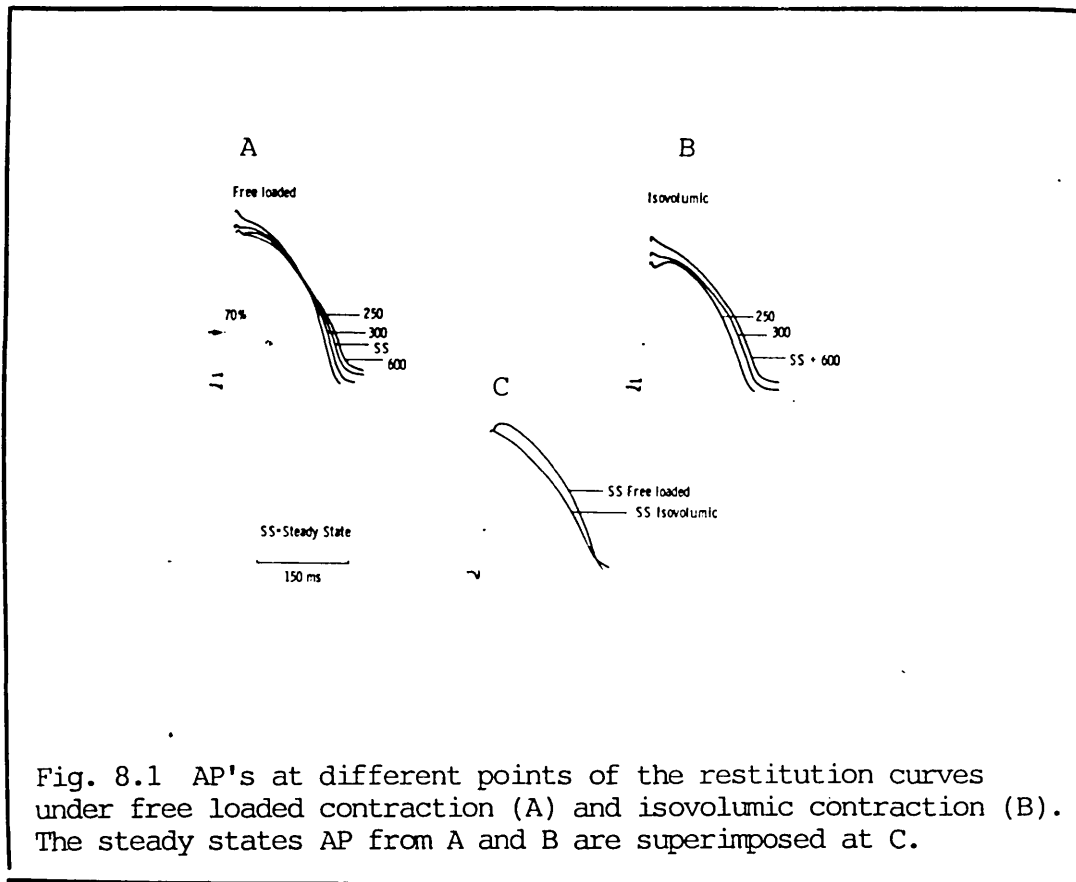
8.2 Results

8.2.1 Isolated Rabbit Heart

8.2.1.1 Area 1 Restitution Curves

A - First Beat

Fig. 8.1 shows AP's at different points of the restitution curves, superimposed at 70% repolarization, under free loaded contraction (A) and isovolumic contraction (B). The figure shows greater shortening at 250ms test pulse interval (TPI), i.e. the interval between the upstroke of the last control AP and of the test AP, under isovolumic compared to free loaded contraction.



Also there is little difference between the steady state APD and that at long intervals under isovolumic contraction, suggesting that the steady state APD is at the plateau of the curve, whereas under free loaded contraction the AP could be prolonged at longer TPI. In agreement with the results in chapter 7 the steady state duration is shorter under isovolumic than free loaded contraction as shown by the superimposition of the two steady states in fig. 8.1C.

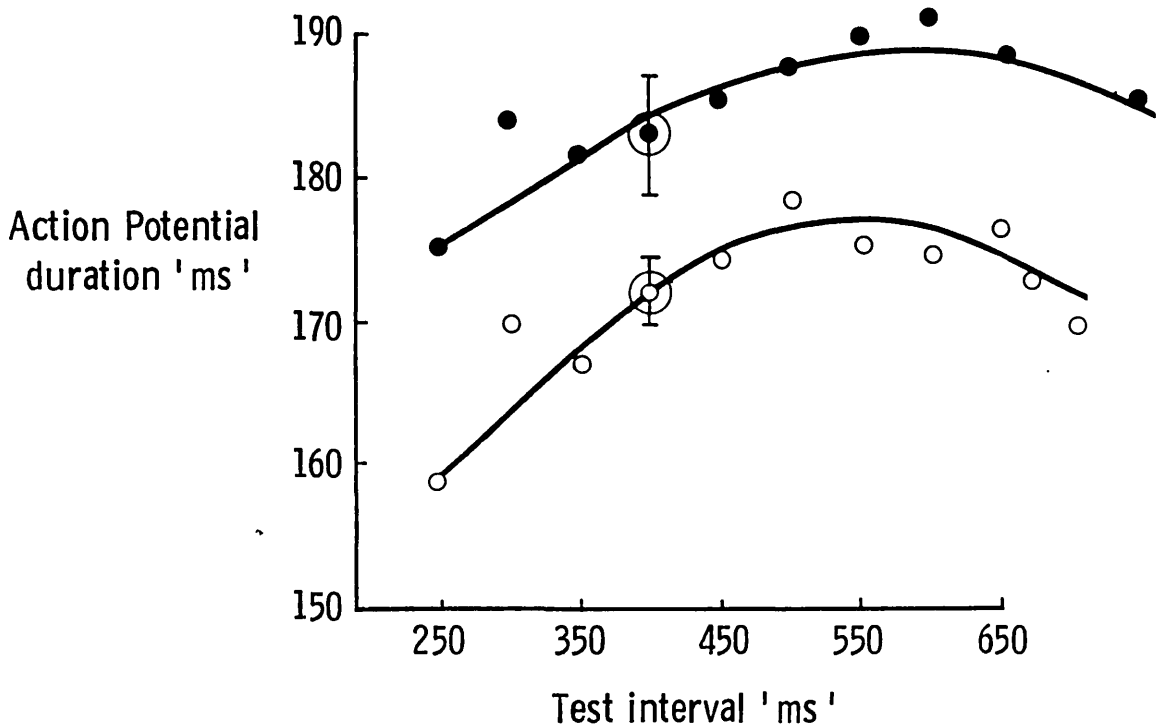


Fig. 8.2 Electrical restitution curves of a single experiment under isovolumic (open circles) and free loaded contraction (closed circle) from area 1 of the isolated rabbit heart. The encircled points are steady state durations, mean ($n = 16$) $\pm$ SD.

The curves of a single experiment are shown in fig. 8.2, and 8.3 are the two curves from 8 preparations (solid curves), the dashed extensions of the curves represent only 6 experiments.

The points at 600ms and 650ms TPI under both mechanical conditions were expected to be lower than they are on the graph - as shown by the dotted lines. The reason for the upward shift at these two points was the fact that 2 experiments which had short steady state action potentials could not be extended beyond 550ms test interval. Therefore the absence of these two experiments resulted in a small shift upwards in the two graphs. Also the standard deviation (SD) at all points of the restitution curves for the 8 experiments ranged from 13.2 ms to 23ms, which resulted in a large standard error (SE). This large SD was due to the large differences in absolute values between different preparations.

Note that the curves of the single experiment show a downward deflection at long intervals, which has been observed in isolated rabbit papillary muscle (Gibbs and Johnson 1961, Wohlfart 1979).

It was not possible to go to long enough TPI in enough number of experiments in order to show it in the absolute values of the grouped data, but when the results were expressed as % of the preceding steady state duration, such deflection is observed as shown in fig. 8.4.

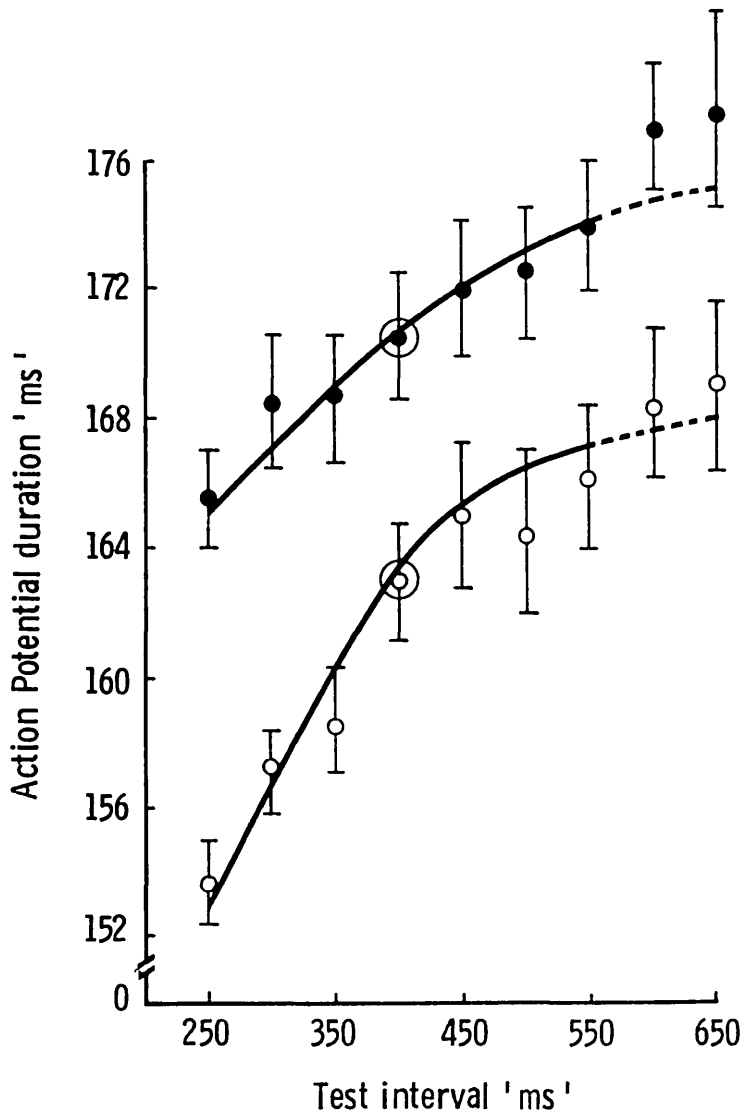


Fig. 8.3 Electrical restitution curves of 8 preparations (solid line), under isovolumic (open circles) and free loaded (closed circles). The dashed lines represent only six experiments. The values are mean $\pm$ SE. The steady states are encircled.

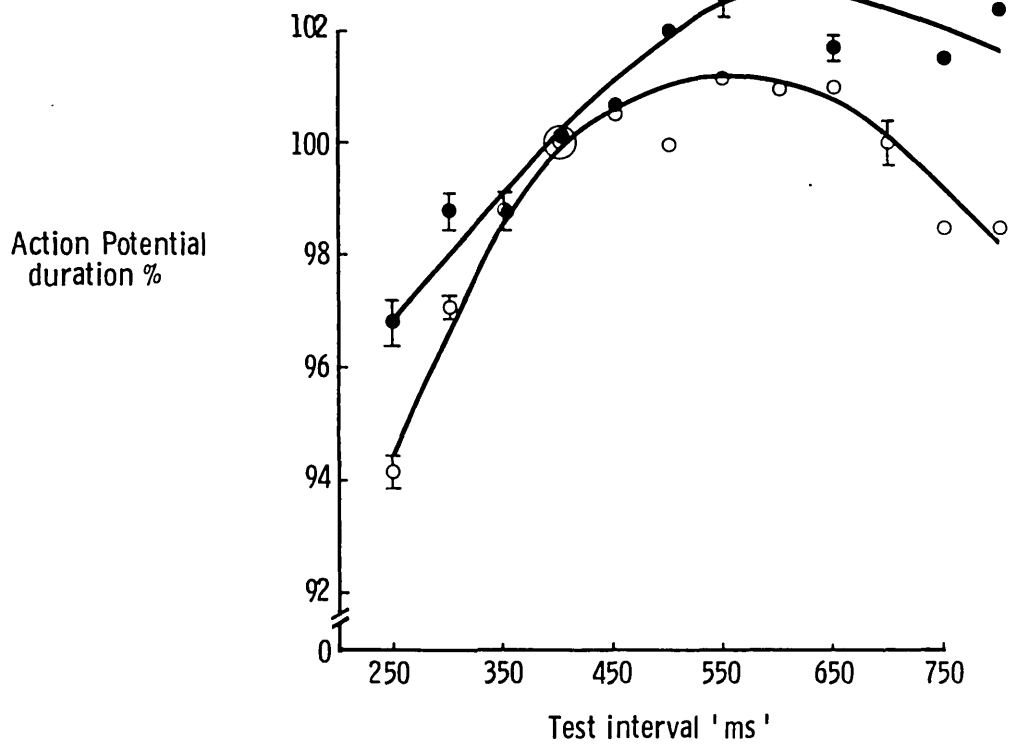


Fig. 8.4 The results of fig.8.3 expressed as % of the preceding steady state (mean + SE). The points with no range have SE within the circle. The steady states are encircled.

On inspection of fig. 8.3 the following features can be seen in the restitution curves;

1 - The average steady state action potential duration was shorter under isovolumic than under free loaded condition. This decrease in the steady state was evident in almost all the experiments. Wilcoxon matched paired test showed this difference to be significant, $P < 0.02$.

2 - If the restitution curve is divided into phase A "the exponential phase", and phase B "the plateau phase", it is clear that the steady state under free loaded contraction falls in the middle of phase 'A'. The free loaded curve of all the experiments in fig. 8.3 do not show a plateau, but in experiments where it was possible to go to longer test intervals a plateau was obtained at 600-650ms. The fact that the steady state AP fell on the exponential phase indicated that under free loaded contraction and at steady state (150/min) there was insufficient time for the steady state action potential to recover between beats, i.e. membrane conductance did not recover between consecutive steady state beats. Under isovolumic contraction the steady state AP was very close to the plateau indicating that the interval between steady state beats was long enough for near complete membrane recovery.

3 - After long test interval the APD under free loaded contraction could be prolonged more than under isovolumic, also the AP remained shorter under isovolumic contraction even at the longest test interval attainable in this study. Therefore, it seemed that the plateau of the isovolumic contraction restitution curve was shifted downwards. I called this shift a 'mechanically dependent downward shift'.

This downward displacement is analogous to the rate dependent and the Ca^{++} dependent downward shift observed by Boyett and Jewell 1978. The Wilcoxon-matched pair test showed this downward shift to be significant at all the points ($P < 0.05$). Student T-test was difficult to apply because of large standard deviation.

4 - The recovery index of the graph under free loaded contraction was calculated from fig. 8.1 to be 670ms and that of isovolumic contraction was 416ms, indicating that the membrane recovery was faster under isovolumic contraction.

5 - The magnitude of the curve under isovolumic contraction i.e. the distance from the plateau to the earliest action potential, was greater than under free loaded contraction, 13 and 9ms respectively.

Two other experiments showed too much scatter to be considered and in one further experiment the isovolumic contraction curve was shifted upwards. The most likely reason is a replacement of suction electrode. Fig. 8.4 shows the values of the above experiments as % of the preceding steady state action potential. The two curves for free and isovolumic contraction show the same features as the absolute values. The percentage results show that the shift in the plateau seen in the absolute values was not due to gradual changes in the steady state APD and, if there was a small shift, it was insignificant.

However, the standard deviations of the steady states of the different preparations under free loaded contraction in 7 out of the 8 experiments ranged from 2-4ms and in one experiment it was 6 ms.

Under isovolumic contraction it ranged from 2-5ms in 7 experiments and 10ms in the eighth. In this last experiment the difference between the two steady state APD was 24ms.

B - Second Beat

In a number of experiments from those shown in figures 7.7 & 7.8 and the above 8.3, the difference in AP shortening of the first beat when increasing the rate between the two mechanical conditions was very much less in the second beat, hence it was of interest to plot the "restitution" curves for the second beat, which could throw some light on possible ion accumulation or increased ion pump activity.

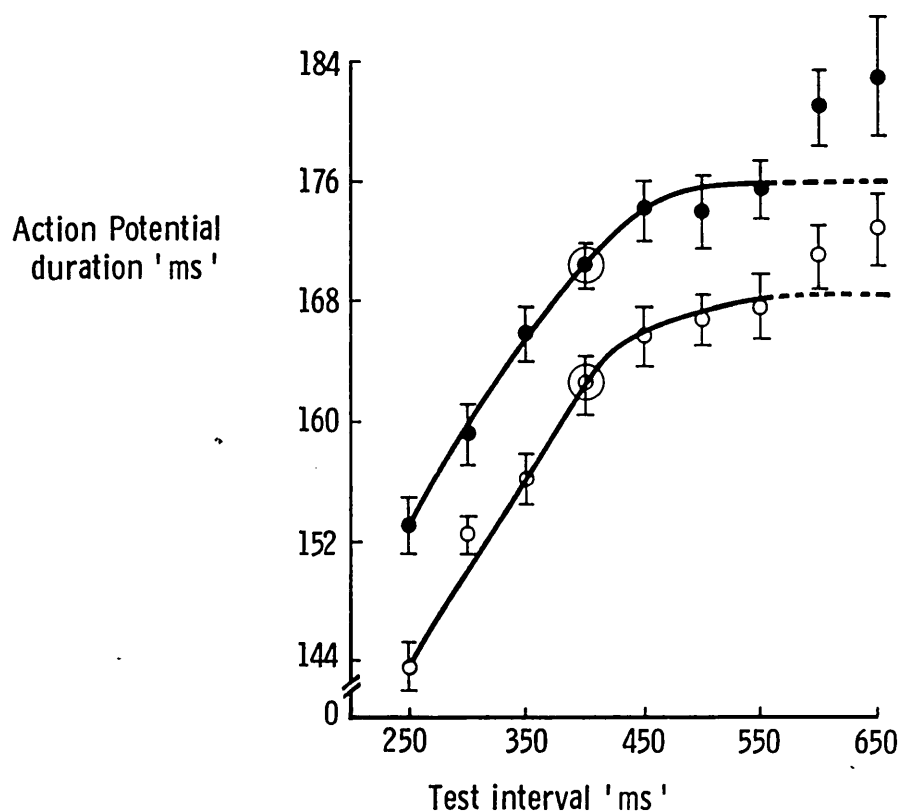
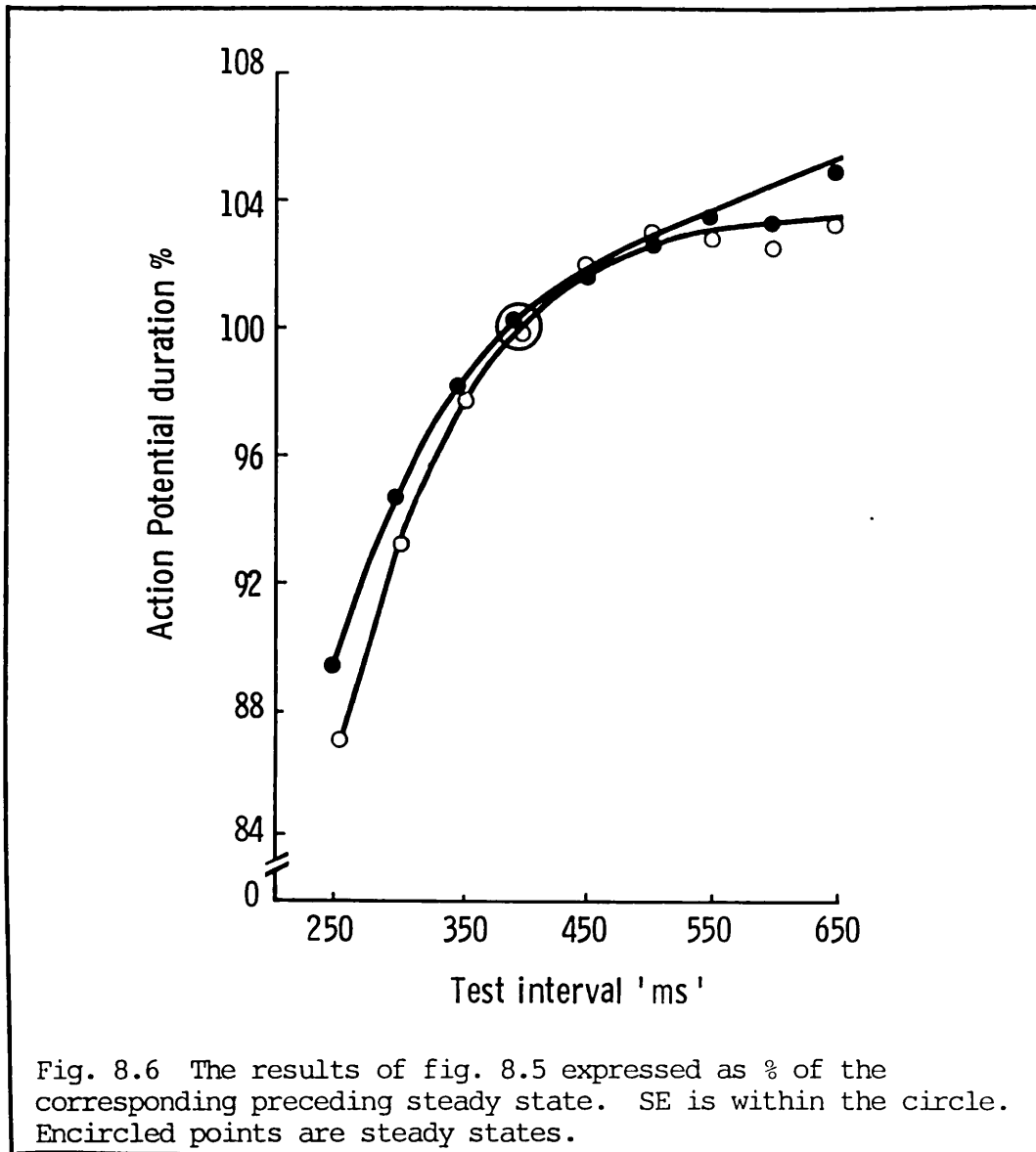


Fig. 8.5 The restitution curves under isovolumic (open circle) and free loaded (closed circle) of the second beat in the same experiments as in fig. 8.3 i.e. 8 preparations (solid line) 6 preparations (dashed lines). The values are mean $\pm$ SE. Steady states are encircled.



Figures 8.5 and 8.6 show the results of the 2nd test beat, of the same experiments of fig. 8.3, in absolute values and as % of preceding steady state respectively. The isovolumic contraction seemed to shift the whole curve down, but the time course was slightly faster than under free loaded contraction. The calculated recovery index 210ms and 224ms for free and isovolumic contraction respectively.

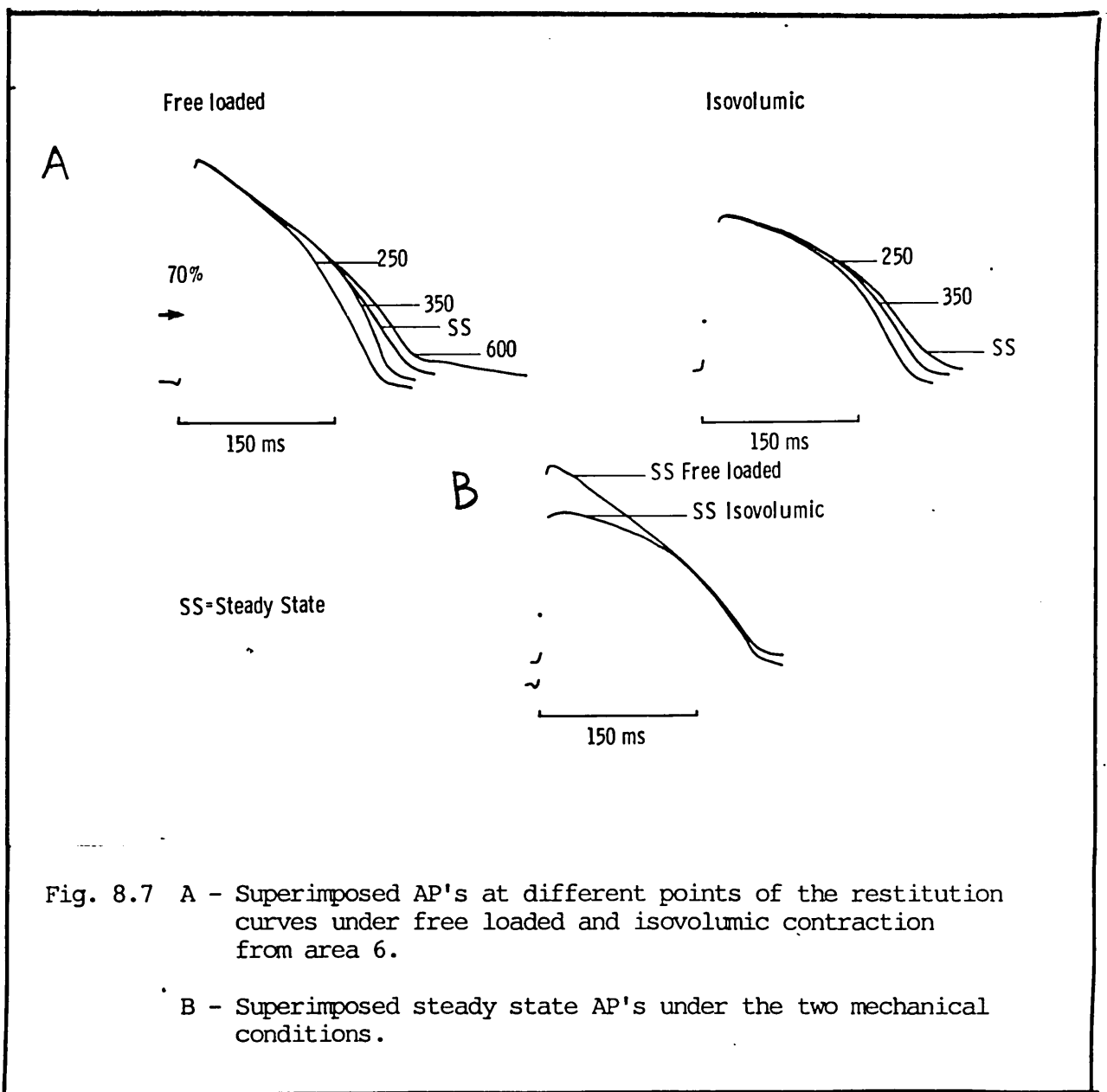
The 2nd beat results, therefore suggest that the difference in the recovery index was present in the first beat only indicating that the mechanism responsible for speeding up of recovery of the first beat was absent or only partially effective in the second beat. Wilcoxon - matched pair test showed the downward shift in the restitution curve was significant at all the points ($p < 0.05$).

8.2.1.2 Area 6 Restitution Curves

A - First Beat

This area was more difficult to investigate because of the close proximity of large vessels and its position on the heart. However, the study was successful in many preparations. Fig. 8.7 shows AP's at different stages of the restitution curves superimposed at 70% repolarization (top panels) and the steady state AP are superimposed in the lower panel. Notice the similarity in APD between the free loaded and isovolumic contraction steady states (fig. 8.7B) and the greater shortening of AP at 250ms TPI under free loaded (fig. 8.7A) compared to isovolumic contraction (fig. 8.7B). Also notice the greater difference between the shortest and longest AP under free loaded than isovolumic contraction. Fig. 8.8 shows the restitution curves of a single experiment for this area under free loaded contraction (closed circles) and isovolumic contraction (open circles) in absolute values.

Fig. 8.9 shows the grouped data of 7 preparations. The SD as in area 1 was large because of large variations in the steady state duration in different preparations.



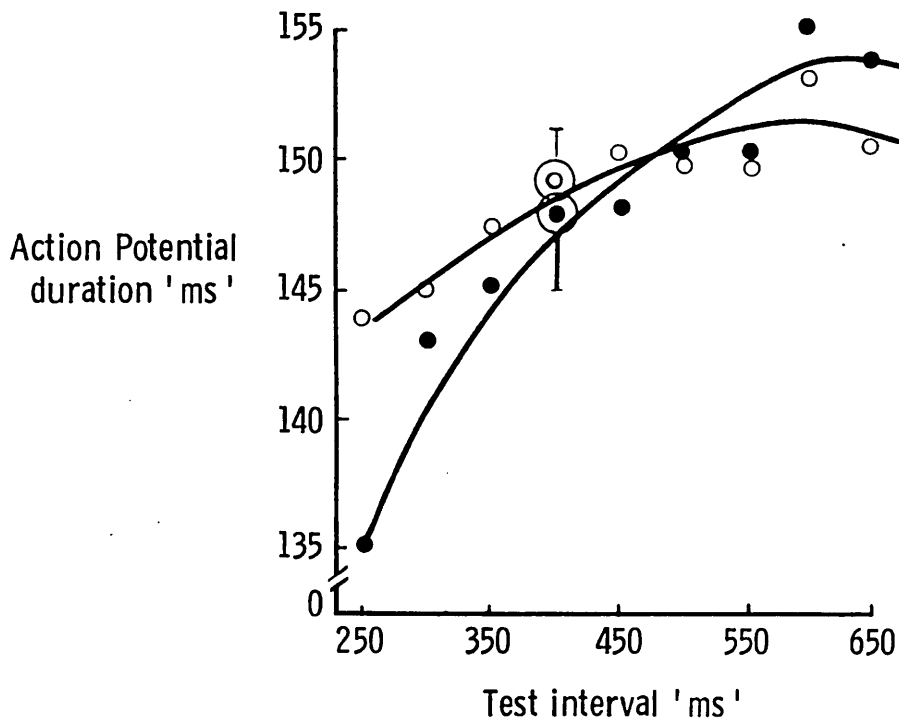


Fig. 8.8 Free loaded (closed circles) and isovolumic (open circles) restitution curves in area 6 of a single experiment. The encircled points are steady state durations, Mean $\pm$ SD.

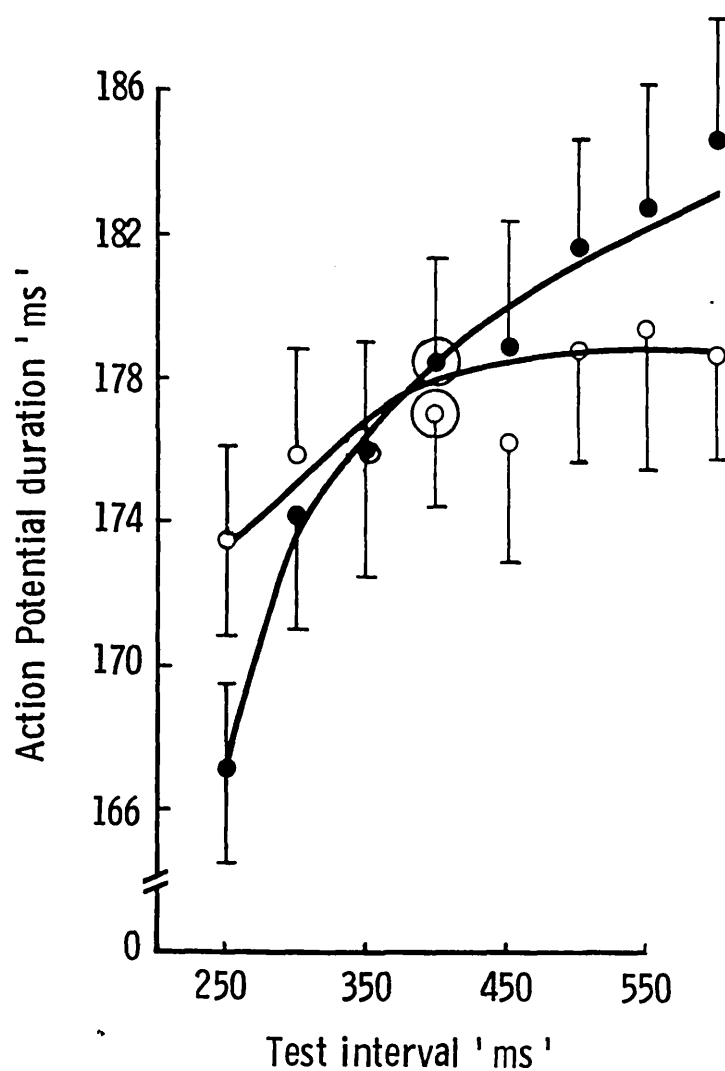


Fig. 8.9. The restitution curves of grouped data $n = 7$, Mean $\pm$ SE. Open circles are under isovolumic contraction and closed circles are under free loaded contraction. The steady state AP's are encircled.

The curves in fig. 8.9 have the following features:-

1 - There seemed to be no consistent difference between the two steady state AP at 400ms TPI. In 3 experiments the steady state APD was significantly shorter under isovolumic and in 2 experiments it was slightly longer. Two of the experiments showed no difference at all.

2 - The steady state duration under isovolumic contraction is very close to the plateau of the curve whereas under free loaded contraction it was on the rising phase of the curve, suggesting more complete membrane recovery between steady state beats under isovolumic contraction but incomplete recovery under free loaded contraction. This is similar to the situation in area 1.

3 - The magnitude of the isovolumic restitution curve was very much smaller than that of free loaded contraction. Different to area 1.

4 - The isovolumic restitution curve has a recovery index of 247ms and the free loaded curve recovery index is 144ms. Therefore isovolumic mode of contraction seems to slow the membrane recovery in this area; opposite in its effect on area 1.

5 - At long (700ms) TPI, the AP under free loaded contraction was prolonged because of longer time being available for membrane recovery, in comparison, the isovolumic AP remained short even at the long intervals.

This suggested a mechanical dependent downward displacement of the restitution curve similar to that seen in area 1. Wilcoxon matched pair test showed the difference between free loaded and isovolumic contraction at TPI of 250, 550 and 600ms to be significant ($p < .05$). At the other points there was no significant difference.

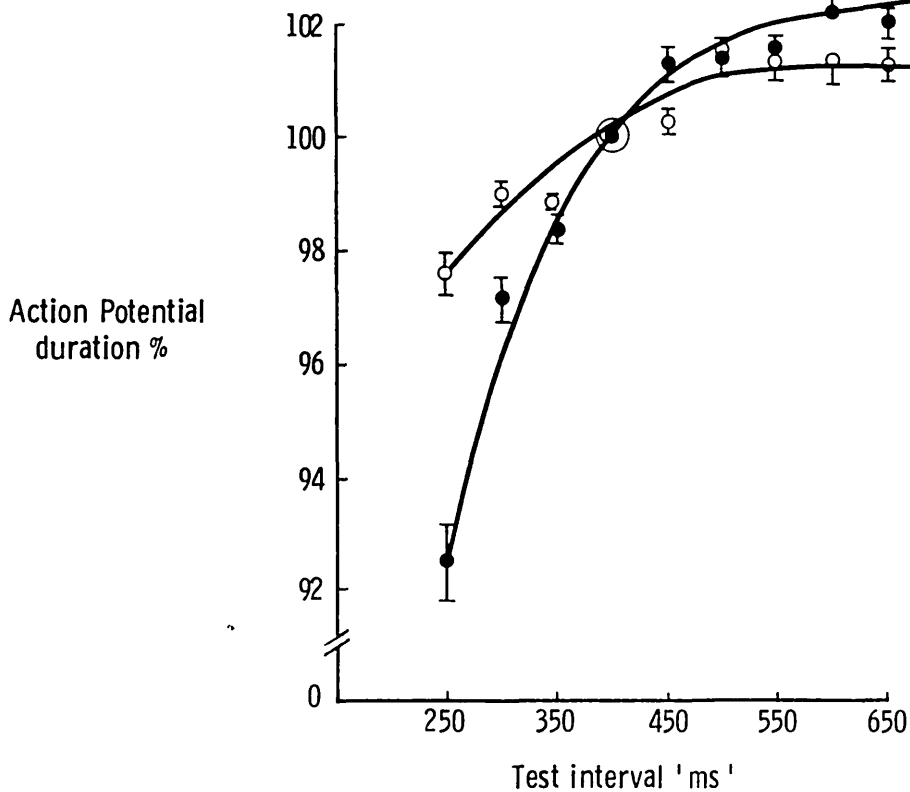


Fig. 8.10 The results of fig. 8.9 expressed as % of the preceding steady state ($n = 7$, Mean $\pm$ SE).

Notice that as in area 1, the restitutions in fig. 8.8 i.e. of a single experiment, deflect downward at long intervals, that is to say the AP shortens at long intervals as described in isolated rabbit papillary muscle by Gibbs and Johnson (1961) and by Wohlfart (1979).

Two other experiments showed too much scattering to be considered and in a further two experiments the whole restitution curve under isovolumic contraction moved upwards. The latter was due to a shift in the steady state APD during the experiment, but the results were comparable to the rest when expressed as a % of the steady state APD. These two experiments were done on the same day with the same Krebs, so it is possible that their different behaviour was due to experimental error.

B - Second Beat

Figures 8.11 and 8.12 show the results for the second beat in absolute values and in percentages respectively. The differences between isovolumic and free loaded contraction are smaller in the second beat, that is similar to area 1.

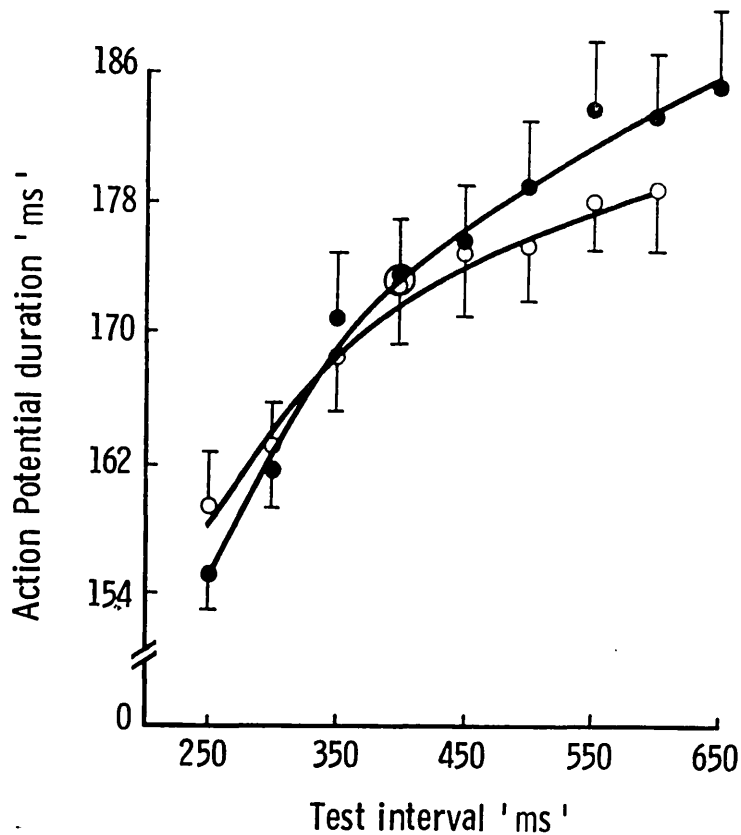


Fig.8.11 Restitution curves in absolute values (Mean + SE) of the second beat in the same 7 experiments of fig. 8.9. Open circle is isovolumic curve and closed circle is free loaded contraction curve.

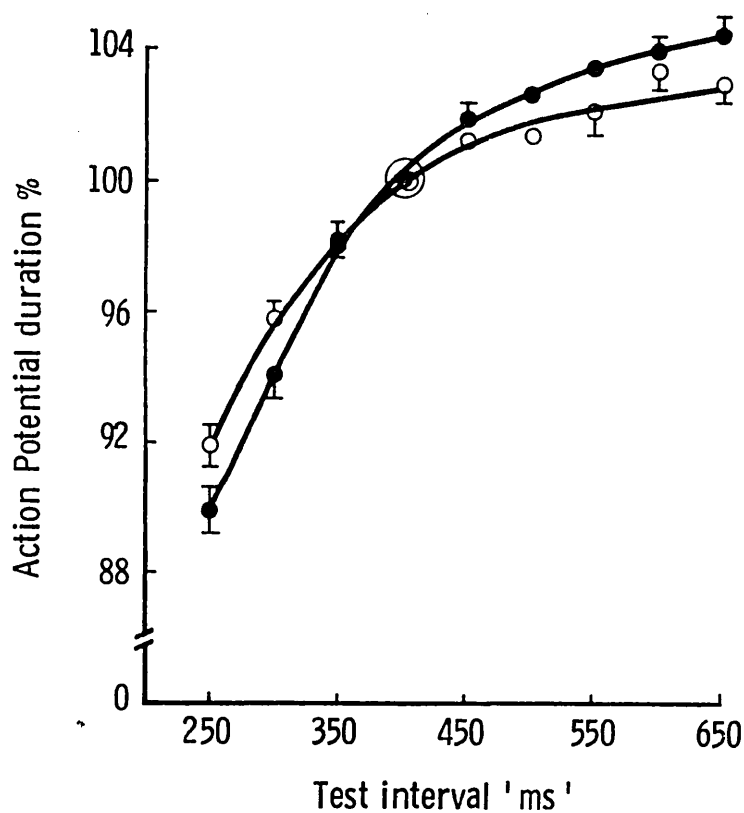


Fig. 8.12 The results of fig. 8.11 expressed as % of the preceding steady state APD. Isovolumic contraction (open circle). Free loaded contraction (closed circle) ($n = 7$ Mean $\pm$ SE). The points with no range had a range within the circle.

8.2.2 Intact Insitu Pig Heart

The restitution curves from the base and apex were constructed under normal and isovolumic contraction. Because of lack of experimental control over the heart, especially the intrinsic rate as in the isolated rabbit heart, it was much more difficult to construct more complete restitution curves.

8.2.2.1 Base Electrical Restitution Curves

Fig. 8.13 shows the electrical restitution curve to be shifted downwards under isovolumic contraction compared to normal contraction similar to that in the isolated rabbit heart. Similar shifts, particularly at long TPI, was seen in two more preparations and not much difference in a third. The recovery index was difficult to calculate, but in the experiment shown in fig. 8.13, it seems to be slower under isovolumic than normal contraction. In one of the other two experiments with a downward shift of the isovolumic curve, there was little difference, by inspection, in the time course between the two mechanical conditions, in fact the curve seemed to be slightly accelerated under isovolumic contraction in this experiment. In the remaining two experiments it is difficult to say because of scattering of the data.

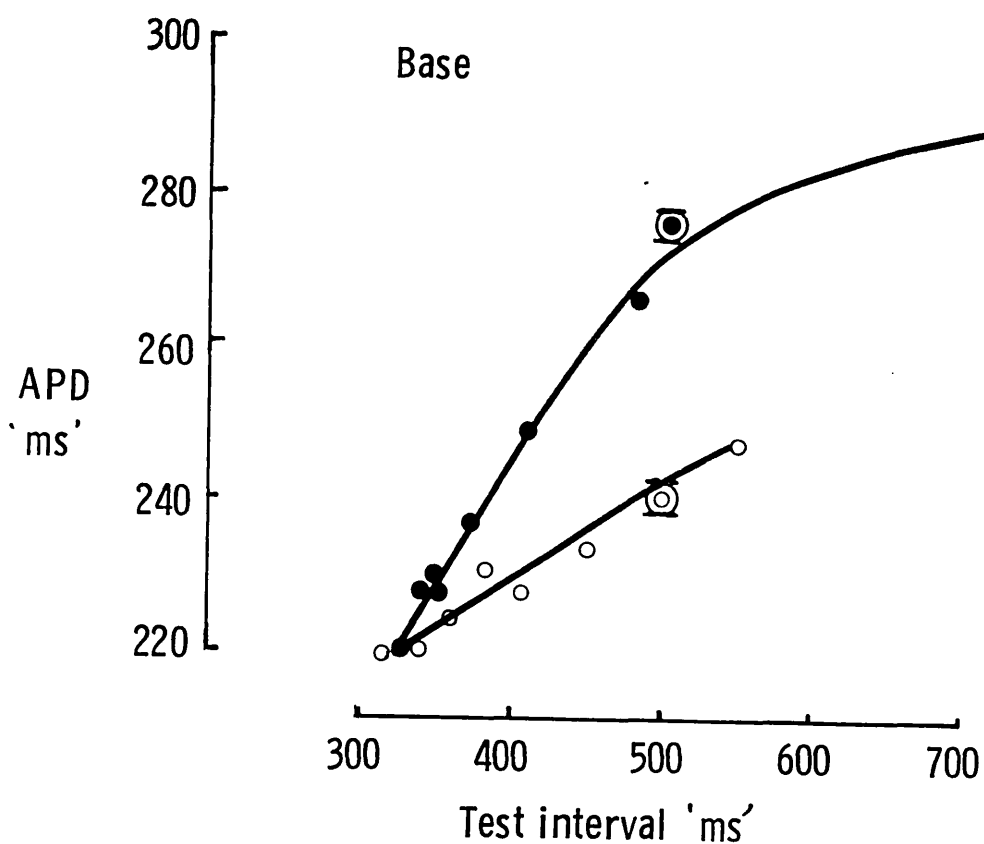


Fig. 8.13 The normal (closed circles) and isovolumic contraction (open circles) restitution curves from the base of an insitu pig heart. The steady state AP's are encircled, (Mean $\pm$ SD, $n = 16$)

8.2.2.2 Apex Electrical Restitution Curves

Similar to the base behaviour, in three preparations the isovolumic restitution curve of the apex was shifted downwards in comparison to the normal contraction curve, fig. 8.14 (this figure and fig. 8.13 are from the same preparation). By eye, the isovolumic curve seems to be slower in the experiment shown in fig. 8.14, and also in another experiment, but difficult to say in the other two.

Notice that in fig. 8.14 the two curves cross over, and this was observed in another two experiments. It was of interest to note that this cross over is similar to that between base and apex restitution curves under normal contraction which was observed by Lab and Yardley (unpublished data) and in the experiment shown in fig. 8.13 and 8.14 (see fig. 8.15, 8.3.2).

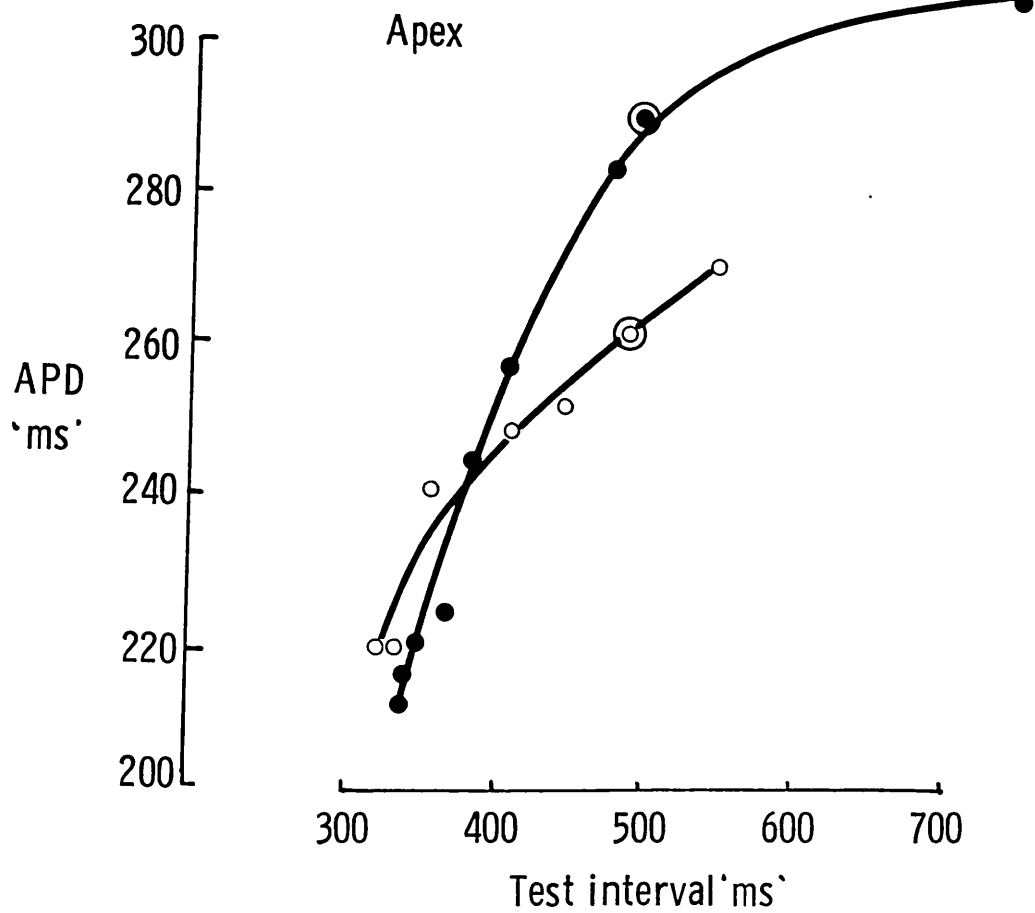


Fig. 8.14 Normal (closed circles) and isovolumic (open circles) contraction curves from a single experiment from the apex of an insitu pig. The steady state AP's are encircled.

CHAPTER 9

Electrical restitution curves at high K^+ (8mM)

9.1 Introduction

9.2 Results

9.2.1 Area 1 restitution curves

9.2.2 Area 6 restitution curves

Chapter 9

Electrical Restitution Curves at high K^+ (8mM)

9.1 Introduction

Increasing extracellular K^+ , $[K^+]_o$, which presumably elevates $[K^+]_e$ in the extracellular spaces has been shown to depolarize the resting membrane potential and to shorten APD (Weidmann 1956, Ellis 1977 Eisner and Lederer 1979a, Attwell et al 1981. See discussion chapter 7 for more references). Increasing $[K^+]_o$ also increases the activity of the Na^+ pump (Ellis 1977, Ruiz-Ceretti et al 1981, Martin and Morad 1982). The possible accumulation of K^+ in the extracellular spaces (Kline and Morad 1976, 1978) has prompted to study the electrical restitution curves under both mechanical conditions of contraction at high K^+ , using the isolated rabbit heart.

9.2 Results

It is well known that increasing the $[K^+]_o$ produces depolarization of the resting membrane potential. Such depolarization was consistently observed in my experiments when changing the perfusing solution from 4mM K^+ to 8mM K^+ .

Fig 9.1 shows action potentials from area 1 'top trace', area 6 'middle trace' and ventricular pressure 'bottom trace' under free loaded contraction recorded simultaneously. The perfusing solution was changed to 8mM K^+ and changed back to 4mM K^+ (control solution). It is clear that the depolarization produced by the 8mM K^+ solution was reversible as would be expected, and it also pointed out that the suction electrode can monitor, qualitatively, changes in resting membrane potential. This depolarization was observed in all the preparations studied under these conditions.

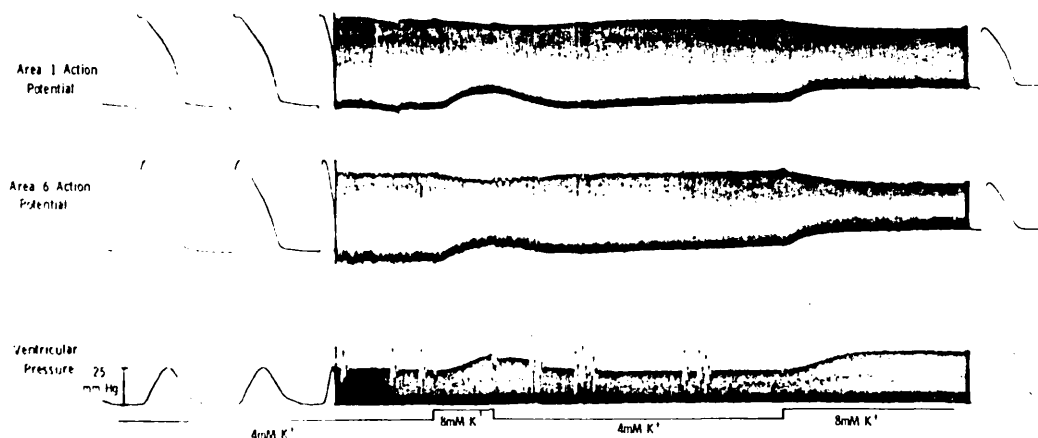


Fig. 9.1 see text for description.

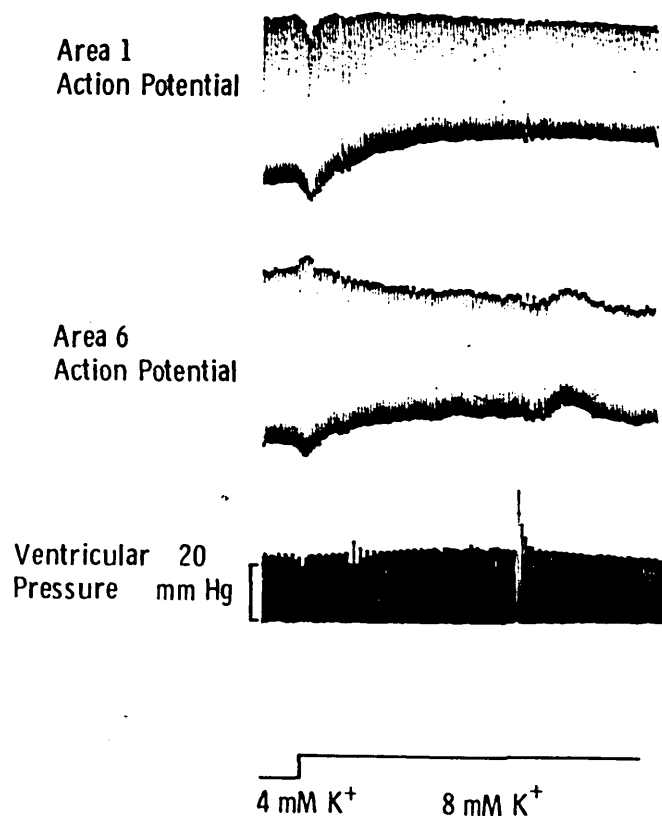


Fig. 9.2 The transient increase in ventricular pressure (bottom trace) under free loaded contraction when changing to 8mM K. It also shows a depolarization of the resting membrane potential in both area 1 (top trace), and area 6 (middle trace).

	<u>Free Loaded</u>		<u>Isovolumic</u>	
	<u>4mMK+</u>	<u>8mMK+</u>	<u>4mMK+</u>	<u>8mMK+</u>
1	26.99±1.4	22.8±1.3	35.5±1.2	35.2±0.8
2	26±1.14	25.2±0.8	42±2	45±1
3	24±0	23±0.9	34.1±0.74	38±2
4	20±1.6	22.2±0.5	31±0.7	32±0
5	22.5±0	20.8±0	36±0.7	40.8±0.7
6	24.2±0	19±0	33.5±1.1	31±0
	23.9±2.5(6)	22.16±2.1(6)	35.3±3.7(6)	37±5,4(6)

MEAN ± SD

n = 15-30

TABLE 9.1 Steady state ventricular pressure(mm Hg) at different
[K⁺]_o .

In addition it shows a rise in ventricular pressure when changing to 8mM K^+ , but this increase was a transient as shown in fig. 9.2. This transient increase in ventricular pressure was seen in four out of six preparations. In all the preparations except one the free loaded contraction steady state ventricular pressure was significantly lower at 8mM K^+ compared to 4mM K^+ as presented in table 9.1.

These observations suggested that intracellular Ca^{++} , $[\text{Ca}^{++}]_i$, increased transiently when changing the perfusion medium, but it was either sequestered into a compartment where it was not available for contraction, or more likely was pumped out of the cell. Under the isovolumic mode of contraction, the case was opposite of that under the free loaded contraction in that the steady state ventricular pressure at 8mM K^+ was higher than that at 4mM K^+ (table 9.1). The ventricular pressure ratios of isovolumic to free loaded contraction at 4 and 8mM K^+ were 1.47 and 1.67 respectively. Another way of comparison is that the ratios of ventricular pressure developed at 8mM K^+ to that developed at 4mM K^+ were 0.927 and 1.05 for free loaded and isovolumic contraction respectively. These results suggest that the isovolumic mode of contraction may somehow increase the intracellular Ca^{++} at 8mM K^+ .

9.2.1. Area 1 Restitution Curve

The hearts were first perfused with control solution (4mM K^+), the electrical restitution curves for areas 1 and 6 were obtained under free and isovolumic contraction before switching to high K^+ (8mM), and repeating the protocol.

Superimposed AP's at different points of the restitution curves at 8mM K^+ are shown in fig. 9.3 . Note the greater shortening at 220ms TPI under isovolumic contraction (top right hand trace) compared to free loaded contraction (top left hand trace) . The steady state AP's are also shown in fig. 9.3 (bottom trace) where the isovolumic AP is shorter than the free loaded contraction AP.

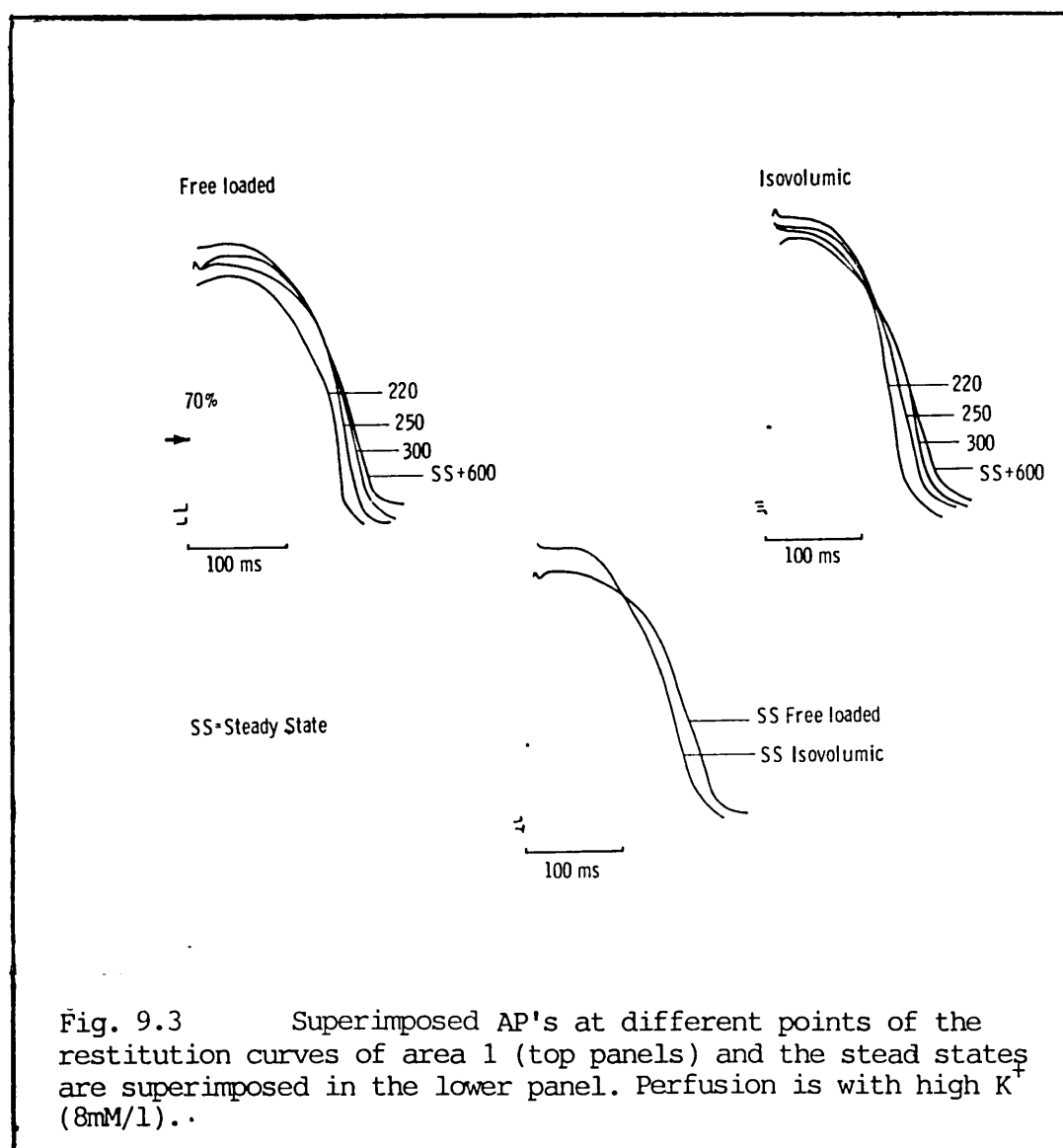


Fig. 9.3 Superimposed AP's at different points of the restitution curves of area 1 (top panels) and the steady states are superimposed in the lower panel. Perfusion is with high K^+ (8mM/l)..

Control restitution curves from 4 out of 6 preparations are shown in fig. 9.5 while the restitution curves of a single experiment under free loaded and isovolumic contraction at high K^+ are shown in fig. 9.4 and the pooled data of the 6 preparations are shown in fig. 9.6.

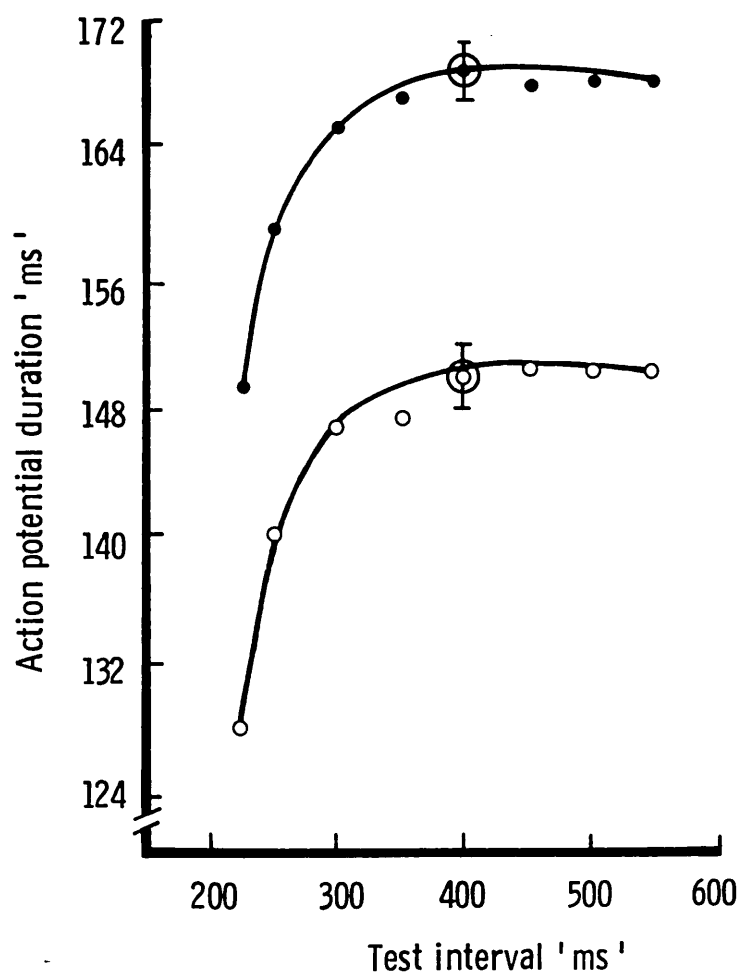


Fig. 9.4. Restitution curves of a single experiment from area 1, at high K^+ . Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled (mean $\pm$ SD).

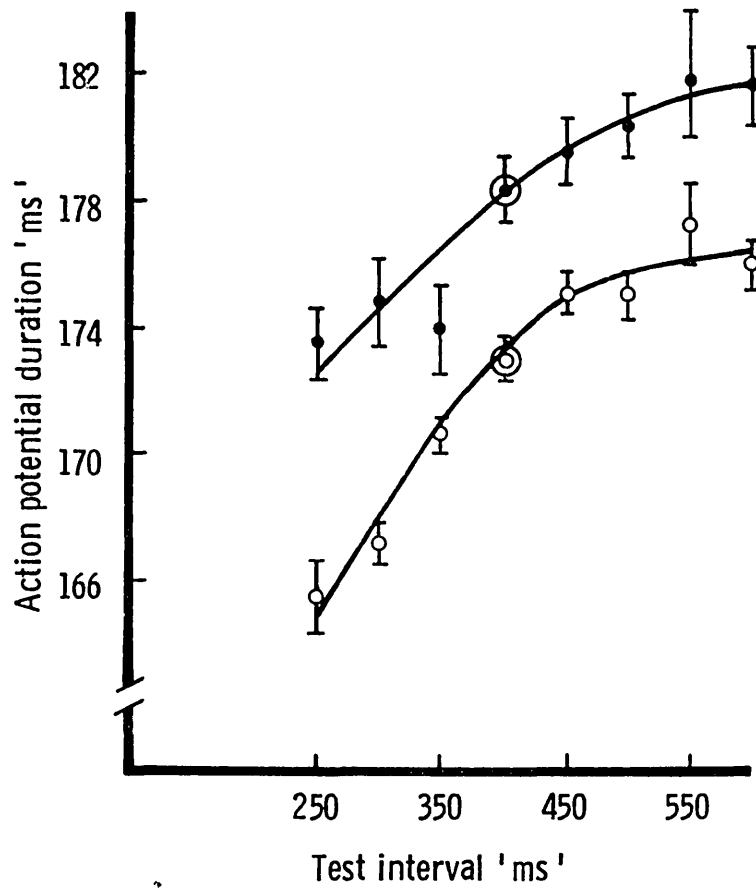


Fig. 9.5 control electrical restitution curves of area 1 under free loaded contraction (closed circles) and isovolumic (open circle) ($n = 4$; mean $\pm$ SE). The steady state AP's are encircled.

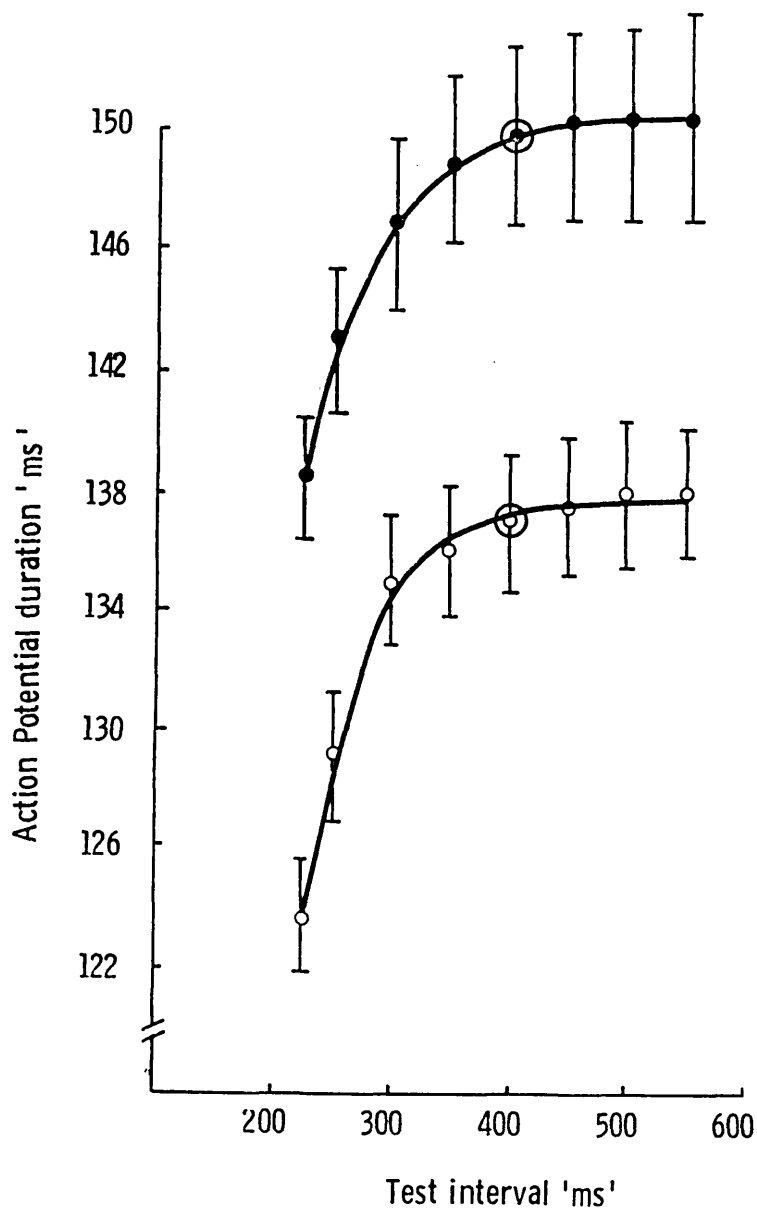


Fig. 9.6 Restitution curves of area 1 constructed from pooled data of 6 preparations (mean + SE) at high K^+ . Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled.

The following features are evident on inspection of the curves in fig. 9.6 for high K^+ . For the control curves see chapter 8.

1. At high K^+ the steady state duration under isovolumic (open circles) was shorter than under free loaded contraction (closed circles). Further, in comparison to the control, the difference between the steady states under the two conditions of contraction with high K^+ was greater. This difference was 13ms as compared to only 5 ms in the control. This suggested that although high K^+ shortened the steady state action potential under free loaded contraction from 178ms (fig. 9.5) in control K^+ to 150ms in high K^+ (fig. 9.6) the effect of isovolumic contraction was to shorten it by a further 13ms. Thus the effect of isovolumic contraction in shortening the action potential was additive to that of high K^+ , suggesting that the further shortening under isovolumic contraction was produced by a different process to that responsible for the AP shortening on elevating $[K^+]_o$.
2. At a stimulus frequency of 150/min there was sufficient time for complete membrane recovery under both conditions of contraction. This was revealed by the fact that the steady state durations fell on the plateau of the curves. If the stimulus interval was prolonged, the action potential under both conditions of contraction lengthened no further.
3. When sufficient time was allowed after a given steady state response, for membrane recovery at high K^+ , the action potential under isovolumic contraction remained shorter than that under free loaded contraction.

This was because of a mechanically dependent downward shift of the restitution curve, similar to that under control. This downward shift under isovolumic was much greater at high K^+ as compared to control, it was 13ms and 5ms respectively.

4. The magnitude of the isovolumic contraction restitution curve was greater than that of free loaded contraction by about 2ms under high K^+ as compared to about 2.5ms between the control curves. Therefore the difference between the amplitudes seems to be very small.

5. At high K^+ the recovery indices of both curves under the two mechanical conditions of contraction approached each other. They were 48ms for free loaded and 59ms for isovolumic contraction. In comparison, a larger difference was seen between the two control curves; 625ms and 454ms for free and isovolumic contraction respectively.

9.2.2 Area 6 Restitution Curves

Depolarization of the diastolic membrane potential in this area was observed in all the experiments when switching to high K^+ . Examples were shown in fig. 9.1 and 9.2 (middle trace in both figures).

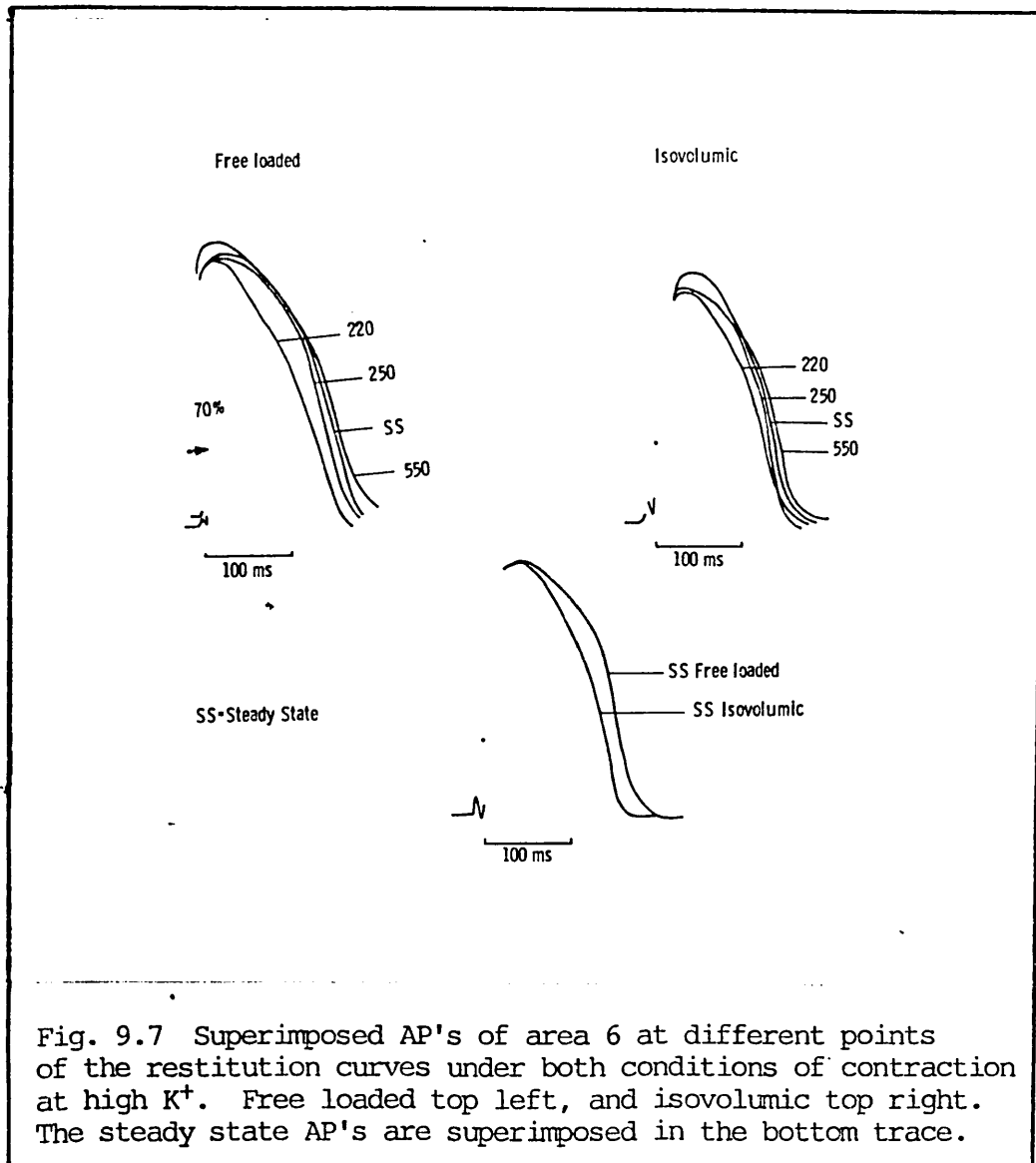


Fig. 9.7 Superimposed AP's of area 6 at different points of the restitution curves under both conditions of contraction at high K^+ . Free loaded top left, and isovolumic top right. The steady state AP's are superimposed in the bottom trace.

The restitution curves of a single experiment under both conditions of contraction at high K^+ are shown in fig. 9.8. They show shorter steady state and smaller amplitude of the curve under isovolumic contraction than free loaded contraction.

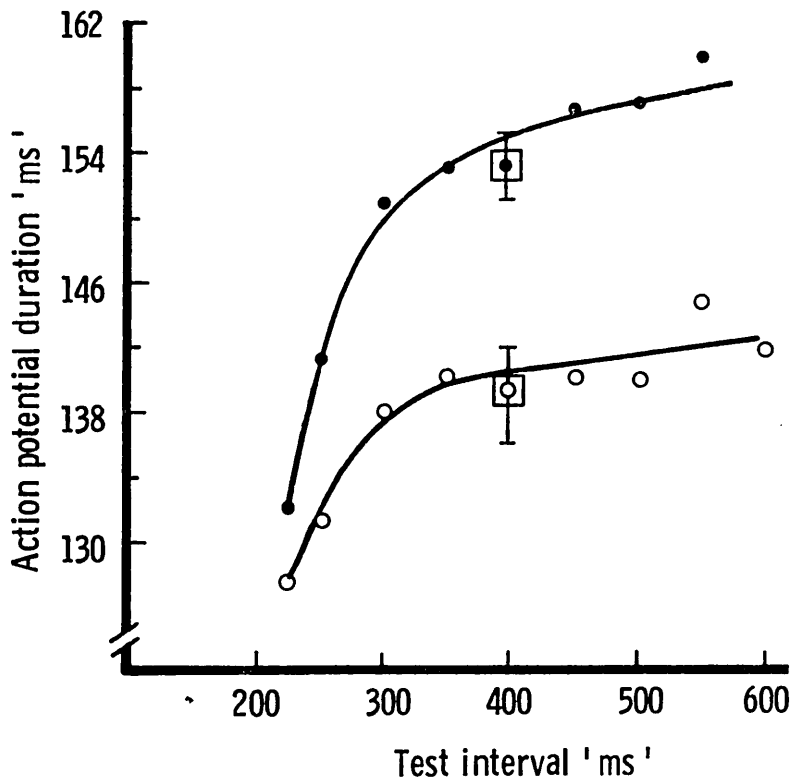


Fig. 9.8. Restitution curves of area 6 in single experiment at high K^+ . Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's (mean $\pm$ SD) are in squares.

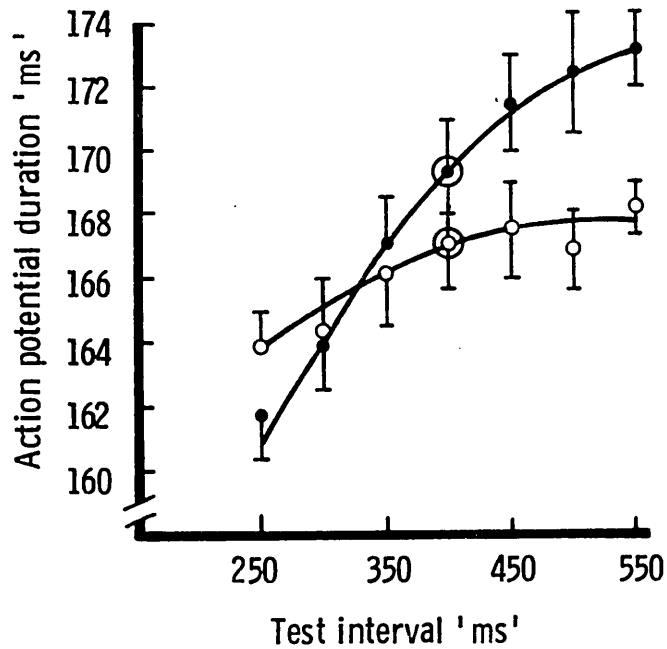


Fig. 9.9 Control restitution curves of area 6 under free loaded contraction (closed circles), isovolumic contraction (open circles). ($n = 4$ mean $\pm$ SE). The steady state AP's are encircled.

Fig. 9.9 and 9.10 show the restitution curves of area 6 obtained at control K^+ , and high K^+ respectively. The following features are evident when inspecting fig. 9.10 i.e. the curves at high K^+ .

The control curves have similar features to those described in chapter 8.

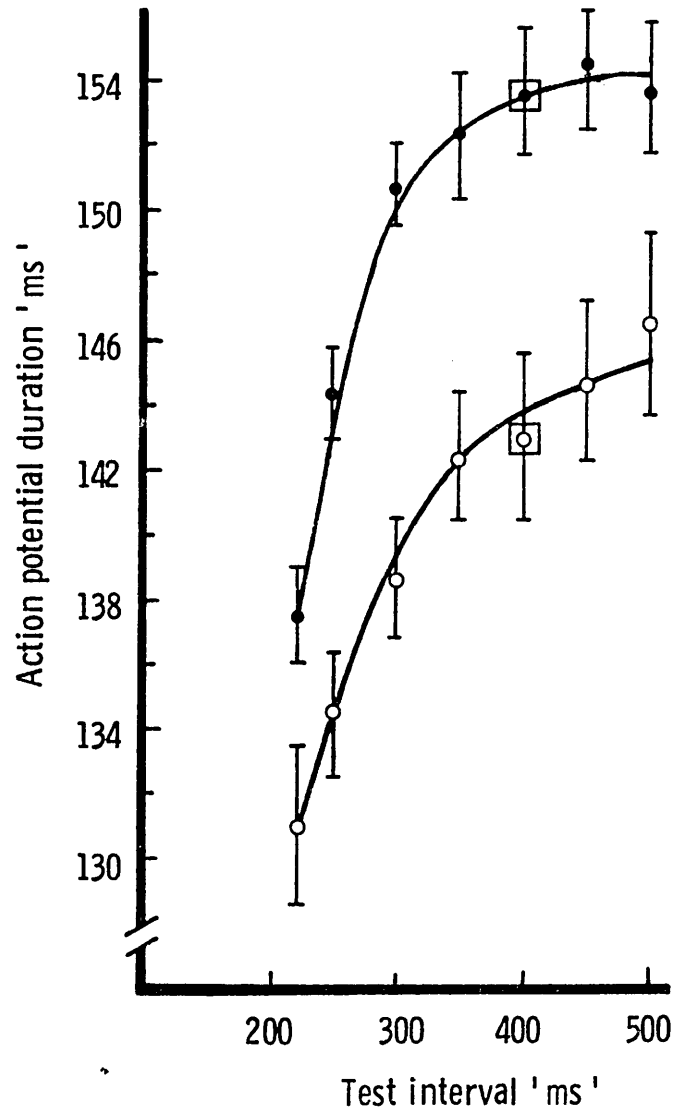


Fig. 9.10 Restitution curves of area 6 constructed from pooled data of 6 preparations (mean $\pm$ SE) at high K^+ . Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are in squares. Area 6.

1. At high K^+ isovolumic contraction resulted in a large shortening of the steady state APD of about 11ms. In the control curves (fig. 9.9) the shortening of the steady state APD produced by isovolumic contraction was not as large as that at high K^+ , fig. 9.10. This, as in area 1, suggested that although high K^+ produced shortening of the steady state APD from about 169ms to 154ms under free loaded contraction, the further shortening of about 11ms under isovolumic contraction was additive to it.
2. It seemed that the membrane was near complete recovery under both conditions of contraction at a steady state rate of 150/min, as shown by the fact that the steady state AP's under both conditions of contraction seem to be close to the plateaux of the restitution curves. It was unfortunate that it was not possible to go to the longer intervals in all the experiments - in only 3 experiments was it possible to go up to 600ms test intervals. The results of all experiments (as % of the preceeding steady state) showed that the steady state durations were indeed very close to the plateaux.
3. The isovolumic contraction, as in area 1, produced greater downward shift at high K^+ than control and even after long intervals the plateau remained shifted shown. Thus changing the mechanics of contraction to isovolumic produced a mechanically dependent downward shift in area 6 similar to that in area 1. The downward shift of the plateau of the isovolumic curve was greater under 8mM K^+ in comparison to control (4mM K^+).

4. The amplitudes of curves at control K^+ were 12ms and 4ms for free and isovolumic contraction respectively. At high K^+ the difference in amplitudes between the two mechanical conditions were not as large as at control. The amplitude under free loaded contraction was 17ms and that under isovolumic contraction was 14ms giving a difference of only 3ms between the two as compared to 8ms between the two curves at control K^+ .

5. Isovolumic contraction slowed the time course at both concentrations of K^+ . In the control curves (fig. 9.9) the recovery indices were 189ms and 247ms for free loaded and isovolumic contraction respectively. At high K^+ (fig. 9.10) they were 50ms under free and 80ms under isovolumic contraction. Therefore, isovolumic contraction slowed the time course of membrane recovery but, this slowing was smaller in high K^+ than in control K^+ .

In summary:-

At 8mM K^+ changing the mechanics of contraction to isovolumic resulted in a large downward shift of the restitution curve, especially at the plateau region of the curve of both areas 1 and 6. This shift was additive to the shift downwards produced by increasing $[K^+]_o$. This suggested the possibility that other different mechanisms were responsible for the shift under isovolumic contraction from that produced by elevating $[K^+]_o$.

The greater AP shortening under isovolumic contraction in area 1, seen

with control Krebs, was maintained at high K^+ , but the time course did not seem to be faster than in free loaded contraction as with control Krebs. Isovolumic contraction compared to free loaded contraction slowed the time course of area 6 at high K^+ but not as much as it did with control Krebs (4mM K^+). Therefore, the effect of isovolumic contraction in slowing the time course of area 6 restitution curve was reduced by increasing $[K^+]_o$, which presumably increased $[K^+]_e$. High K^+ also reduced the difference between the amplitudes of the restitution curves seen at 4mM K^+ in area 6. Notice also that high K^+ accelerated the time course of restitution curves of both areas compared to control which is in agreement with Lab and Yardley (unpublished data).

CHAPTER 10

Electrical restitution curves at low Ca^{++} (0.5mM)

10.1 Introduction

10.2 Results

10.2.1 Area 1 restitution curves at low Ca^{++}

10.2.2 Area 6 restitution curves at low Ca^{++}

Chapter 10

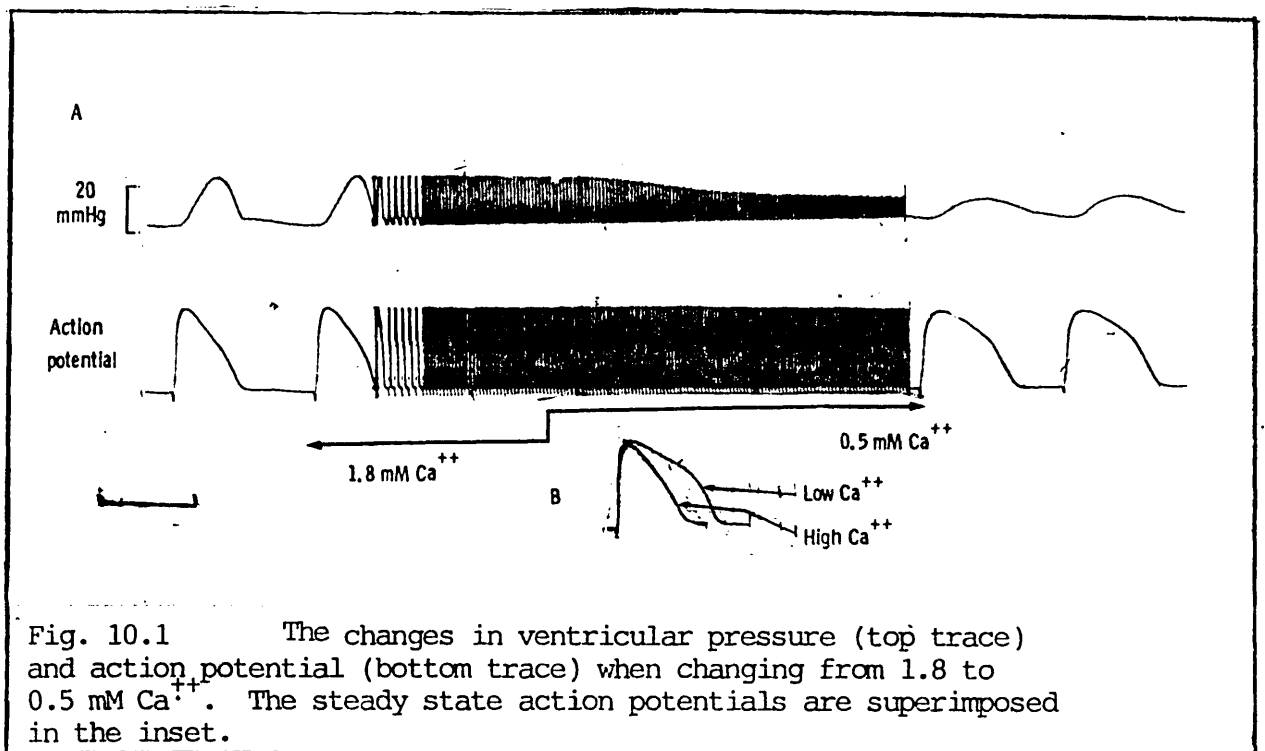
Electrical Restitution Curves at low Ca^{++} (0.5mM)

10.1 Introduction

Lakatta and Jewell (1977) and Lakatta and Spurgeon (1980) suggested an increase in Ca^{++} influx to be responsible for the increased tension in a stretched isolated cat papillary muscle. Allen and Kurihara (1982) have verified this suggestion by the use of aequorin. Elevating $[\text{Ca}^{++}]$ in the perfusing medium of many cardiac preparations has been shown to shorten APD (see discussion 10.3). Bass (1975) showed a downward displacement of the plateau part of the electrical restitution curves at high Ca^{++} , while Boyett and Jewell (1978) discussed the possibility of $[\text{Ca}^{++}]_i$ accumulation being responsible for the downward shift of the electrical restitution curve at higher rates of stimulation. The possible accumulation of $[\text{Ca}^{++}]_i$ under isovolumic contraction, may be due to mechanical stretch or another unknown mechanism. This could be responsible for APD shortening and the downward shift of the electrical restitution curves. It was therefore decided to investigate AP behaviour and the restitution curves at low Ca^{++} , which would reduce, if not abolish the changes induced by isovolumic contraction. Elevating $[\text{Ca}^{++}]$ in the Krebs to 3.6mM was also attempted, but the coronary flow under such conditions was halved. This could result in hypoxia, furthermore the AP was not steady at all and gradually shortened over long periods of time.

10.2 Results

Lowering the extracellular Ca^{++} concentration had the expected effect of reducing the ventricular pressure and prolonging the APD. Fig. 10.1 shows the changes in ventricular pressure 'top trace' and in action potential duration 'lower trace' when switching from 1.8mM Ca^{++} control to 0.5mM Ca^{++} under free loaded contraction. The ventricular pressure gradually decreased and settled at a new steady state which was much lower than the control steady state and the action potential of both areas prolonged. The steady state AP under low Ca^{++} and under control Ca^{++} of this preparation are superimposed in fig. 10.1 (inset). Table 10.1 has the steady state ventricular pressure under both mechanical conditions at 1.8mM and 0.5mM Ca^{++} .



<u>At 1.8mM Ca⁺⁺</u>		<u>At 0.5mM Ca⁺⁺</u>	
<u>Free Loaded</u>	<u>Isovolumic</u>	<u>Free Loaded</u>	<u>Isovolumic</u>
24.9 ± 3.7(7)	38.3 ± 8(7)	13.65 ± 2.8(7)	28.8 ± 7(7)

TABLE 10. 1 Ventricular pressure (mm Hg) at different [Ca⁺⁺]_o

10.2.1 Area 1 Restitution Curves at Low Ca⁺⁺

Superimposed AP's from area 1 at different points of the restitution curves at low (0.5mM) Ca⁺⁺ under both mechanical conditions are shown in fig. 10.2 (top traces).

The figure shows the similarity in the extent of AP shortening at 300ms TPI between free loaded and isovolumic contraction, and also the similarity of the steady state APD under the two mechanical conditions at low Ca⁺⁺. Fig. 10.3 shows the restitution curves of a single experiment obtained from area 1 at low Ca⁺⁺. The two curves virtually superimpose.

The curves in fig. 10.4 are controls from 4 preparations showing similar features as already described in chapter 8, whereas those in fig. 10.5 are the curves at low Ca⁺⁺ constructed from pooled data of 10 preparations. When inspecting fig. 10.5 the following can be deduced.

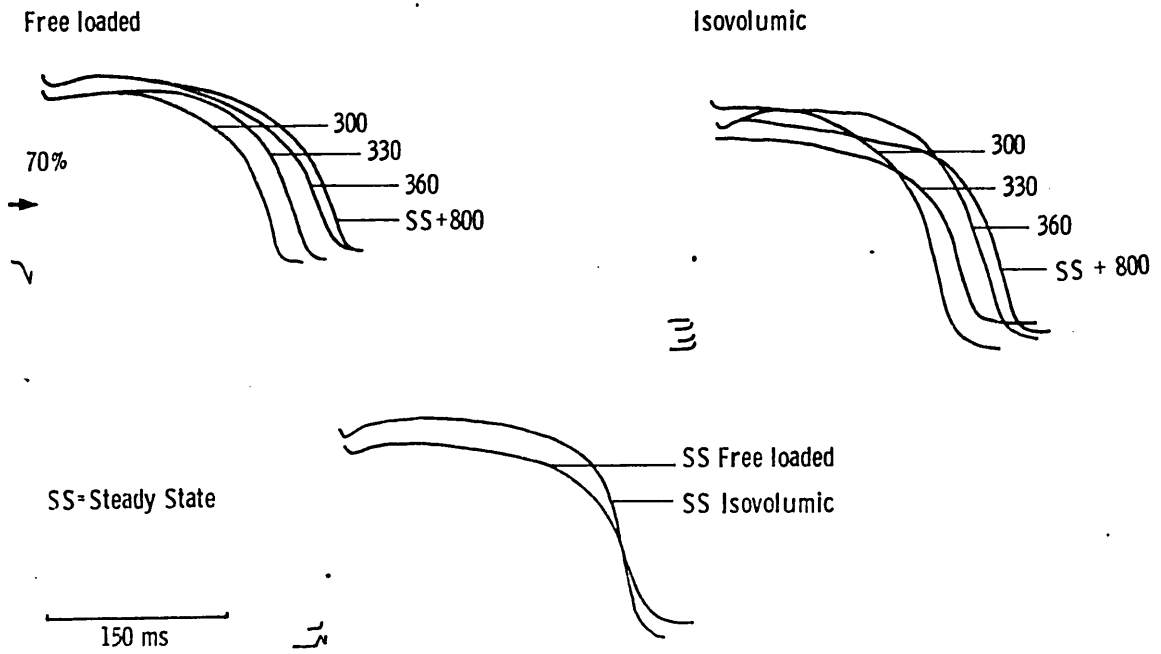


Fig. 10.2 . Superimposed AP's at different points of the restitution curves of area 1 (top trace) at low Ca^{++} , and the steady state AP's are superimposed in the lower panel.

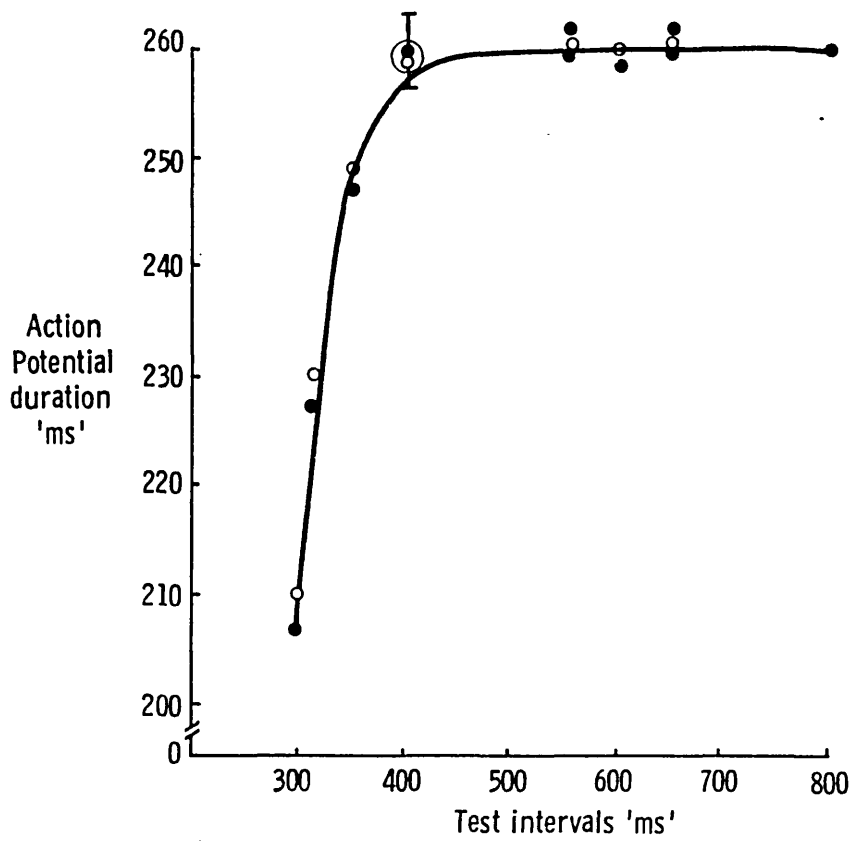
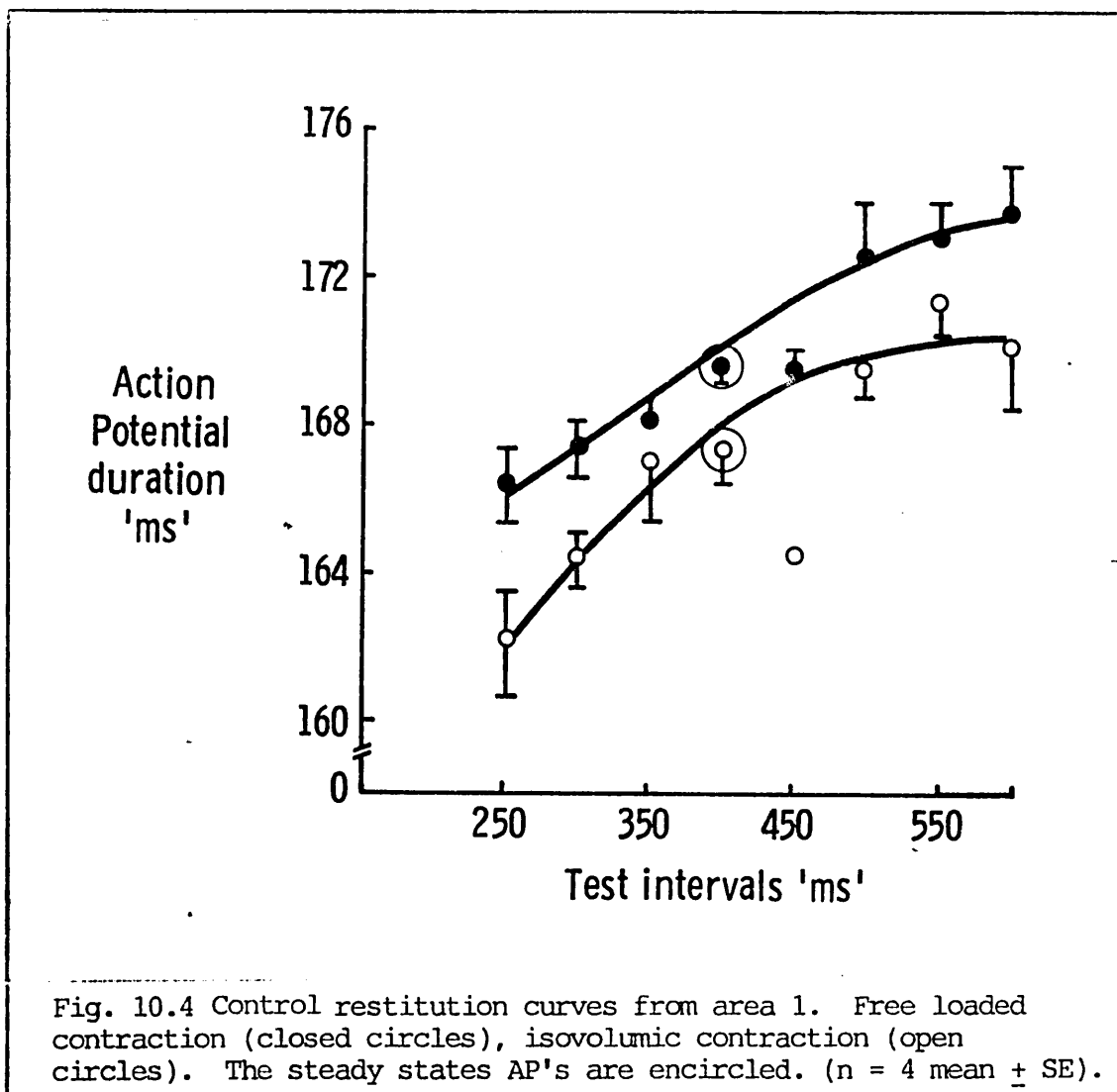
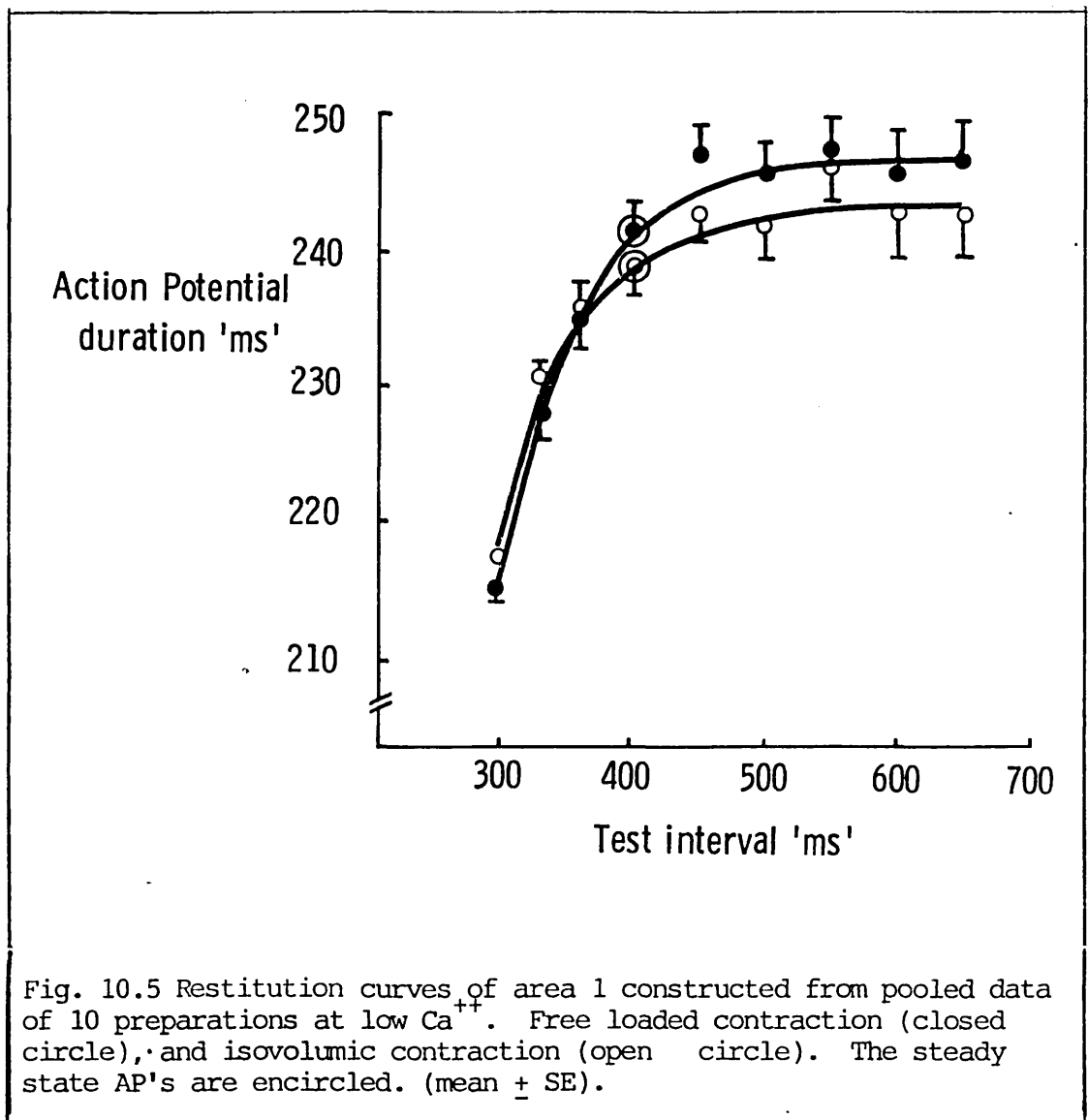


Fig. 10.3 Electrical restitution curves of area 1 from a single preparation at low Ca^{++} . Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's (mean $\pm$ SD) are encircled.

1. There was no significant difference between the steady states and they fell close to the plateaux of the corresponding curves, suggesting that the steady state intervals were long enough for complete membrane recovery under both mechanical conditions.
2. The magnitude of free loaded and isovolumic restitution curves were close, although the magnitude of the isovolumic curve seemed to be slightly smaller, 39ms as compared to 46ms for the free loaded, but this was not significant (see 3 below).



3. The plateau of the isovolumic contraction curve was slightly lower than that of free loaded curve, but this was due to the fact that in two preparations the plateau of the curve had large downward shifts. The overall difference shown in fig. 10.5 was not significant (Wilcoxon-matched pair test). In one experiment only, the whole curve shifted upwards about 10ms.



4. The recovery indices at low Ca^{++} were 43ms for free loaded contraction and 30ms for isovolumic contraction as compared to 520ms and 343ms for free loaded and isovolumic contraction respectively under control, 1.8mM Ca^{++} . Note that at low Ca^{++} the restitution curves under both contraction conditions were markedly accelerated compared to control Ca^{++} .

10.2.2. Area 6 Restitution Curves at low Ca^{++}

Superimposed AP's at different points of the restitution curves under both mechanical conditions are shown in fig. 10.6 top panels, whereas in the lower panel are steady state AP's showing the similarity in APD between the free loaded and isovolumic at low Ca^{++} .

The restitution curves in fig. 10.7 are of a single preparation, from area 6 at low Ca^{++} showing the similarity between the two curves, namely the free loaded contraction curve (closed circles) and the isovolumic contraction curve (open circles).

Figs. 10.8 and 10.9 show the restitution curves of area 6 under free loaded and isovolumic contraction at control (1.8mM) and low (0.5mM) Ca^{++} respectively.

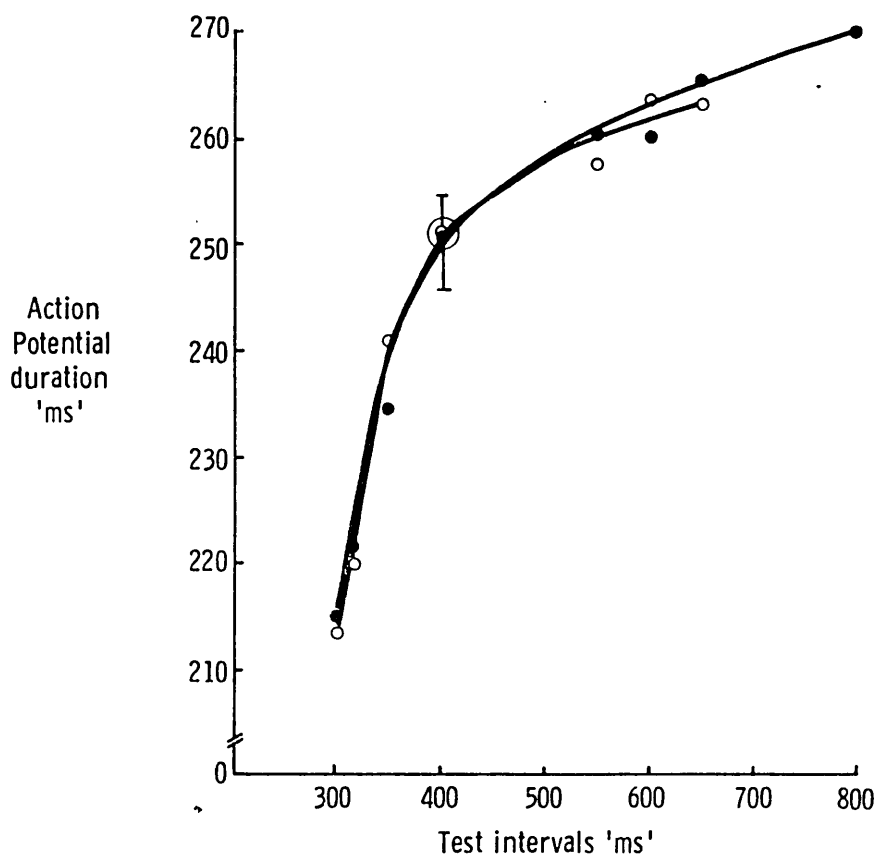


Fig. 10.7 Restitution curves of a single preparation from area 6 at low Ca^{++} (from the same preparation as that in fig. 10.3 for area 1). Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled; (mean $\pm$ SD).

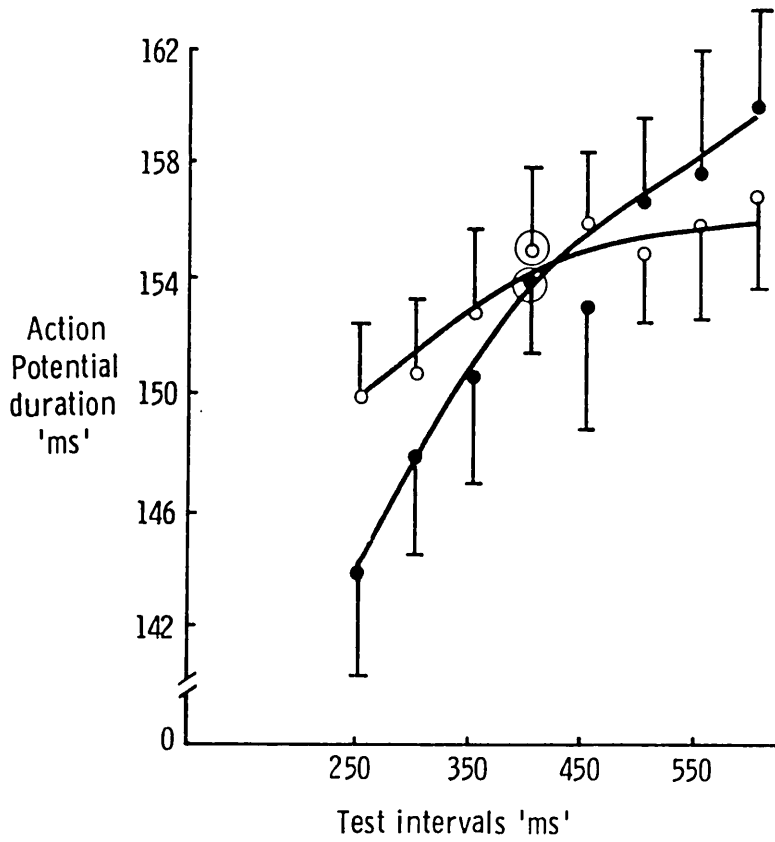


Fig. 10.8 Control restitution curves from area 6. Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled. ($n = 5$ mean $\pm$ SE).

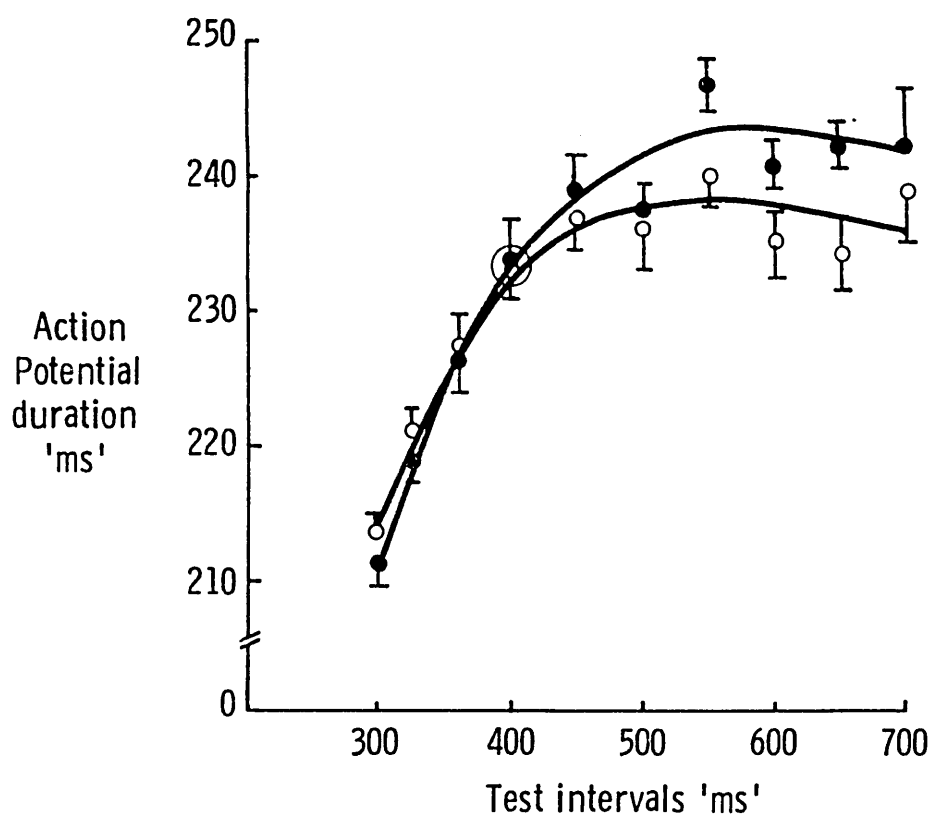


Fig. 10.9 Restitution curves of area 6 at low Ca^{++} constructed from pooled data of 10 preparations (the same preparations as those in fig. 10.5). Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled (mean $\pm$ SE).

The control curves have already been described in chapter 8, but the curves at low Ca^{++} have the following features.

1. For free loaded and isovolumic contraction respectively, the magnitudes of the curves at low Ca^{++} are 30ms and 25ms as compared to 16 and 6ms under control Ca^{++} .

2. The two steady state APD's are virtually identical under both mechanical conditions.

3. The recovery indices at low Ca^{++} are 77ms for free loaded and 58ms for isovolumic contraction whereas at control Ca^{++} they were 164ms for free loaded and 247 for isovolumic contraction. While low Ca accelerated both recovery indices the results suggest that isovolumic contraction compared with free loaded contraction seemed to accelerate the recovery index of restitution of area 6 at low Ca^{++} rather than slowing it as under control Ca^{++} .

4. At low Ca^{++} , the steady state APD's were very close to the plateaux under both mechanical condition of contraction, suggesting that the intervals between steady state beats were long enough for near complete membrane recovery. Although the plateau under isovolumic contraction seemed to shift down, a Wilcoxon-matched pair test showed this shift to be insignificant.

Thus at low Ca^{++} the free loaded and isovolumic contraction restitution curves, as in area 1, are very close and the difference between them seen at control Ca^{++} is almost absent.

In one preparation only, the isovolumic contraction curve was shifted upwards compared to free loaded contraction. In the same preparation the isovolumic contraction of area 1 was also shifted upwards which suggests a possible experimental error.

Summary:-

Low Ca^{++} reduced the differences in restitution curves and in APD's between free loaded and isovolumic contraction of both areas. The effect of isovolumic contraction in slowing the recovery index of restitution curve of area 6, when compared with free loaded contraction under $1.8\text{mM } \text{Ca}^{++}$ was not only reduced but reversed, i.e. isovolumic contraction seemed to slightly accelerate the recovery index.

CHAPTER 11

Electrical restitution curves at low temperature, 24°C

11.1 Introduction

11.2 Results

11.2.1 Area 1 restitution curves at 24°C

11.2.2 Area 6 restitution curves at 24°C

Chapter 11

Electrical Restitution Curves at Low Temperature 24°C

11.1 Introduction

Lowering the temperature has been demonstrated to prolong the APD in various cardiac preparations like sheep purkinje and ventricular, calf purkinje fibre; guinea pig atrial muscle, cat papillary and rabbit ventricular muscles. (Coraboeuf and Weidmann 1954, Thyrum 1974, Cohen et al 1976, Boyett and Jewell 1978, Benndorf and Nilius 1982). Lowering the temperature has also been shown to slow the kinetics of the slow Ca^{++} inward current and the K^+ outward repolarizing current, thus it would be expected that the temperature would slow the restitution of APD. However, more importantly, for the purpose of this study, lowering the temperature is expected to reduce the activity of the $\text{Na}^+ - \text{K}^+$ pump. From the results of the previous chapters it was thought the effects of isovolumic contraction might be due to two separate mechanisms - one responsible for the downward shift and the other one is responsible for the changes in the restitution curve time course as indicated by the recovery index. The fact that the restitution curves were accelerated when $[\text{K}^+]$ was elevated in the perfusing medium (chapter 9) and the possibility that the Na^+/K^+ pump activity was increased under such conditions (Ellis 1977, Ruiz-Ceretti et al 1981, Martin and Morad 1982) suggested that the Na^+/K^+ pump may play a role in determining the time course of AP recovery. I therefore decided to construct the electrical restitution curves under both conditions of contraction at low temperature under which the pump is expected to be reduced if not inhibited.

11.2 Results

11.2.1 Area 1 Restitution Curves at 24°C

The restitution curves of a single experiment are plotted in fig. 11.1 showing the similarity in the rising phase of the curves between free loaded contraction and isovolumic contraction but the plateau under isovolumic contraction was shifted downwards.

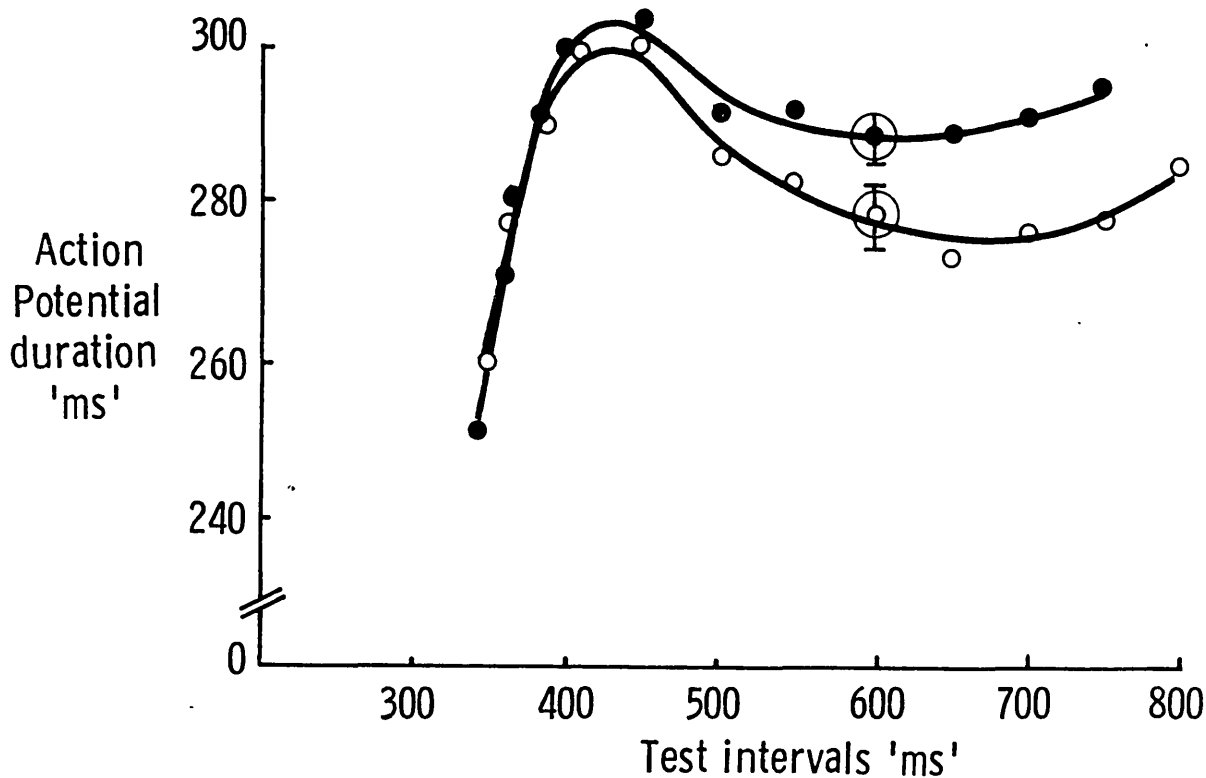
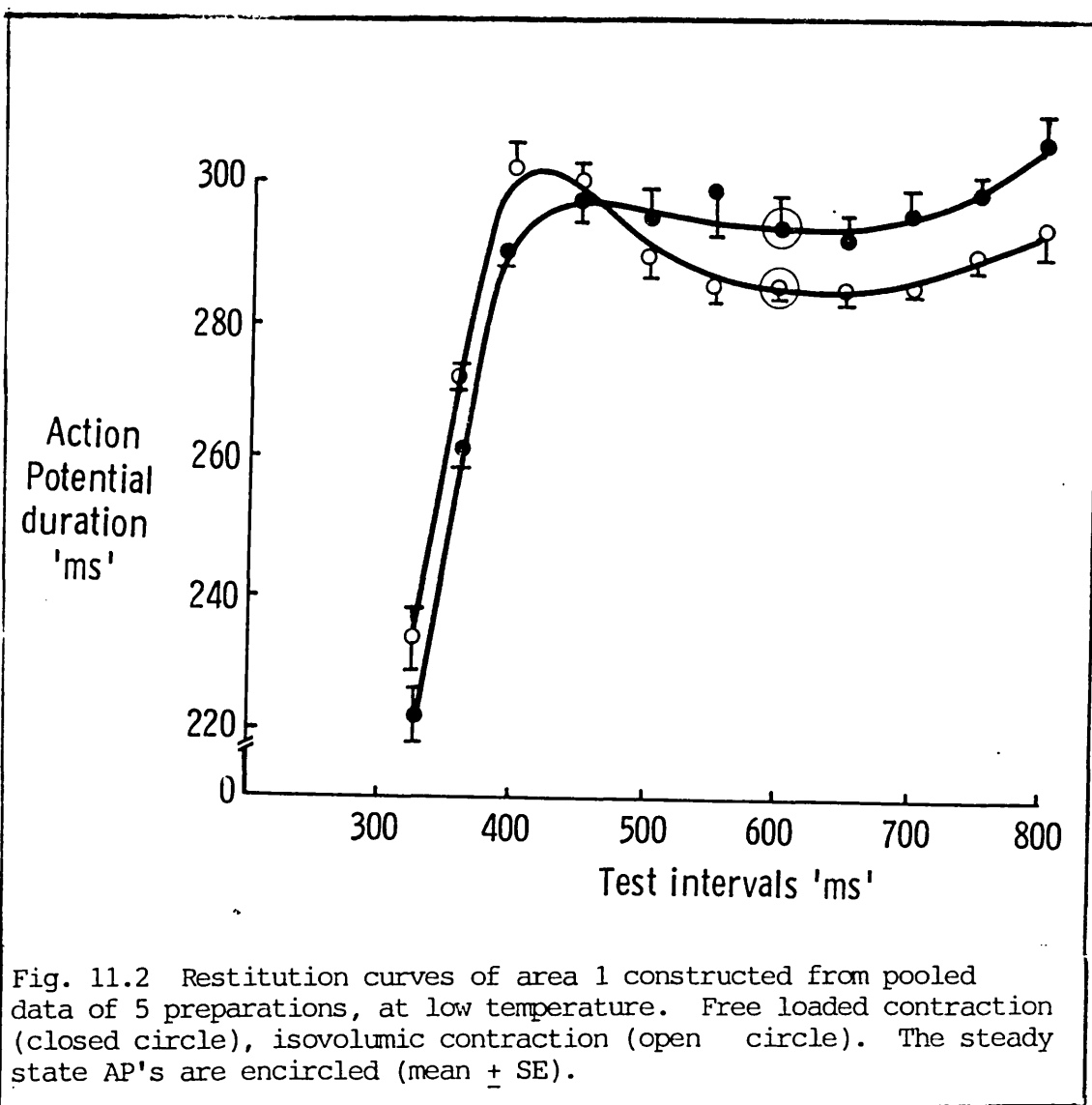


Fig. 11.1 Electrical restitution curves of area 1 at low temperature. Free loaded contraction (closed circle), isovolumic contraction (open circle). The steady AP's (mean $\pm$ SD) are encircled.

The pooled data of 5 preparations, at steady state pacing rate of 100/min (i.e. 600ms TPI) are shown in fig. 11.2.



The following features can be seen in fig. 11.2.

1. The rising phase of both isovolumic and free loaded contraction were close and had recovery indices of 35ms and 33ms respectively. Compared to the restitution curves at 34°C (the control curves in the previous chapters) the recovery index seemed to be accelerated at low temperatures.
2. The rising phase of the isovolumic restitution curve was shifted upwards specially at 360ms and 400ms TPI. This shift was due to a shift in two preparations only but the shift was large enough to reflect a shift in the pooled data.
3. The steady state APD was significantly shifted downward under isovolumic contraction in three preparations.
4. Under both mechanical conditions the steady state APD fell on the plateaux of the curves indicating complete membrane recovery between steady state responses.
5. The AP under isovolumic contraction, even after long enough TPI, remained shorter than that under free loaded contraction thus showing that the mechanically induced downward shift, described in the previous chapters, was still present at low temperature. However in two preparations (the same preparation in which the end of the rising phase (400ms) was shifted upwards under isovolumic contraction) there was no difference between the plateaux under both condition of contraction.

The steady state APD under free loaded contraction in the middle of one of these experiments gradually got shorter whereas under isovolumic contraction it gradually got longer. The end result was no difference between the plateaux of the two mechanical condition of contractions.

11.2.2 Area 6 Restitution Curves at 24°C

Fig. 11.3 show the restitution curves under free loaded and isovolumic contraction of area 6 obtained from a single preparation (the same as that shown in fig. 11.1 for area 1). It shows the rising phases to be very similar and superimposed, while the plateau of curve under isovolumic contraction was shifted downward.

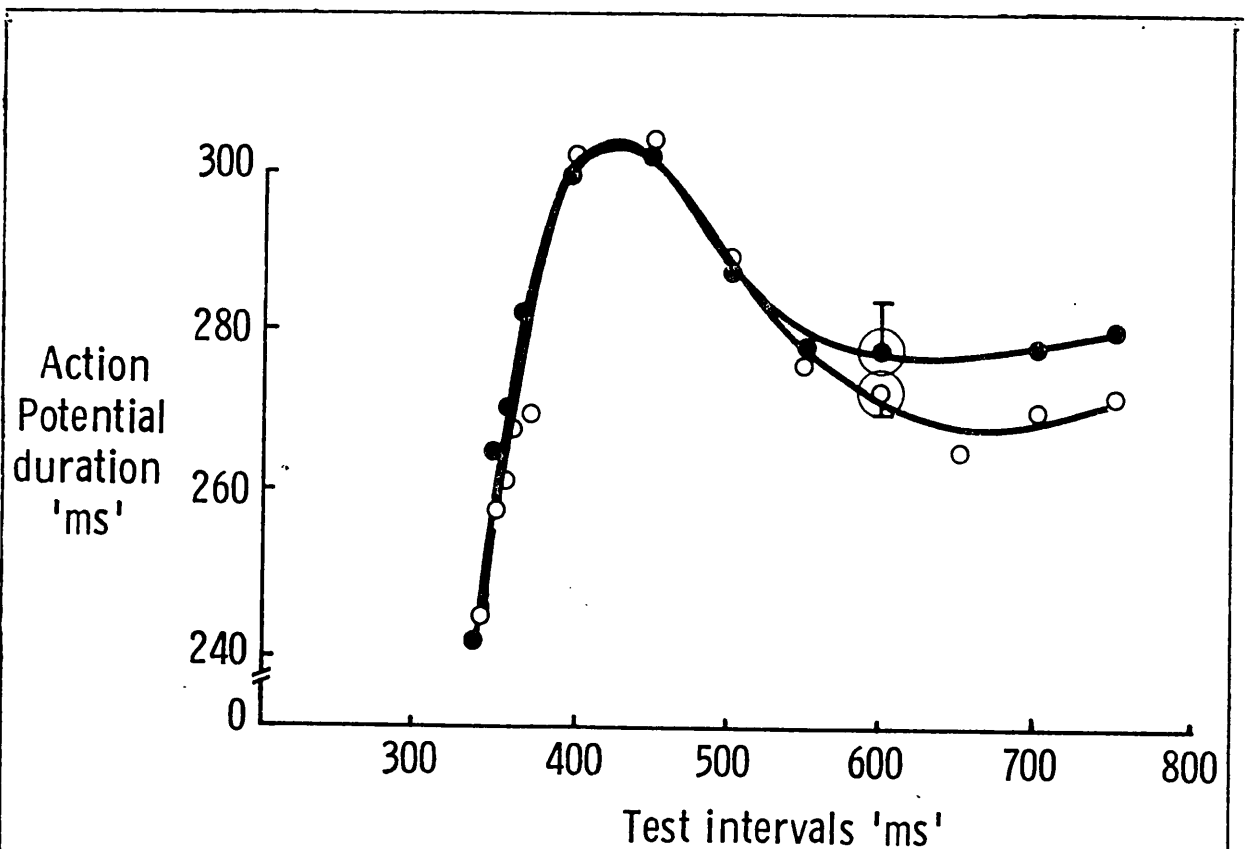


Fig. 11.3 Electrical restitution curves of area 6 at low temperature. Free loaded contraction (closed circle) isovolumic contraction (open circle). The steady state AP's (mean $\pm$ SD) are encircled.

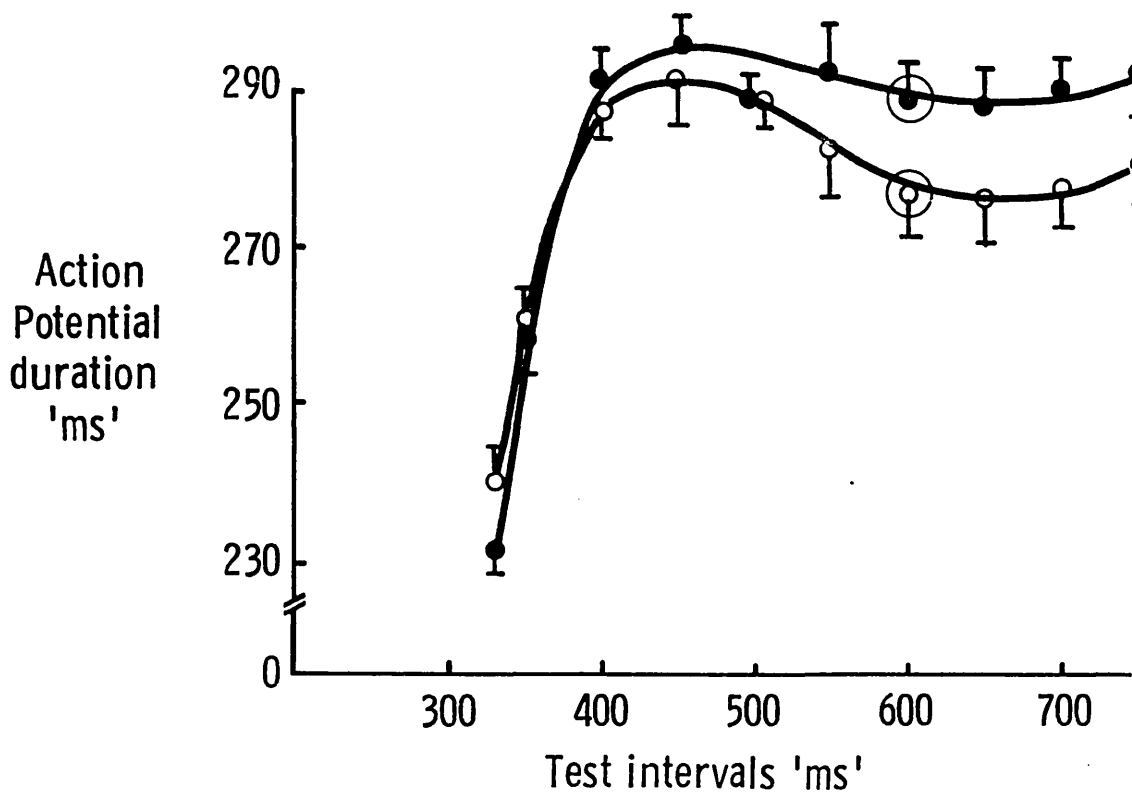


Fig. 11.4 Restitution Curves of area 6 constructed from pooled data of 5 preparations (the same preparations as those shown in fig.11.2 for area 1) at low temperature. Free loaded contraction (closed circles), isovolumic contraction (open circles). The steady state AP's are encircled. (mean $\pm$ SE).

The following features can be seen when inspecting fig. 11.4.

1. The recovery indices were very close, 77ms and 65 for free loaded and isovolumic contraction respectively.

This is shown in the figure by the similarity and the close proximity of the rising phases of the two curves. Note that the recovery of APD in this area is also faster at low temperature compared to at 34°C (as shown by the control curves in the previous chapters), similar to that in area 1.

2. The steady state APD under both condition of contraction fell on the plateaux , as for area 1, suggesting complete membrane recovery between steady state beats.

3. The steady state APD was greatly shifted downward under isovolumic contraction in three preparations only. In two more preparations there was no difference between free loaded and isovolumic contraction, in fact in the same preparations there was no difference between free loaded and isovolumic contraction of area 1 too, which could suggest an experimental error.

3. It is clear that at long TPI the APD under isovolumic contraction remained shorter than that under free loaded contraction. Hence the plateau part of the restitution curve under isovolumic contraction was shifted downward (i.e. mechanically dependent downward shift).

Summary:-

Low temperature accelerated the time course of the restitution curves of both areas. The effect of changing the mechanics of contraction to isovolumic at low temperature only shifted the plateau of the

restitution curves of both areas down, but there seemed to be no effect on the recovery indices, hence on the time course of recovery.

CHAPTER 12

Some correlative studies. The effect of changing the mechanics of contraction on the T-wave of ecg

12.1 Introduction

12.2 Results

12.2.1 The effects of changing the mechanics on T-wave and the Q-T interval

12.2.2 The behaviour of the T-wave when increasing the heart rate under free loaded and isovolumic contraction

Chapter 12

Some Correlative Studies. The effect of changing the mechanics of contraction on the T-wave of the Electrocardiogram (ecg)

12.1 Introduction

The T-wave of the mammalian ecg is almost synchronous with the repolarization phase of the ventricular action potential and is derived from differences in action potential durations in different regions of ventricle. But there is still controversy in defining the normal distribution of ventricular repolarization and their relation to body surface T-wave.

I have shown above that the mechanics of contraction can effect the action potential duration and there seems to be regional differences in this effect. I, therefore, expect that changing the mechanics of contraction would have an effect of some kind on the T-wave of the ecg. In consequence I have carried out one or two confirmatory studies on the effect of changing the mode of contraction on the Q-T interval, a reflection of APD, and the T-wave, a reflection of regional differences in repolarization.

The ecg was recorded by placing two cotton wool wick electrodes on the epicardium of the rabbit Langendorff preparation and the input was fed into a high gain AC amplifier. Later two silver electrodes were hooked in place, and the ecg was recorded after the resulting damage (raised S-T segment) was healed, (reduction of, or flat S-T segment).

12.2 Results

The effect of mechanics of contraction on the T-wave is divided into two sections.

1. Describes the changes in the T-wave and the Q-T interval when changing the mechanics of contraction from free loaded to isovolumic contraction under normal, 8mM K^+ and 0.5mM Ca^{++} Krebs and also at low temperature (24°C) perfusion. There was no change in rate of stimulation.
2. Describes the differences in T-wave behaviour between free loaded and isovolumic contraction when changing the rate of beating from 150 to 240/min under normal, 8mM K^+ and 0.5mM Ca^{++} Krebs and at low temperature where the rate change was from 100 to 182/min.

12.2.1 The effects of changing the mechanics on the T-Wave and the Q-T interval

a. With control Krebs

The T-wave in fig. 12.1 was gradually depressed when the mechanics of contraction was changed to isovolumic, and reached steady state within about 2-3 mins. This was observed in four other preparations. In a fifth preparation the T-wave amplitude got bigger under isovolumic contraction. These results are in keeping with the previous suggestion that isovolumic contraction has a regional difference in its effect on the duration of the action potential. This is shown when comparing the steady state under free loaded and isovolumic contraction of area 1 and area 6 in fig. 8.3. In area 1 the steady state duration was shortened under isovolumic contraction in most preparations, whereas in area 6 it was generally not different from free loaded, with only a few exceptions.

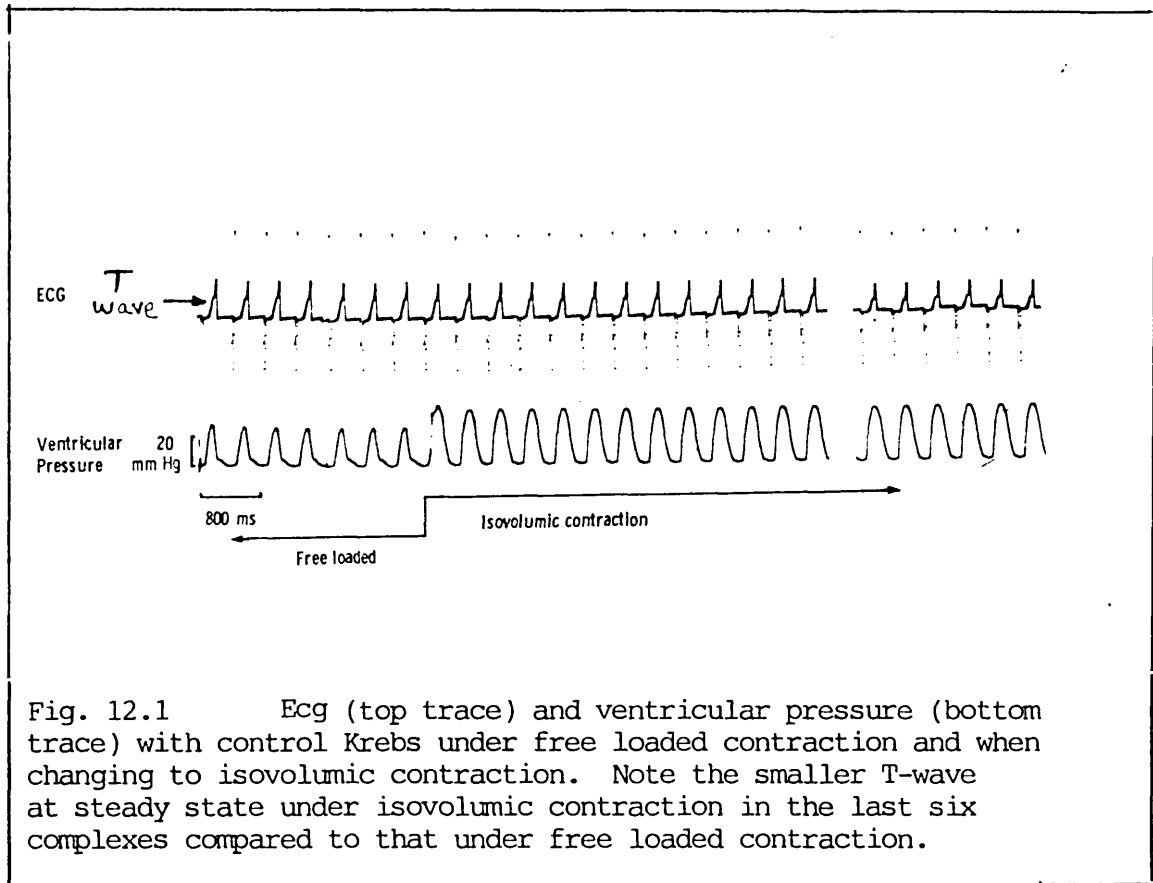


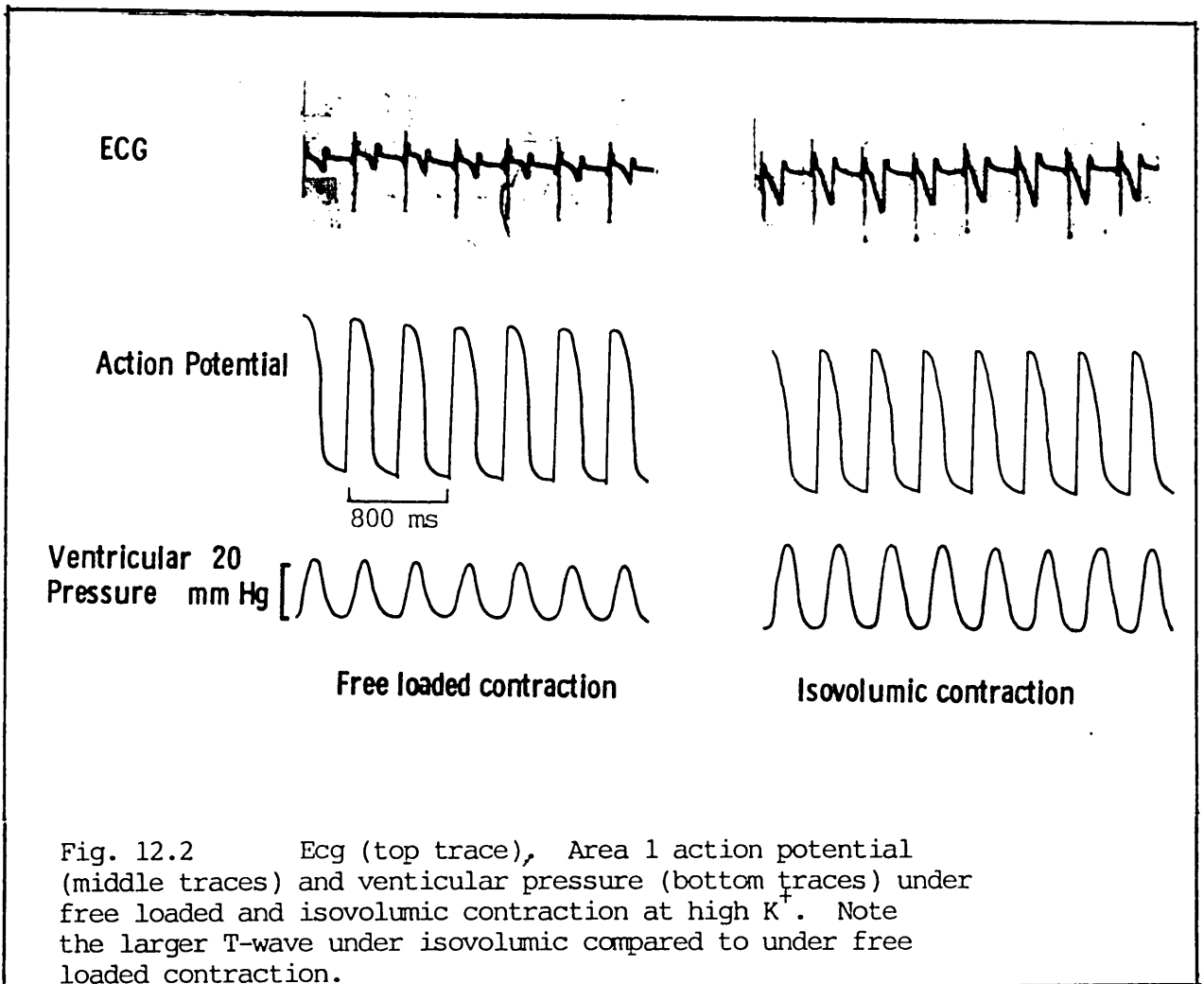
Fig. 12.1 Ecg (top trace) and ventricular pressure (bottom trace) with control Krebs under free loaded contraction and when changing to isovolumic contraction. Note the smaller T-wave at steady state under isovolumic contraction in the last six complexes compared to that under free loaded contraction.

The Q-T interval is expected to be shorter under isovolumic contraction if APD is shorter. Unfortunately, in the experiments from which ecg was recorded at the same time as the AP, the shortening in APD under isovolumic contraction was not large enough to reflect clearly shortening of the Q-T but at high K^+ perfusion the shortening of Q-T interval of the ecg and the AP's of both areas can be seen in (fig. 12.6).

b. With high K^+

Fig. 12.2 shows the difference in the T-wave (top trace) under free loaded and isovolumic contraction with 8mM K^+ . The middle traces are the AP's (from area 1) and the bottom trace is the ventricular pressure. From the restitution curve studies, it was shown in fig. 9.6 for area 1 and in fig. 9.9 for area 6 that under isovolumic contraction the steady state APD's were shifted in both areas down more when compared with free loaded contraction under 8mM K^+ than when perfusing with control K^+ . This is supported by the fact that there was a large change in the T-wave when changing the mechanics of contraction from free loaded to isovolumic contraction and the T-wave actually increased in amplitude when changing to isovolumic contraction under high K^+ rather than being depressed as in fig. 12.1 with control (4mM K^+). Although the two figures, that is fig. 12.1 and 12.2 were obtained from different preparations this type of change in the T-wave under isovolumic contraction was observed in the other two preparations from which the ecg was recorded. This effect was also reversible.

The larger shortening in APD when changing to isovolumic contraction is supported by the large decrease in the Q-T interval as shown in fig. 12.6.



c. With low Ca^{++}

I have shown in figs. 10.5 and 10.9 that the isovolumic mode of contraction at low Ca^{++} had little effect on the steady state

APD of both areas. The support for this from the T-wave studies with low Ca^{++} is shown in fig. 12.3. This figure shows (from top downward) AP from area 1, ecg, ventricular pressure, and AP, from area 6. The first two beats on the left hand side are control beats under free loaded contraction. It was switched to isovolumic contraction at the arrow.

The figure shows no difference in either the magnitude of the T-wave or the Q-T interval between the two mechanical conditions. In three more preparations isovolumic contraction had no effect on the T-wave or the Q-T interval at low Ca^{++} perfusion. In only one further preparation the T-wave inverted from slightly positive under free loaded contraction to slightly negative under isovolumic contraction.

d. At low temperature

Changing the mechanics from free loaded to isovolumic contraction at low temperature shortened the Q-T intervals and the T-wave seemed to change little as shown in fig. 12.4, occasionally the T-wave was depressed. The change in V Q-T interval is in keeping with the shortening of APD under isovolumic contraction compared to free loaded contraction in both areas. It is also in keeping with the similar behaviour of areas when changing from free loaded to isovolumic contraction as shown in figs. 11.2 and 11.4.

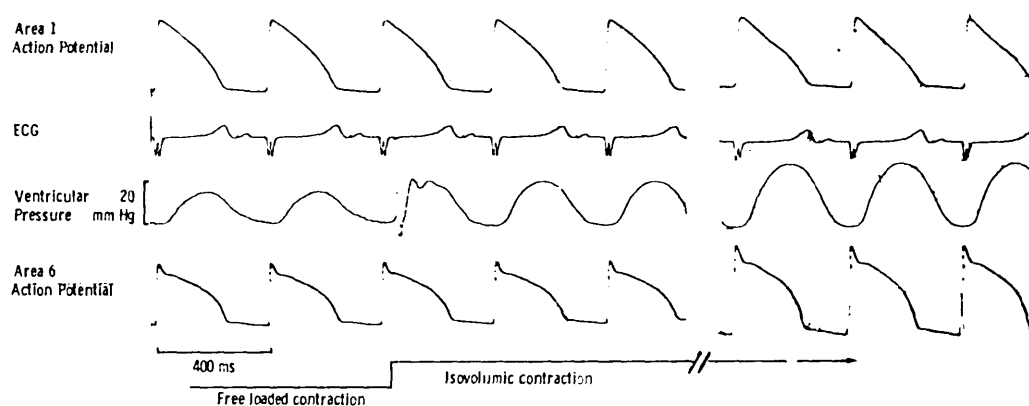


Fig. 12.3 From top down, area 1 AP, ecg, ventricular pressure and area 6 AP. The last three complexes on the right hand side are at steady state condition under isovolumic contraction (under low Ca^{++} perfusion).

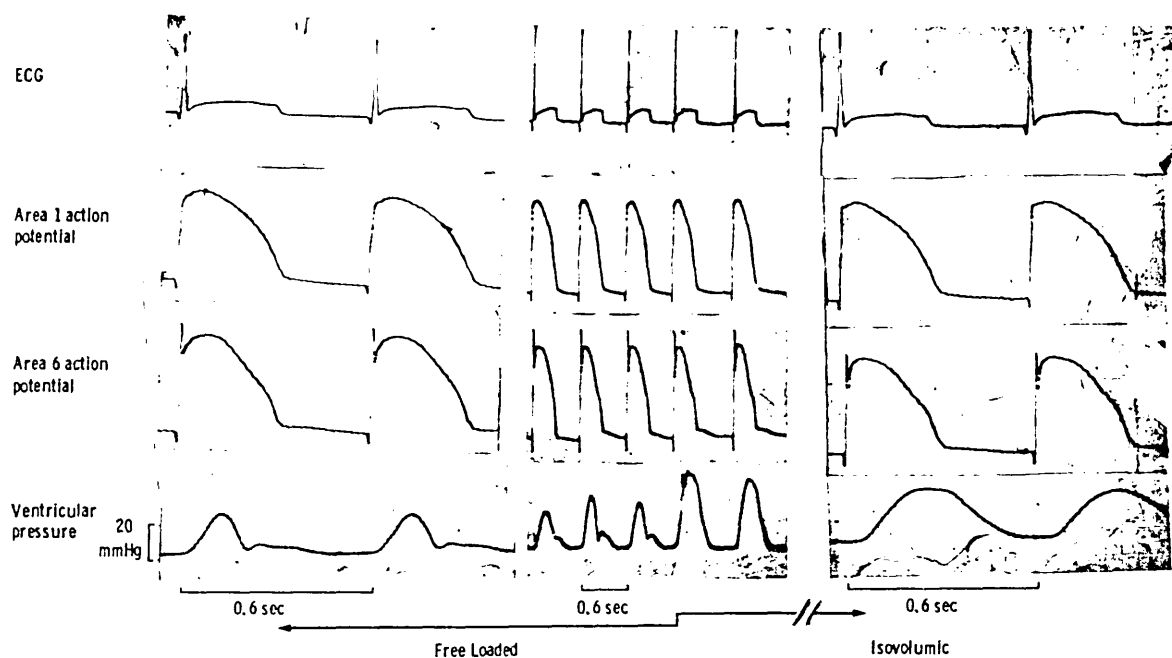


Fig. 12.4 Changes in ecg, AP's of area 1 and 6 and in ventricular pressure at low temperature when changing from free loaded to isovolumic contraction. The far right panel containing two complex of each of the above parameter represent the steady state under isovolumic contraction.

12.2.2. The behaviour of the T-wave when increasing the heart rate under free loaded and isovolumic contraction

There were regional differences in the AP behaviour observed when examining the first beat of a rate change during isovolumic compared with free loaded contraction (fig. 7.9). Therefore, one can predict the T-wave of the first beat when increasing the rate should also behave qualitatively differently under isovolumic compared to under free loaded contraction.

a. With control Krebs

There were clear differences in the behaviour of the T-wave when increasing the rate of pacing in one step under free loaded contraction compared with that under isovolumic contraction.

Fig., 12.5 shows records of an ecg (top trace) and ventricular pressure (bottom trace) under free loaded and under isovolumic contraction. The rate was increased in one step from 150 to 240/min. The first beat under both conditions of contraction is indicated in the figure by an arrow.

The T-wave amplitude of the first beat as % of the steady state T-wave amplitude is 0.56 under isovolumic contraction and 0.72 under free loaded contraction. This is in keeping with the previous observations that AP shortening when increasing the rate is different under the two mechanical conditions of contraction, and that there are regional differences in the behaviour of the AP.

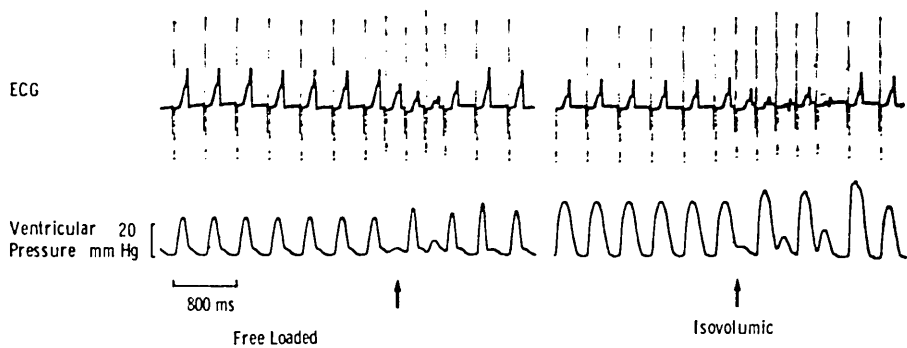


Fig. 12.5 The change in the T-wave of the first beat (indicated by the arrow) when increasing the rate in one step from 150 to 240/min under free loaded and isovolumic contraction, under control K perfusion.

In the same preparation two more attempts under each of the two mechanical conditions gave similar results. This was also the case in four more preparations including that in which the T-wave got bigger under isovolumic contraction. The results in fig. 12.5 and those in fig. 12.1 were obtained from the same preparation.

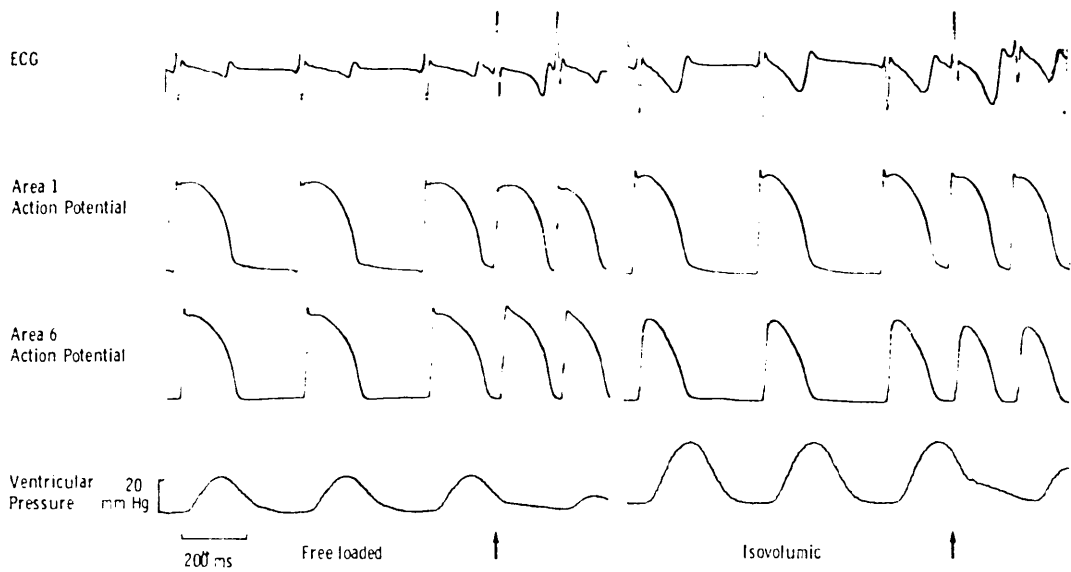


Fig. 12.6 The change in the T-wave (top traces), areas 1 and 6 AP's (second and third traces down respectively) and ventricular pressure (bottom traces) when increasing the rate in one step from 150 to 270/min (at the arrow) under free loaded and isovolumic contraction at high K^+ .

b. With 8mM K^+

The restitution curve studies showed that the differences in AP shortening, when increasing the rate between isovolumic and free loaded contraction to be greater with high K^+ than the differences with control K^+ . These results are supported by the large differences in T-wave behaviour shown in fig. 12.6.

In the figure the differences between the steady state and first T-wave under free loaded contraction is greater than the differences between the steady and the first T-wave seen under isovolumic contraction. Three more attempts under free and two more attempts under isovolumic in the same preparation showed similar differences between free and isovolumic.

Similar large differences in the behaviour of the T-wave with high K^+ between free loaded and isovolumic contraction was seen in another preparation in which I carried out rate changes while recording the ecg. Fig. 12.6 also shows shorter steady state Q-T interval under isovolumic compared to under free loaded contraction supporting the finding that APD is shorter under isovolumic compared to free loaded contraction which can be seen in fig. 12.6 when comparing the AP of area 1 and 6 under the two mechanical conditions.

Note that there was some conduction change when increasing the rate of beating as shown by the changes in the QRS complex.

Despite this change the comparison between free loaded and isovolumic contraction as shown in fig. 12.6 is still valid because an almost identical change has taken place under both mechanical conditions.

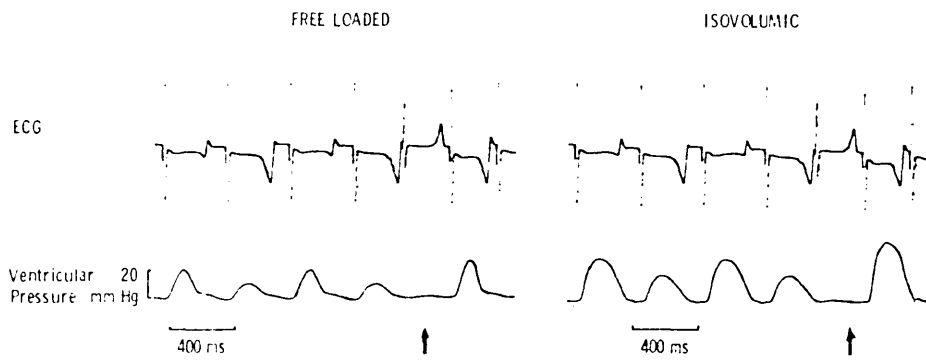


Fig. 12.7 The change in the T-wave (top trace) and ventricular pressure (bottom trace) when increasing the rate (at the arrow) under free loaded and isovolumic contraction at low Ca^{++} .

b. With 0.5mM Ca^{++}

The restitution curves with 0.5mM Ca^{++} showed no significant difference in the degree (extent) of AP shortening between free loaded and isovolumic contraction in both areas when the rate of beating was increased from steady state of 150 to 200/min. Fig. 12.7 shows the T-wave of the first beat after increasing the rate under free loaded and under isovolumic contraction. Note the alternans under both conditions of contraction, the corresponding T-waves under both conditions are almost identical; similar biphasic waves alternating with large negative waves. The increase in rate was carried out after the large negative T-wave under both free loaded and isovolumic contraction for valid comparison. It is evident in the figure that the changes in T-wave were identical under both mechanical conditions of contraction. The T-wave under both contractions of fig. 12.7 inverted and have the same amplitude.

An increase in the rate after the small biphasic T-wave also gave identical results under free and isovolumic contraction, but this was not followed by inversion, instead it got smaller in amplitude. In the same preparation four more attempts under each mechanical condition gave identical results including those carried out after a small T-wave. No differences in T-wave behaviour between free and isovolumic contraction when increasing the rate was seen in three more preparations.

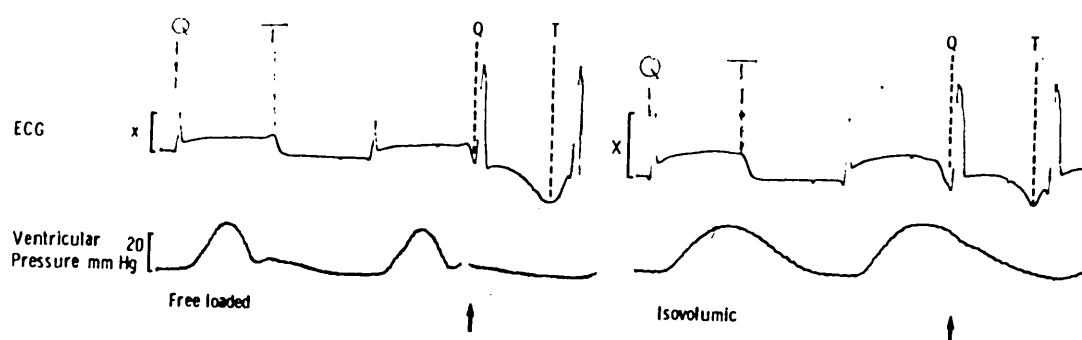


Fig. 12.8 The change in the T-wave (top trace) and ventricular pressure (bottom trace) when increasing the rate (at the arrows) under free loaded and isovolumic contraction at low temperature.

In one further experiment there was a small difference in the same preparation as described in 12.2.1 (c). The T-wave inverted under isovolumic contraction. Also notice the similarity of the corresponding steady state Q-T interval under both conditions of contraction, suggesting no change in APD on changing from free loaded to isovolumic contraction.

d. At low temperature

The restitution curves at low temperature showed that both areas shortened more under free loaded contraction when the rate was increased from 100 to 180/min. The changes in the T-wave of the first beat were also different under the two mechanical conditions. Fig. 12.8 shows this difference in behaviour of the T-wave under free loaded and under isovolumic contraction at 24°C. Note that the gain was doubled on the ecg under isovolumic contraction. T-wave of the first beat after increasing the rate was wide and large under free loaded contraction. It was sharp and small under isovolumic contraction. The figure also shows shorter steady state Q-T interval under isovolumic compared to under free loaded contraction supporting the shortening in APD observed when changing to isovolumic contraction as shown by the downward shift of the restitution curves (chapter 11). Two more rate changes under each mechanical condition in the same preparation gave similar results.

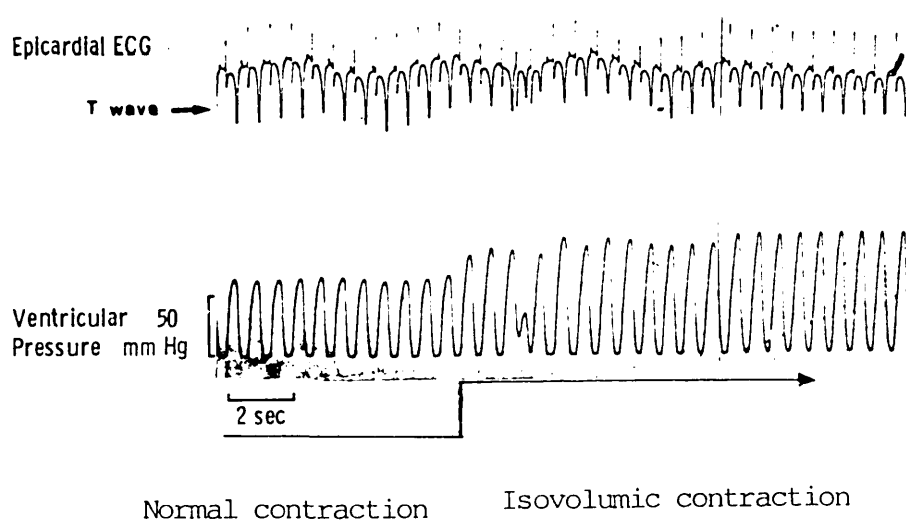


Fig. 12.9 The epicardial ecg from insitu pig heart (top trace) and ventricular pressure (bottom trace) when changing from normal to isovolumic contraction. The T-wave was gradually depressed. The last 9 complexes on the far right show the steady state under isovolumic contraction.

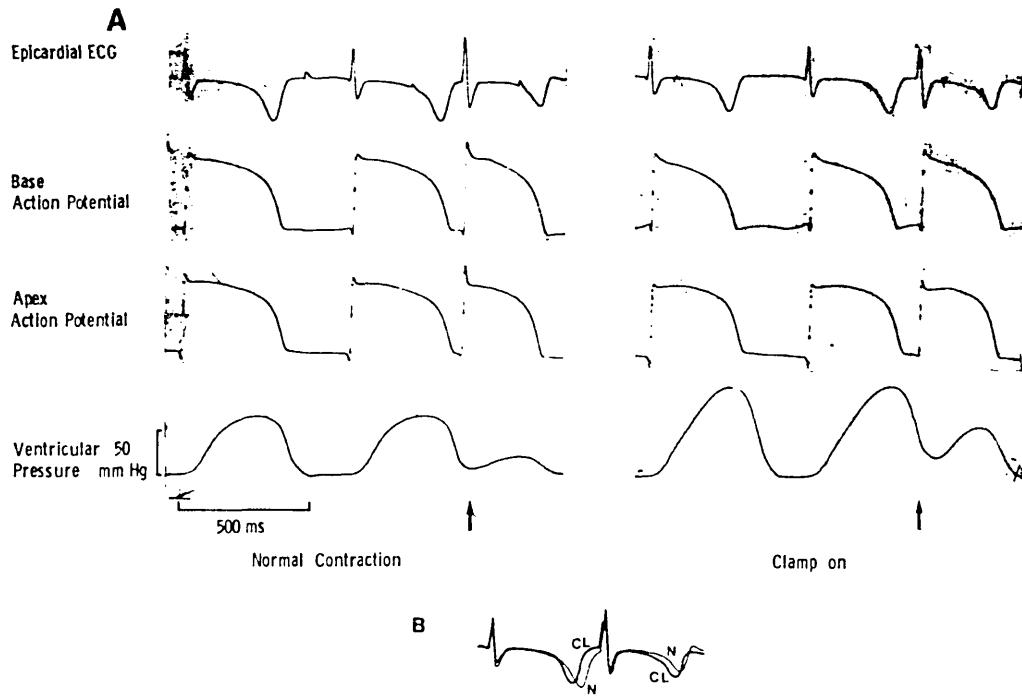


Fig. 12.10 A. From top, epicardial ecg, base AP, apex AP and ventricular pressure under normal and isovolumic contraction. The rate was changed from 96 to 142/min at the arrows.

B. Superimposed epicardial ecg of the first beat increasing the rate and the immediately preceding control under normal contraction (N) and isovolumic contraction (CL).

In the intact insitu pig heart the T-wave of the epicardial ecg was gradually depressed when clamping the aorta as shown in fig. 12.9. The T-wave also behaved differently when increasing the rate under normal contraction from that under isovolumic contraction fig. 12.10 a,b. The amplitude of the T-wave of the first beat was more depressed with respect to the steady state under normal compared to under isovolumic contraction. In addition the Q-T interval was shorter under isovolumic compared to under normal contraction as demonstrated by the superimposition of the ecg's in fig. 12.10b.

Summary

The Q-T interval shortened, in line with the shortening of APD shown in the previous chapter, when changing from free loaded to isovolumic contraction with control Krebs, high K^+ and at low temperature, but no change with low Ca perfusion. The regional differences in AP behaviour is supported by the differences in the T-wave behaviour under free loaded from that under isovolumic contraction when increasing the rate. Also the changes in the T-wave amplitude when changing to isovolumic contraction support the regional differences in AP changes on changing the mechanics of contraction.

Chapter 13 Discussion

- 13.1 Mechanically induced depolarization of the resting membrane potential.
- 13.2 Mechanical dependent shortening of the AP.
 - 13.2.1. Explanatory mechanisms.
 - 13.2.1.1 Extracellular $[K^+]$ and $Na^+ - K^+$ pump.
 - 13.2.1.2 Intracellular $[Ca^{++}]$.
 - A. Mechanical stretch under isovolumic contraction.
 - B. Effect of stretch on $[Ca^{++}]_i$.
 - C. Effects of increased $[Ca^{++}]_i$.
 - i) Increased Na^+/Ca^{++} and Na^+/K^+ .
 - ii) Increase in Ca^{++} dependent K^+ current.
 - iii) Reduced I_{si} .
 - 13.2.1.3 Increased Na^+_i under isovolumic contraction.
 - 13.2.1.4 Other mechanism
 - 1. Reduced I_{si}
 - 2. Hypoxia
 - 3. Ca^{++} inward current
- 13.3 Regional differences in the effects of mechanics on AP shortening in response to a change in rate.

- 13.3.1 Isolated rabbit
 - A. Area 1
 - B. Area 7
 - C. Area 6
- 13.3.2 Intact pig.
- 13.4 APD vs shortening
- 13.5 Electrical restitution curve
 - 13.5.1 Control conditions of perfusion.
 - 13.5.1.1 A. Isolated rabbit
 - i. Area 1
 - ii. Area 6
 - 13.5.1.2 B. Intact pig
 - i. Base
 - ii. Apex
 - 13.5.2 High K^+ perfusion.
 - i. Area 1
 - ii. Area 6
 - 13.5.3 Low Ca^{++}
 - i. Area 1
 - ii. Area 6
 - 13.5.4 Low temperature
 - Area 1
 - Area 6
- 13.6 T-wave of the ecg.

13.1 Mechanically dependent shortening of the action potential

Changing the mechanics of contraction to isovolumic contraction produced a reversible shortening of the steady state APD in all areas investigated except area 2 (table 7.1) and area 6 which was found later to be inconsistent in its behaviour. The change in APD was slow in the isolated rabbit but fast in the insitu pig heart which has long APD and a change was visible clearly in the first two beats (fig. 7.16). The shortening of the AP under isovolumic contraction is in agreement with results from various ventricular preparations. Stauch (1966) reported shorter AP in isovolumically contracting frog ventricle as compared to isotonic contraction. Lab (1968, 1978a, 1980) found similar results in frog ventricle and, more recently (1982) he reported shortening of the AP in the left ventricle of insitu pig heart on constricting the aorta. Kaufmann et al (1971) showed an immediate shortening of the AP when an isolated cat papillary muscle was made to contract isometrically. They also showed this shortening to be reversible when going back to isotonic contraction. Also quick release, near peak tension, of an isometrically contracting cat papillary muscle (Kaufmann et al 1971, Hennekes et al 1977) and isovolumically contracting frog ventricle (Lab 1980) prolonged APD. It has been also reported that stretch caused shortening of the APD in various preparations, in cat papillary muscle (Dudel and Trautwein 1954, quoted in Lab 1982), in mammalian papillary muscle (Lepeschkin 1976b, figure 3) and in frog ventricle (Lab 1978a, 1980). Several reports showed no change in APD as a consequence of stretch. Lab (1980), Hennekes et al (1977, 1981) found no effect in cat papillary muscle, but Gennser and Nilsson (1968) and Allen (1975) found inconsistent results in human papillary and cat papillary muscle respectively.

Although the above results showed inconsistencies in the effect of stretch, they showed an agreement in that the mode of contraction affects the APD. The available evidence therefore indicates that the mechanics of contraction can effect the AP as well as the resting membrane potential.

13.1.1 Explanatory Mechanisms

The above changes in APD and the base-line shift of membrane potential could be due to one or more of the following possible mechanisms:-

1. Accumulation of K^+ in the extracellular clefts, $[K^+]_e$, and the activation of the $Na^+ - K^+$ pump.
2. Intracellular accumulation of Ca^{++} , $[Ca^{++}]_i$.
3. Intracellular accumulation of Na^+ , $[Na^+]_i$.
4. Other mechanisms, e.g. hypoxia, changes in kinetics of the depolarizing or repolarizing currents responsible for the genesis of the AP.

It must be borne in mind that the above mechanisms and any other mechanisms suggested later to explain the restitution curves results are speculative.

13.1.1.1 Extracellular Accumulation of K^+

Direct effect on membrane

Lab (1982) put forward the possibility that mechanical changes could distort the extracellular spaces which could effect the movements of K^+ during activity which has been demonstrated to fluctuate during activity in ventricular and purkinje fibres (Kline and Morad 1978 Martin & Morad 1982, Cohen & Kline 1982). The result of restricting the K^+ movement could be its accumulation in the extracellular spaces.

This K^+ accumulation can explain the observed depolarization of the resting membrane potential under isovolumic contraction (Kline and Morad 1978, Cohen and Kline 1982, see section 13.1 for more references). It can also explain the shortening of the AP under isovolumic contraction. Action potential shortening produced by increasing $[K^+]_o$ has been observed in various isolated cardiac preparations and intact hearts from turtle, rabbit, dog, sheep, cat and frog (Weidmann 1956, Surawicz et al 1959, Kline and Morad 1978, Noble 1981, Hennekes et al 1981, Kline and Kupersmith 1982, Lee and Dagostino 1982, Tsutsumi et al 1983, Lab and Yardley unpublished data, this study chapter 9). This shortening of the AP has been explained by increased outward K^+ current due to increased membrane conductance to K^+ under high $[K^+]_o$ and possibly to increased activation of the $Na^+ - K^+$ pump (see chapter 2).

It is unlikely that increased $[K^+]_o$ is the major cause for the above changes seen under isovolumic contraction because activation of the $Na^+ - K^+$ pump would decrease the amount of K^+ accumulated which should reverse or at least reduce the depolarization and thus remove the effect of elevated $[K^+]_o$ on membrane conductance. Ellis (1977) found a significant increase in the pump activity, as indicated by the changes in $[Na^+]_i$ measured with ion selective glass microelectrodes in cardiac Purkinje fibres, when raising $[K^+]_o$ to 10mM. Martin and Morad (1982) found that increasing the concentration of K^+ in the perfusate from 3 to 5mM increased the rate of uptake of K^+ from the extracellular space of frog ventricle which they demonstrated to be due to Na^+ -pump activation. Ruiz-Ceretti et al (1981) showed a decrease in $[Na^+]_i$

when increasing $[K^+]_o$ to 5mM in rabbit ventricular muscle. The depolarization of the resting membrane potential observed on increasing the rate of stimulation was reversed during continued stimulation in rabbit atrial muscle (Kunze 1977) in frog ventricular muscle (Martin and Morad 1982) and in dog Purkinje fibre (Kline et al 1980, Kline and Kupersmith 1982). This repolarization of the resting potential was shown to be dependent on the activity of the Na^+/K^+ pump which suggests that the Na^+ -pump plays a role in maintaining $[K^+]_o$ in the extracellular clefts.

It can, therefore, be said that increasing the membrane conductance to K^+ and/or increasing a K^+ outward current as a consequence of increased $[K^+]_e$ alone is unlikely mechanism for explaining the maintained shortening of the steady state AP under isovolumic contraction.

However, increasing the activity of the $Na^+ - K^+$ pump is an alternative explanation. The pump could generate an outward current that may affect the AP. It is therefore possible that an increase in the $Na^+ - K^+$ pump activity, which may be due to accumulation of K^+ in the extracellular spaces, might contribute to the shortening of the steady state APD under isovolumic contraction by generating an outward current at the plateau of the AP (see 2.3.1).

The above explanation of AP shortening with isovolumic contraction means that the mechanical distortion responsible for the initial accumulation of $[K^+]_e$ continues throughout the period of isovolumic contraction with the tendency to increase $[K^+]_e$ in the face of maintained $Na^+ - K^+$ pump activity to try to keep $[K^+]_e$ constant. The possible activation of the $Na^+ - K^+$ pump, as a result of an increase

in $[K^+]_e$, is however unlikely to be of large magnitude because the experimental evidence shows the pump to be near maximum activation at $[K^+]_o$ of 4-5 mM. The half maximum activation of the pump has been estimated to be at about 1.5mM $[K^+]_o$ in guinea pig atria (Glitsch 1982) and at about 1mM in dog Purkinje fibre (Gadsby et al 1981). Increasing $[K^+]_o$ up to 16.5mM had little effect on the pump activity in cardiac Purkinje fibres (Bosteele and Carmeliet 1972). While Ellis (1977) found a significant increase in the pump activity when raising $[K^+]_o$ to 10mM. Ruiz-Ceretti (1981) observed little change when increasing $[K^+]_o$ beyond 5mM, also Eisner et al (1981a) found that nearly maximum pump activity at extracellular $[Rb^+]$ of 4mM and higher concentration had no significant effects on the pump activity.

Finally, the suggestion that mechanical distortion of the extracellular spaces can affect the K^+ movement or accumulation loses some credibility when considering the results in figures 7.1, 7.2, 7.3a and 7.4a which show slow changes of APD and resting potential. If the changes seen under isovolumic contraction were due solely to K^+ accumulation in the extracellular spaces and activation of the $Na^+ - K^+$ pump, the reverse changes when going back to free loaded contraction would have been faster than observed. Attwell et al (1981), Gadsby and Cranefield 1979, Eisner and Lederer 1979b, Gadsby 1980). all showed fast return to normal conditions brought about by the $Na^+ - K^+$ pump when the physiological manoeuvre is removed. Lab (1982) reached similar conclusion. Furthermore, the results of high K^+ (described in chapter 9) suggest that the mechanism/s responsible for AP shortening when changing from free loaded to isovolumic contraction is/are independent of those responsible for the shortening at high K^+ (see later section).

13.1.1.2 Intracellular Accumulation of $[Ca^{++}]$

The first question is can an increase in intracellular $[Ca^{++}]$ due to increased Ca^{++} influx as a result of epicardial stretch in this study, explain the resting membrane depolarization and the shortening of the steady state action potential duration under isovolumic contraction?

A . Mechanical Stretch under Isovolumic Contraction

Le Winter et al (1975) by means of ultrasonic crystals implanted in the epicardium showed epicardial stretching when occluding the aorta at the base, middle and apex of the intact left ventricle in dog heart. Olsen et al (1981) also, found changes in the dog left ventricular shape and dimensions when occluding the aorta consistent with myocardial stretching. In the present study, using the the isolated rabbit heart, a stretch took place when changing the mode of contraction to isovolumic. The stretch occurred during the fast rising phase of the ventricular pressure. Both stretch and pressure slowly increased until they reached a steady state roughly at the same time (fig. 7.18) in the isolated rabbit heart. The most likely cause of the slow change in size of this stretch is the filling from the thebesian drainage into the left ventricle. Mechanical stretch has been reported in insitu pig heart when occluding the aorta(Lab 1982).

B. Effect of Stretch on intracellular Ca^{++} concentration

Lakatta and Jewell (1977) have shown that when a cat papillary muscle was stretched, the mechanical performance (as shown by the developed tension) increased immediately after the stretch and continued to increase slowly for a few minutes. Similarly, two phase increases in tension were observed by Allen (1977) and Allen and Kurihara (1982). Lakatta and Spurgeon (1980) showed a two phase increase in tension when increasing the frequency of beating, but the rate and magnitude of the increase was significantly larger at a longer muscle length. In both studies of Lakatta et al (1977, 1980) increasing the rate of stimulation or raising the bathing $[\text{Ca}^{++}]$ increased the rate and magnitude of the slow rise in tension, but verapamil (slow inward Ca^{++} current blocker) dramatically reduced both. It was, therefore, suggested that the immediate rise in tension was due to an increase in the amount of Ca^{++} released from an internal store (Lakatta and Jewell 1977) which was supported by the observation of increased myoplasmic $[\text{Ca}^{++}]$, as measured by aequorin, in skeletal muscle when it was stretched (Gordon and Ridgway (1976). To explain the slow rise in tension when the muscle was stretched or when the rate of beating was increased, Lakatta and Jewell (1977) and Lakatta and Spurgeon (1980) have suggested increased transsarcolemma influx of Ca^{++} . This would increase the Ca^{++} load of an internal store from which more Ca^{++} will be released by an action potential. In support of this Allen and Kurihara (1982) showed the slow decrease in tension, when a cat papillary muscle is released and held at short length was accompanied by a decrease in the aequorin signal and when the

muscle is stretched again and held at long length the slow increase in tension was accompanied by an increase in the aequorin signal which suggested elevated sarcoplasmic $[Ca^{++}]$. The verapamil results support the suggestion that the increased transsarcolemmal influx of Ca^{++} at longer length was partly mediated through increased Ca^{++} inward current (I_{si}) but Allen (1977) found inconsistencies in the changes of the action potential duration when stretching cat papillary muscle, in one case the duration prolonged in a second it shortened and in a third it was unchanged. These results might indicate that length dependent Ca^{++} influx is not due to just increased I_{si} , but some other mechanism possibly, passive influx might be more important.

c) Effects of increased $[Ca^{++}]_i$

An increase in $[Ca^{++}]_i$ can modulate the AP and possibly the resting membrane potential through one or more of the following mechanisms.

- i) Its effect on the $Na^+ - Ca^{++}$ exchange and $Na^+ - K^+$ pump.
- ii) An increase in Ca^{++} dependent K^+ outward current/s.
- iii) A decrease in slow inward Ca^{++} current.

- i) The effect of increased $[Ca^{++}]_i$ on $Na^+ - Ca^{++}$ Exchange and $Na^+ - K^+$ pump

There is good deal of evidence for the existence of a $Na^+ - Ca^{++}$ exchange in the mammalian as well as the amphibian heart, which contributes towards maintaining low $[Ca^{++}]_i$ by exchanging about 3 external Na^+ for 1 internal Ca^{++} ion (Horackova and Vassort, 1979; Pitts, 1979; Chapman and Tunstall, 1980;

Coraboeuf et al 1981, Sheu and Fozzard 1982). The exchange is reversible (depending on the membrane resting potential and the electrochemical gradient for Na^+) whereby external Ca^{++} can be exchanged for internal Na^+ (Busselen et al 1978, Mullins 1979, 1982, Horackova and Vassort 1979, Burt 1982, Sheu and Fozzard 1982, Bridge and Bassingwaight 1983). A good deal of evidence shows that the exchange is electrogenic, generating a current, and is affected by the membrane potential (Niedergerke 1963, Horackova and Vassort 1979, Caroni et al 1980, Reeves and Sutko 1980, Chapman and Tunstall 1980, Chapman et al 1981b, Busselen 1982, Coraboeuf et al 1981, Mullins 1982, Philipson and Nishimoto 1982, Sheu and Fozzard 1982).

The currents generated can effect the action potential duration in a simulated model of purkinje fibre (Fischmeister and Vassort 1981). When Ca^{++} extrusion was reduced or inhibited by reducing or removing external Na^+ (that will reduce or inhibit the exchange process) a reversible hyperpolarization of the resting membrane potential was observed (Horackova and Vassort 1979, Coraboeuf et al 1981, Sheu and Fozzard 1982). When the membrane was depolarized by extracellular K^+ , the $\text{Na}^+ - \text{Ca}^{++}$ exchange was reversibly inhibited and was shown to generate an outward hyperpolarizing current (Niedergerke 1963, Chapman and Tunstall 1980, Busselen 1982, Sheu and Fozzard 1982). These results suggest that at normal extracellular $[\text{Na}^{++}]$ and at high resting membrane potential the exchange generates an inward depolarizing current which contributes to the resting membrane potential (Mullins 1979, 1982).

Increased $[Ca]_i$ has been shown to activate the Na^+-Ca^{++} exchange. Caffeine, for example, which is known to produce an increase in $[Ca^{++}]_i$ probably by releasing Ca^{++} from an internal store, increased $^{45}Ca^{++}$ efflux in guinea pig atrial muscle (Jundt et al 1975). But in the absence of extracellular Na^+ caffeine caused contracture, presumably as a result of increasing $[Ca^{++}]_i$ because the exchange is inhibited in the absence of external Na^+ . Similar results were obtained by Chapman and Tunstall (1981a and 1981b). It is, therefore, possible that increased $[Ca^{++}]_i$ under isovolumic contraction could activate the exchange to extrude more Ca^{++} during diastolic interval and this would generate an inward depolarizing current carried by inward moving Na^+ ions which can depolarize the resting potential. The accumulation of $[Ca^{++}]_i$ would be expected to be slow in reaching steady state and so would be the resting membrane depolarization; consistent with the results shown in figure 7.1. When the mode of contraction is changed back to free loaded contraction, the influx would stop and $[Ca^{++}]_i$ would slowly fall because of a net outward flux and this is also consistent with the slow hyperpolarization observed on changing back to free loaded contraction fig. 7. 2.

Since internal Ca^{++} is exchanged for external Na^+ , the increased activation of the Na^+-Ca^{++} exchange would be expected to increase $[Na^+]_i$, the consequences of which would be to increase activation of the Na^+-K^+ pump (Glitsch 1972, 1982; Eisner and Lederer 1980, Gadsby and Cranefield 1979) which would generate an outward repolarizing current (section 7.5.3.1) which may also contribute to the shortening of the steady state action potential duration under isovolumic contraction. During diastole this current would tend to oppose the inward depolarizing current generated by the

$\text{Na}^+ - \text{Ca}^{++}$ exchange. This could explain why the resting membrane depolarization was not observed in all preparations, because the extent of the depolarization would depend on the extent of activation of both opposing currents by $\text{Na}^+ - \text{Ca}^{++}$ exchange, and $\text{Na}^+ - \text{K}^+$ pump, and the balance between them.

ii) The activation of Ca^{++} dependent K^+ outward currents

The other mechanism by which increased $[\text{Ca}^{++}]_i$ can effect the action potential is by activating a Ca^{++} dependent outward current.

Increasing extracellular $[\text{Ca}^{++}]$ is well known to change the shape and shorten the duration of the action potential in dog, rabbit, and cat (Hoffmann and Suckling 1956, Temte et al 1967, Kass and Tsien 1976, Boyett and Jewell 1978, Siegal and Hoffmann 1980, Johannsson and Wolhfart 1980, Colatsky and Hogan 1980, Urata and Goto 1982). To explain this effect of increased $[\text{Ca}^{++}]_o$ Kass and Tsien (1976) suggested an increased activation of the background time-independent K^+ outward current (I_{k1}) in purkinje fibre.

Similarly Bassingthwaighete et al (1976) suggested a feed-back loop between APD and $[\text{Ca}^{++}]$ under the membrane in ventricular muscle. They suggested that accumulation of Ca^{++} ions under the membrane causes an increase in I_{k1} . Experiment evidence from other studies seems to support the above suggestion (See w. 3.1 for more details).

Recently Urata and Goto demonstrated increased I_{k1} under Na-deficient condition in frog atrial muscle. They also observed a fast activation of the delayed outward current (I_X). Urata and Goto attributed the shortening of the AP under Na-deficient condition to

iii) Modulation of I_{si} by $[Ca^{++}]_i$ (Ca^{++} induced inactivation of I_{si})

Several investigators have shown I_{si} (the slow inward current) to depend on $[Ca^{++}]_o$ in a way which suggests that it is dependent on the inward driving force. When $[Ca^{++}]_o$ is increased the magnitude of the current increased in cat papillary muscle, in cow ventricular trabeculae, dog ventricular muscle, guinea pig atrial muscle, and Purkinje fibres (Beeler and Reuter 1970a) Kohlhardt 1975, Reuter and Scholz 1977, Thyrum 1974 and Mascher and Peper 1969).

Consistent with this idea Beeler and Reuter (1970a) observed a reduced I_{si} after removal of Na^+ from the bathing solution which they attributed to the increased internal accumulation of Ca^{++} which in turn resulted in a decrease in the inflow driving force. Simurda et al (1976) showed a progressive decrease in the slow inward current in cat trabeculae and papillary muscle during repetitive stimulation by voltage-clamp depolarization in spite of several fold increase in contractility. They suggested that the decrease in I_{si} was due to decrease driving force as a consequence of intracellular accumulation of Ca^{++} which produced the observed increase in contractility.

If the fast Na^+ inward current is abolished, slowly rising action potentials which are mediated by I_{si} can be produced upon large enough excitation. The rate of rise and the overshoot of these Ca^{++} mediated action potentials have been used as indices of I_{si} . Tritthart et al (1976) found the rate of rise, overshoot and duration of these action potentials to be markedly reduced when the frequency of stimulation was increased. It was suggested that increasing $[Ca^{++}]_i$ decreased the driving force for the inflow of Ca^{++} which in turn resulted in decreased I_{si} . When the rate was decreased, the rate of rise, overshoot and duration returned slowly to normal. Another possible mechanism through which increased $[Ca^{++}]_i$ can reduce I_{si} is mediated by CAMP.

An inverse relationship between $[Ca^{++}]_i$ and cAMP has been suggested in many types of cells including cardiac cells (Wollenberger et al 1973, Harary et al 1976). Endoh et al (1976) found intracellular level of cAMP and contractile force of isolated rabbit papillary muscle to have a reciprocal relationship during induction of different contractile states by changing frequency of stimulation. These observations are compatible with the view that the intracellular concentration of free Ca^{++} regulates tension as well as [cAMP] (Flitney and Singh 1979). Sperelakis and Schneider (1976), Reuter (1979) and Lindemann et al (1982) have suggested that the number of functional slow inward channels is regulated by cAMP which presumably involves phosphorylation of a membrane protein associated with the slow channels. Decreased [cAMP] as a consequence of elevated $[Ca^{++}]_i$ could thus reduce the functional number of slow channels which in turn reduces I_{si} and vice versa. In support of this Azuma et al (1981) found a close correlation between the rate of rise of the slow action potentials in chick heart and the level of cAMP. So it seems that the magnitude of I_{si} is controlled by the level of cAMP which is in turn could be regulated by $[Ca^{++}]_i$. Flitney and Singh (1980) suggested that Ca^{++} can change the level of cAMP by affecting the activity of cAMP-phosphodiesterase which is responsible for the destruction of cAMP.

The hypothesis that cAMP modulates I_{si} predicts, therefore, an increase in I_{si} , hence a prolongation of the action potential, when intracellular [cAMP] rises.

Recently Trautwein and Tanigauchi (1982) have been cited by Osterrieder et al (1982) to have shown increased I_{si} when cAMP is

injected into a single myocyte. Osterrieder have demonstrated prolonged action potential duration and increased height of the APD as well as increased I_{si} amplitude when cAMP was injected into isolated ventricular cells from guinea pig.

It is, therefore, conceivable that an increased $[Ca^{++}]_i$ under isovolumic contraction would have decreased the level of cAMP which in turn would result in a smaller number of functional slow channels, thus reducing I_{si} . This in turn would result in a shorter APD. The slow shortening of the action potential upon changing to isovolumic contraction shown in fig. 7.3 can be explained by a gradual increase in $[Ca^{++}]_i$ and thus a gradual changes in cAMP levels and the number of functional slow channels. The slow recovery shown in fig. 7.4a could be the result of the slow reverse of the above processes.

Inconsistent with the above hypothesis that I_{si} and thus APD and amplitude are regulated by cAMP which is in turn regulated by $[Ca^{++}]_i$, Noble and Shimoni (1980a, b) showed a progressive increase in the slow inward current on repetitive voltage clamp depolarizations after a rest period and when the frequency was changed during constant stimulation in frog atrial muscle. Urata and Goto (1982) found increased action potential plateau amplitude due to enhanced I_{si} under Na^+ deficient conditions also in frog atrial muscle, while Isenberg (1977b) found that injection of Ca^{++} into sheep Purkinje fibre increased I_{si} and EGTA injection reduced it. These differences could have been due to species differences between mammalian and amphibian hearts and tissue differences between Purkinje and ventricular muscle fibres.

The result of increased Ca^{++} influx would be to increase $[\text{Ca}^{++}]$ in the storage compartment from where more Ca^{++} can be released during excitation: increasing $[\text{Ca}^{++}]$ in the store would increase $[\text{Ca}^{++}]$ in the membrane compartment which has been suggested to inversely determine the action potential duration (Johannsson and Wohlfart 1980). Lullmann and Peters (1977, 1979) have identified a plasmalemma membrane from which Ca^{++} is released on depolarization, and is rebound on repolarization. Therefore, the increase influx of Ca^{++} could load the storage site as well as this membrane compartment with Ca^{++} . However, disagreement with the above suggestion of increased $[\text{Ca}^{++}]_i$ under isovolumic contraction has been obtained by Allen et al (1983,) who found the peak amplitude of the aequorin signal from the isometrically contracting cat papillary muscle not to be different from that when the muscle was allowed to contract isotonically, but the time course of decline of the aequorin signal was shorter under isometric contraction. However, in their protocol the muscle was allowed to contract isotonically once after a train of six isometric beats and the cycle was repeated. If the processes of accumulation of $[\text{Ca}^{++}]_i$ and its decrease, when changing to isometric contraction and back to isotonic contraction respectively, were slow processes, no change would be expected in peak $[\text{Ca}^{++}]_i$ in their preparation until several beats after changing the mode of contraction. However, Allen and Kurihara (1982) showed previously that peak $[\text{Ca}^{++}]_i$ increased with length after steady state conditions were allowed to be reached.

13.1.1.3 Increasing $[\text{Na}^+]_i$ under isovolumic contraction

An alternative to the suggestion of increasing $[\text{Ca}^{++}]_i$ under isovolumic contraction, is increased $[\text{Na}^+]_i$. It was suggested above that a partial inhibition of the Na^+/K^+ pump as a result of hypoxia could be responsible for the resting membrane depolarization but the possibility of hypoxia was discounted, (see next section). However, a partial inhibition of the Na^+ pump is possible as a consequence of a conformational change of the ATPase molecule, which is located in the sarcolemma (Philipson and Nishimoto 1982) and is responsible for the Na^+ pump activity. The conformational change could be produced by increased tension in the ventricular wall and/or muscle stretching under isovolumic contraction. High hydrostatic pressure exerted on an isolated frog heart has been observed to change the activity of acetylcholinesterase, probably as a result of a conformational change in the enzyme molecule (Young 1973). Therefore, an analogous change in the Na^+/K^+ ATPase as a direct result of membrane stretching and/or increased tension producing a partial inhibition of the pump is a possibility that cannot be ruled out. Partial inhibition of the pump would be expected to increase $[\text{Na}^+]_i$, the consequence of which would be an increase in $[\text{Ca}^{++}]_i$ through $\text{Na}^+-\text{Ca}^{++}$ exchange (see above). Philipson and Nishimoto (1982) demonstrated the Na^+ binding sites of the $\text{Na}^+-\text{Ca}^{++}$ exchange to be different on the opposite sides of the sarcolemma and they concluded from the results obtained that a small shift in $[\text{Na}^+]_i$ in the physiological range would have marked effects on Ca^{++} influx i.e. increased $[\text{Na}^+]_i$ would increase Ca^{++} influx.

As a result of the Na^+ pump inhibition, the accumulation of K^+ in the extracellular spaces can explain the resting membrane depolarization and increasing $[\text{Ca}^{++}]_i$ through one or more of the processes described above, and can modulate the action potential duration.

However, inhibition of the $\text{Na}^+ - \text{K}^+$ pump as discussed later is not favoured.

13.2.1.4 Other Possible Mechanism

1. Reduced I_{si}

Gettes and Reuter (1974) demonstrated reduced I_{si} and plateau duration when the resting membrane potential was depolarized. Also the recovery of I_{si} was slowed. If the change in the base-line of the AP is true depolarization, it might suggest reduced I_{si} and its recovery, hence shorter AP under isovolumic contraction, but the depolarization is unlikely to be of large magnitude to have a significant effect on I_{si} . Only depolarization to -40 mV or higher produces a significant affect (Gettes and Reuter 1974), which is unlikely to happen under isovolumic contraction.

2. Hypoxia

It is possible that under isovolumic contraction the myocardium developed hypoxia which is well known to be accompanied by shortening of the APD and a decrease in the resting potential in cardiac tissues (Carmeliet 1978, Fozzard 1980, Horio et al 1982, Weiss and Shine (1982). The source of hypoxia could be the cessation of the thebesian circulation, a reduction of coronary flow, and an increase in O_2 consumption following the increase in wall force. However, several arguments mitigate against this explanation. The thebesian circulation was small and even absent in many experiments. The largest flow from the thebesian circulation was 2ml/min in 3 Kg rabbit which had 35ml/min total coronary flow.

Further, measuring the coronary flow with measuring cylinder showed no change in flow under isovolumic contraction from that under free loaded contraction. Moreover, reduced coronary flow and the development of hypoxia would be expected to be accompanied by a reduction in the developed ventricular pressure (Harding and Poole-Wilson 1980, Bourdillon and Poole-Wilson 1981) but this was not observed in any experiment during isovolumic contraction.

3. Ca^{++} dependent inward current

Colquhoun et al (1981) described inward channels which were abundant in cardiac cells from neonatal rat heart and were activated by intracellular Ca^{++} , with a reversal potential at -5mV. Kass et al 1978) described a transient inward current in Purkinje fibres which was induced by strophanthidin and was similar to that described by Colquhoun et al (1981). This current could have been a leak current or an inward current generated by the $\text{Na}^+ - \text{Ca}^{++}$ exchange. This current can explain the depolarization of the resting membrane potential seen under isovolumic contraction if there was intracellular accumulation of Ca^{++} .

One further possibility which cannot be ruled out is the direct effect of mechanics on the kinetics of the ionic currents which are responsible for the generation of the resting and the action potential (chapter 2). Changes in the kinetics and in amplitudes of the fast Na^+ inward current and the voltage dependent K^+ outward currents in giant squid axon have been observed when the hydrostatic pressure in a chamber containing the nerve is increased (Conti et al 1982a, & b).

13.2 Regional Differences in the effects of Mechanics on AP shortening in response to a change in rate

Regional differences in steady state action potential duration have been recognised since 1882 by Burden, Sanderson and Paige (cited in Noble and Cohen 1978) and have been demonstrated in left ventricle of dog (Autenrieth 1975), sheep (Cohen et al 1978), pig (Lab and Yardley 1979) and in rabbit heart (Lab and Yardley unpublished data). Regional differences in dynamic electrophysiological behaviour have also been seen in sheep heart (Cohen et al 1976) and in left ventricle of pig in situ (Lab and Yardley 1979). The possible mechanisms for these regional differences have been discussed in chapter 4 and will not be discussed any further because the evidence is very limited for them.

Instead the discussion will concentrate on the mechanisms which could be responsible for the mechanical dependent electrophysiological differences in a single region with a brief discussion on the possible mechanisms responsible for the regional differences in the effects of changing the mechanics to isovolumic contraction.

13.2.1 Isolated rabbit heart

A. Area 1

Two possible mechanisms could be responsible for the greater, shortening in APD under isovolumic contraction than under free loaded contraction and these are intimately related to the rate dependent shortening and APD changes with mechanics described in chapter 3

They will, therefore, be briefly described.

1. In chapter 3 incomplete recovery of I_{Si} or maintained activation of the K^+ time-dependent outward current were described as the major cause of shortening of a premature AP. Either a further decrease in I_{Si} through increasing its rate of inactivation or through slowing its rate of recovery, or a further increase in rate of activation of the K^+ currents during the premature AP under isovolumic contraction could result in greater shortening. It is unlikely that a decrease in I_{Si} is responsible for this difference because a reduction of I_{Si} by selective blockers (e.g. verapamil and manganese) reduce the extent of shortening of the first beat in dog Purkinje fibre in response to a sudden increase in rate (Colatsky and Hogan 1980).

From the study of Browning and Strauss (1981) a shorter AP in Purkinje and ventricular muscle was accompanied by greater magnitude and rate of K^+ efflux which was supported by Kline and Kupersmith (1982). Therefore, larger increase in K^+ outward current under isovolumic compared to under free loaded contraction in the first beat after a sudden change in TPI to 250ms is favoured to explain the greater shortening observed under isovolumic contraction. The increase in K^+ efflux could be mediated through a possible elevation $[Ca^{++}]_i$. Bassingthwaighe et al (1976) found a greater shortening in steady state APD when the rate was increased from 5/min to 30 and 60/min at higher $[Ca^{++}]_o$, which they attributed to increased I_{K1} , this is in keeping with the above suggestion.

2. Increased Na^+-Ca^{++} Exchange and Na^+-K^+ Pump activity

Greater activation of the Na^+-Ca^{++} exchange in the reverse direction at the plateau of AP (i.e. extracellular Ca^{++} is exchanged for intracellular Na^+), and of the Na^+-K^+ pump during the premature AP could be responsible for the greater shortening in AP because both processes generate an outward current carried by Na^+ and the activities of both

have already been suggested to be higher under isovolumic contraction. The results in figure 7.8, and a later study, fig. 8.5, when constructing electrical restitution curves, show the difference in shortening between free loaded contraction and isovolumic contraction to decrease in the beats following the first one, which supports the above suggested mechanisms because of possible accumulation of $[Na^+]_i$ after the first beat. There is some further evidence from this study and other studies to support both mechanisms. For example, increased rate of K^+ efflux during a premature AP has been observed to be accompanied by a shorter APD in dog Purkinje fibres (Kline & Kupersmith 1982) and most of the change takes place in the first and second beats (Kline and Morad 1978). If increased K^+ outward current due to accumulation of $[Ca^{++}]_i$ is responsible for this difference between free loaded and isovolumic contraction then reducing or preventing this accumulation would predict no difference between free loaded and isovolumic contraction. This is shown in the restitution curves in figure 10.3, where there was no significant difference between the two mechanical conditions.

Iinuma and Kato (1978) found larger shortening of a premature AP in the presence of caffeine in dog ventricular muscle, while Urata and Goto (1982) reported shortening of the steady state APD on reducing external Na^+ , in order to increase $[Ca^{++}]_i$, in frog ventricle. This shortening was due to increased K^+ outward current whereas reducing external Ca^{++} prolonged the APD by delaying the activation of i_x current and suppressing time-independent K^+ current. Other evidence from the present investigation favour the increased activation of the $Na^+ - K^+$ pump. For example, slowing the pump by lowering the temperature to $24^\circ C$ abolished the greater shortening under isovolumic contraction in area 1, despite the fact that the steady state AP was shorter under isovolumic contraction (Chapter 11).

This might suggest that the possible Ca^{++} accumulation under isovolumic contraction is still present. But because the pump activity is reduced, any indirect influence of a rise in $[\text{Ca}^{++}]_i$ on the pump activity is absent.

Increasing $[\text{K}^+]$ in the perfusate from 4 to 8 mM did not only maintained, but exaggerated the mechanically induced shortening as shown by the restitution curves (figure 9.6), perhaps because K^+ stimulates the $\text{Na}^+ - \text{K}^+$ pump activity.

B. Tip of the Apex

The similar behaviour of this area under isovolumic and free loaded contraction could be due to the lack of changes in the mechanics of contraction, after all there is little contraction in this area because of the fibre structure (Greenbaum et al 1981a). Although a stretch is possible under isovolumic contraction which could explain the shortening of the steady state AP in some experiments, the absence of contraction is probably the cause of the similar behaviour in AP shortening between the two mechanical conditions. Also note that the absence of contraction could mean absent or reduced stress and strain in the myocardium in this area which has been suggested to be the cause of regional differences in blood flow, glycogen metabolism, and metabolic rate as explained later in chapter 4. It is possible that in this area the muscle can not be made to contract isometrically under isovolumic contraction, as might be the case in other areas (e.g the base), because it has nothing to pull against. Therefore, there will be no difference between the mechanical conditions of contraction in this area as outlined above.

c. Area 6

My later studies concentrate on area 6, the following discussion will therefore refer to this area, but similar arguments apply to area 5. When comparing area 6 to area 1 a striking observation is that the extent of shortening of a premature AP, i.e. at 250ms TPI, is greater under free loaded contraction than under isovolumic contraction (figure 7.9, A6) and that is opposite the behaviour in area 1 (figure 7.9, A1).

If we now consider the isovolumic contraction only in the first group of experiments (Table 1), the steady state APD of area 6 under isovolumic contraction was much shorter than free loaded contraction in comparison to later experiments and to other areas. A possible explanation for this is the inadvertent placing of the suction electrode close to or on a coronary vessel. Hypoxia developing as a result could contribute to this shortening of the steady state action potential duration (Fozzard 1980). It is unlikely that the different behaviour of this area was due to a hypoxia-induced large shortening of AP because later experiments showed differences in behaviour under the two mechanical conditions even when there was little shortening in the steady state APD under isovolumic contraction. In support of this, is the similar behaviour of the area 5. Hypoxia damage to this area is unlikely. The small change in steady state APD seen later during the restitution curve studies (e.g. figure 9.9) and the change in the time course of the restitution curves under isovolumic contraction were very similar to those produced by manganese and verapamil, used to block I_{si} in the study of Colatasky and Hogan (1980) where the APD '0' at -60mV was plotted against the diastolic interval.

It is, therefore, possible that in this area the effects of mechanics are mediated through reducing I_{si} . To invoke this proposal, area 6 should behave differently mechanically from say areas 1, 2 and 3.

Anatomically area 6 is directly above the wall of the septum and stretching under isovolumic contraction may not be as large as in the free wall, as in areas 1, 2 and 3. Moreover, the right ventricle itself may splint these areas because of its close proximity. An interaction between the two ventricles has been demonstrated (Weber et al 1981, Badke 1982). According to the proposed stretch dependent increase in intracellular Ca^{++} and assuming that the magnitude of the influx is a function of the degree of stretch, the increase in $[Ca^{++}]_i$ may not be as large as in other areas on the free wall. Therefore, the increase in $[Ca^{++}]_i$ will depend on the magnitude of the intracellular Ca^{++} increase and the extent of reduction in I_{si} . How reduced I_{si} can decrease the extent of AP shortening is not clear. One possibility is by the reduction of time-independent K^+ outward current.

The magnitude of I_{si} has been suggested to have a feed-back on the K^+ current of the same (i.e. accompanying) AP (Bassingthwaight et al 1976). Reduced I_{si} could mean less accumulation of Ca^{++} at the inner side of the membrane during an AP which would activate less K^+ outward current or would reduce the rate and degree of I_{si} inactivation (Isenberg 1977b). One thing the results seem to suggest is that the reduced AP shortening is independent of changes in the $Na^+ - K^+$ pump activity because it is present at low temperature (fig. 11.4) when the $Na^+ - K^+$ pump activity is expected to be reduced, and at high K^+ (fig. 9.10) when the pump activity is expected to be increased.

Under low Ca^{++} perfusion there was no significant difference between free loaded and isovolumic contraction (fig. 10.9) which might support the above suggested mechanism of reduced K^+ outward current or slow I_{si} inactivation in the first beat (i.e. at 250 ms TPI) under isovolumic mode of contraction in this area.

The results of this investigation do not provide information about the possible causes of the above cellular mechanisms underlying the mechanical dependent regional electrophysiological differences. One possibility may be different mechanical changes occurring in different regions. LeWinter et al (1975) and Olsen et al (1981) reported regional differences in the degree of stretch and the mechanics of contraction in response to changing the mechanics of contraction from normal to isovolumic contraction in insitu dog left ventricle (chapter 5). In this study area 6 is located above the septum which may suggest some resistance to stretch in this area. The close proximity of the right ventricle may provide an additional resistance to stretch under isovolumic contraction. Therefore, at steady state, the affect of changing to isovolumic contraction in APD would be expected to be small in this area. This is consistent with the findings as shown by the difference between steady state APD's in figs. 8.9, 9.9 and 10.8. The results of this area in table 7.1 do not agree with this, but an explanation has been suggested above which implies placement of suction electrode on large blood vessels.

From the above suggestion of regional differences in stretch, it would expected to see the largest changes in steady state APD in those areas where stretch is expected to be largest. The results of areas 3, 4 and 5, which are located on the free wall and expected to have large stretch, seem to be in agree-

ment with this hypothesis. The shortening of steady state APD under isovolumic contraction in these areas is larger than in other areas. However, further study on these areas is required to confirm these results. The observation of regional differences in mechanical contraction in response to changing from normal to isovolumic contraction in Le Winter et al and Olsen et al studies may also apply to the rabbit heart in this study. As for example, area 1 may be contracting isometrically whereas shortening of muscle may be maintained at area 6, but to a lesser degree than in area 7 (the apex) because the apex has no resistance to pull against. It is possible, therefore, that the regional differences in the affects of mechanics on

AP shortening in response to a change in rate to be attributed to regional differences in mechanical shortening of the epicardium. It was unfortunate that the tripod needed to investigate this point in the rabbit heart is too small to be constructed reliably with the facilities available but an ideal method would be using ultrasonic crystals.

In conclusion, it is suggested that regional differences in the extent of muscle stretch, which may result in different degrees of intracellular Ca^{++} accumulation, is responsible for the regional differences in steady state APD shortening, while regional differences in muscle shortening may be responsible for the regional electrophysiological differences after a sudden change in rate.

13.2.2 Intact pig heart insitu

In agreement with the results obtained by Lab and Yardley (1979) the AP shortening at the base was less than that at the apex under normal conditions of contraction in response to an increase in rate. (tables 7.2 & 7.3). But this was true when the APD was longer at the apex. Some other areas near the apex (close to the LAD) have shorter AP's and the shortening in these areas was less than that in the base (pigs 6 & 7, table 7.2 & 7.3). In general, therefore, it seems that the longer the steady state APD the greater the change in duration with a rate change under normal contraction.

Under isovolumic contraction, i.e. when the aorta is clamped, the extent of shortening of the first beat when increasing the rate is less in both base and apex, than under normal conditions of contraction, that is the behaviour was similar to area 5 and 6 in the isolated rabbit heart. Note that the first group of experiments (number 1, 2 and 3, table 7.3) the shortening was greater under isovolumic contraction in the apex. This is probably due to replacement of the electrode laterally where the APD was longer. Because of the longer AP's in these areas, there was greater shortening in the premature AP.

A possible cause of the difference in AP behaviour in both areas of the intact pig heart under the two mechanical conditions is a reduction in I_{si} under isovolumic contraction, i.e. similar to the explanation for area 6 in the rabbit heart.

Possible mechanisms responsible for reducing I_{si} have already been described in section 7.5.3.2, iii. A possible reduction in I_{K1} due to a decrease in $[Ca^{++}]$ at the inner side of the membrane under isovolumic contraction could also explain the differences in AP behaviour under the two mechanical conditions of contraction in the pig heart. However, a reduction in I_{K1} may be unlikely because increased Ca^{++}_i has been suggested to explain the steady state APD under isovolumic contraction.

In the above context the following studies may be relevant. In the study of Bassingthwaigthe (1976) on calf and sheep ventricular bundles, when increasing the rate of stimulation, the extent of shortening in the steady state AP was greater at high bathing $[Ca^{++}]$. Boyett and Jewell (1978) found reduced extent of shortening in the first beat in response to increase in rate at high $[Ca^{++}]$. Similarly, reduced extent of shortening was seen by Colatsky and Hogan (1980) in dog Purkinje fibre at high $[Ca^{++}]$ but, at higher steady state stimulation (60/min) the extent of shortening was greater at high $[Ca^{++}]_o$ in absolute values as well as when the APD is expressed as % of the corresponding steady state APD. This greater shortening under high $[Ca^{++}]_o$ compared to that under normal $[Ca^{++}]_o$ was similar to the shortening under isovolumic contraction compared to free loaded contraction seen in area 1 of the rabbit heart. This is compatible with the proposed hypothesis of an indirect effect of elevated $[Ca^{++}]_i$.

The above results seem to suggest that the greater shortening of APD under isovolumic contraction is also dependent on the steady state rate of stimulation. The results from one pig lends some support to the suggestion that the rate of stimulation might have a role in the extent of changes produced by changing the mode of contraction. In pig number 3 the steady state rate was high (145/min) and the AP of both base and apex were short. In this animal the extent of shortening in response to a sudden increase in rate (to 200/min) was greater when the clamp was on in both areas but especially at the apex.

In keeping with the above proposal, Attwell et al (1981) suggested a possible rate dependent changes in an intracellular chemical concentration that can control membrane currents either via membrane depolarization or changes in metabolic activity.

13.3 The relation between regional action potential and fractional shortening

The results in fig. 7.17 shows the regional APD plotted against the fractional shortening in the intact pig heart. It shows no correlation between them at all if all the points are considered together (correlation coefficient 0.11). These results suggest that the factors responsible for regional differences in APD are more complicated than just regional differences in mechanics, and this could be the reason why no correlation was seen in fig. 7.17. Other possible reasons are errors like unreliable tripod recording or measurement error because of the noisy base line of the tripod recording.

13.4 Electrical restitution curves

13.4.1 Control conditions of perfusion

13.4.1.1 Isolated Rabbit

i Area 1 (Base).

The discussion on the effect of the mode of contraction on the restitution curves will be divided into two aspects.

- A. Mechanical dependent downward shift.
- B. The effect on the time course of the curve.

A. Mechanical Dependent Downward Shift

Under isovolumic contraction the whole of the restitution curve was displaced downwards in area 1. The steady state duration was longer under free loaded contraction and fell on the shoulder of the curve, which suggests incomplete membrane recovery between steady state beats. Under isovolumic contraction the steady state duration was near the plateau which suggests greater degree of membrane recovery at steady state as compared to free loaded contraction. The downward shift of the plateau is similar to a Ca^{++} -dependent downward shift observed in cat papillary muscle (Bass 1975, Boyett and Jewell 1978) and in dog Purkinje fibre (Colasky and Hogan 1980). The shift is also similar to the rate dependent shifts seen in isolated preparations (Boyett and Jewell 1978, Colatsky and Hogan 1980) and in intact isolated rabbit heart (Lab and Yardley unpublished data) as well as in insitu dog and pig left ventricles (Elzinga et al 1981, Lab and Yardley unpublished data). Boyett & Jewell (1978, 1980) proposed that this shift is produced by intracellular Ca^{++} accumulation which may increase the activation of the time-independent

K^+ outward current (Bassingthwaite et al 1976, Isenberg 1977c,d) and/or speed the faster activation of the time-dependent K^+ current (Urata and Goto 1982). In support of the above proposal increased $[Ca^{++}]_i$ after increasing the rate of stimulation has been demonstrated by Lado et al (1982), Cohen et al (1982) and Morgan and Blinks (1982) by Ca^{++} selective microelectrodes and Ca^{++} -sensitive aequorin. Colatsky and Hogan (1980) also came to the conclusion that $[Ca^{++}]_i$ plays a role in the downward shift of the electrical restitution which was produced by increasing the steady state rate of stimulation of dog Purkinje fibres. It is possible, therefore, that the downward displacement of the electrical restitution curve seen under isovolumic contraction is produced by elevated $[Ca^{++}]_i$, that has already been suggested to explain the shortening of the steady state action potential duration. Even at the longest test interval possible (figs 8.2, 8.4) the duration of the AP under isovolumic contraction was shorter than free loaded contraction which supports the suggestion of some type of ion accumulation.

The other possible mechanism is the accumulation of K^+ in the extracellular spaces. Although this might contribute to the changes, it is unlikely to be major mechanism as discussed at the beginning of the discussion.

The possibility that this downward shift is due to the modulation of I_{si} by $[Ca^{++}]_i$ cannot be ruled out. Colatsky and Hogan (1980) reported a shift in the plateau of APD vs diastolic interval curve when I_{si} was blocked by verapamil and manganese, but in their experiments there was little shortening if any at all in APD at -60mV, which I calculated from the height of their AP to be 72% repolarization.

It is difficult to reconcile this with shortening of the steady state duration under isovolumic contraction. However, Lab and Yardley (unpublished data) observed large shortening of the AP produced by 1 mg/L verapamil in isolated Langendorff rabbit heart.

It is interesting to note that adrenaline (known to increase Ca^{++} conductance probably by increasing the number of functional channels Reuter 1979) has been demonstrated to shift the electrical restitution curve downward in insitu dog heart (Elzinga et al 1981) in a similar way to that seen in area 1 under isovolumic contraction. Ouabain also shifts the plateau of the restitution curve downwards in sheep purkinje fibre (Noble and Cohen 1978) and in intact rabbit heart (Lab and Yardley unpublished data). This downward shift by Ouabain could also be attributed to the accumulation of $[\text{Ca}^{++}]_i$, as an indirect effect of the Na^+ pump inhibition. The latter has been demonstrated by using ion selective microelectrodes, and aequorin (Bers and Ellis 1982, Sheu and Fozzard 1982, Lee and Dagestino 1982, Morgan and Blinks 1982).

Other processes that can contribute to the downward shift of the plateau under isovolumic conditions are the proposed increased activation of the $\text{Na}^+-\text{Ca}^{++}$ exchange and the Na^+-K^+ pump. The results obtained by Colatsky and Hogan (1980) which showed the rate dependent shortening of the steady state APD not to be appreciably affected by verapamil and manganese, might lend some support to the above suggestion of increased activity of both processes. In contradiction to this, Bassingthwaigthe et al (1976) found that verapamil and D600 reduced a frequency-dependent increase in an outward current.

However, they did not measure the APD when I_{si} was blocked, and the contradiction between the two studies remains unconfirmed. They have nevertheless shown that the steady state rate dependent changes in APD to depend on the bathing $[Ca^{++}]$, there was greater shortening when the rate was increased from 5/min to 60/min at higher $[Ca^{++}]$.

B. Acceleration of the time course as indicated by a smaller recovery index

Under isovolumic contraction the time course of the curve was speeded up by approximately 200ms. Boyett and Jewell (1978) observed acceleration of the curve when raising $[Ca^{++}]$ in the bathing solution of a cat papillary muscle. They attributed this to increased speed of recovery of the slow inward current from inactivation which was reported by Kohlhardt et al (1975) in cat ventricular muscle and was attributed to increased $[Ca^{++}]_i$. Colatsky and Hogan (1980) reported no significant differences in the rate of recovery of their restitution curve between normal (1.8mM) and high (7.2mM) Ca^{++} . However, their graphs in figure 6B seems to show slight acceleration under high Ca^{++} , in fact the curve under high Ca^{++} in relation to that under normal Ca^{++} seems to be qualitatively quite similar to the isovolumic contraction curve in relation to that under free loaded contraction (fig. 8.3). Adrenaline was seen to accelerate the time course of the electrical restitution curve in insitu dog heart (Elzinga et al 1981), however this was not consistent in all their experiments. It is, therefore, possible that increased rate of recovery of I_{si} under isovolumic condition to be partly responsible for the acceleration of the curve.

Other mechanisms that may contribute to the faster recovery are increased activation of the Na^+ pump and $\text{Na}^+/\text{Ca}^{++}$ exchange, since both processes are involved in restoring pre-stimulus conditions. The results with high K^+ , low Ca^{++} and low temperature (chapters 9, 10 and 11 respectively) are in keeping with this suggestion (see later). Other possible mechanism/s that cannot be ruled out is/are faster activation and recovery of the K^+ outward currents (I_x , I_{kl}).

The above leads to another possible explanation, which is a faster inactivation and reactivation of the K^+ outward currents (I_x and I_{Kl}). Faster and greater K^+ currents activation has already been suggested as a possible mechanism to explain the greater shortening in APD under isovolumic contraction. A faster rate of activation could mean faster recovery from inactivation thus a faster restitution curve. Urata and Goto (1982) observed faster activation of I_x in frog atrial muscle in Na^+ - deficient conditions, a situation under which it is expected to have elevated $[\text{Ca}^{++}]_i$.

A further possible mechanism contributing to the faster time course of recovery is the faster and increased activation, under isovolumic contraction, of the $\text{Na}^+ - \text{K}^+$ pump. When inhibiting the pump with ouabain the electrical restitution curve was shifted downward and its time course was slowed down in the base and apex of an isolated rabbit heart perfused at 34°C (Lab and Yardley unpublished data). Cohen et al (1976) observed only a downward shift in the interval-duration curve of an isolated sheep purkinje and ventricular fibres produced by ouabain, but it is not known at what temperature.

(i) . Area 6

The electrical restitution curves of area 6 unlike that of area 1, cross over, and under isovolumic conditions it has slower time course of recovery compared to that under free loaded contraction (figs. 8.9, 8.10). 8.9 & 8.10).

A. Mechanical Dependent Downward Shift of the Plateau

The plateau of the curve is shifted downward under isovolumic contraction at test intervals greater than 450ms, and the rising phase is shifted upwards. The isovolumic curve, in relation to free loaded contraction curve, is very similar to that obtained by Colatsky and Hogan (1980) when I_{si} was blocked by verapamil in relation to control. The shift downward of the plateau might be related to some intracellular accumulation of Ca^{++} , similar to that seen in area 1, but this cannot explain the small shortening in the steady state duration unless some other mechanism is/are also present in this area, at steady state and shorter test intervals that would tend to prolong the AP. As for example reduced activation of the $Na^+ - K^+$ pump. Another possible explanation is a reduced activation of the $Na^+ - Ca^{++}$ exchange because of possible lack of $[Ca^{++}]_i$ accumulation. Blocking I_{si} by manganese or verapamil had very little effect on APD at -60mV which is about 72% repolarization in their results, but shortened the duration at the plateau. The small AP shortening at -60mV is in agreement with the effect of isovolumic contraction in this area if it was due to the blocking of I_{si} . Bassingthwaite et al (1976) found that blocking I_{si} with verapamil reduced an outward current only at high rates of stimulation. It follows, therefore, if a similar situation exists in this area of the isolated rabbit heart at long test intervals i.e. lower heart rate, the effect of reduced I_{si} would be more important than other mechanisms in determining the APD and would result in shortened duration, and thus the curve is shifted

downwards. The difference between the free loaded contraction AP and that of isovolumic contraction at the corresponding test intervals becomes greater at longer intervals (fig. 8.9) which might emphasise the importance of I_{Si} determining the APD under isovolumic contraction since shortening at this stage would be independent of time needed for membrane recovery because the steady state duration is almost at the plateau. At shorter intervals than steady state, i.e. at higher rates of stimulation the degree of activation of the K^+ current/s might become increasingly more important. The shorter the test interval (i.e. the higher the rate) the less activation, or more suppression of the K^+ current (Bassingthwaite et al 1976). Thus under isovolumic contraction, a possible reduction in I_{Si} and hence in I_K compared to free loaded contraction could explain the shift upwards of the rising phase of the restitution curve, i.e. towards less shortening, under isovolumic contraction. Also a reduction in the activation of the $Na^+ - K^+$ pump and the $Na^+ - Ca^{++}$ exchange might contribute to this upward shift because in both cases an outward current will be absent. The cause of the reduction in the activity of both processes in this area is not clear but could be due to regional differences in their activities or their densities. Cohen et al (1976) suggested differences between base and apex Na^+ pump activity to explain the electrophysiological differences between these two areas in sheep ventricular muscle, while Peters et al (Personal Communication) found differences in its density between left atrium and ventricle in guinea pig heart.

Kass and Tsien (1975) blocked I_{Si} by manganese, lanthanum and D600 and found a net increase in a time-dependent outward current in purkinje fibres. Whether this is the case in ventricular muscle is not known and whether this is due to I_{Si} being blocked or to a specific effect of the blockers on the current is not clear either. If this is present in the rabbit myocardium it can explain the shorter AP at the

plateau of the restitution curve but cannot explain the shift upwards at the rising phase at shorter TPI. In rabbit papillary muscle Johannsson and Wohlfart (1979) reported D600 to flatten the restitution curves in similar but not identical way to that observed by Colatsky and Hogan (1980), and suggested D600 to inhibit the inverse relation between the APD and the $[Ca^{++}]$ in the hypothesized membrane compartment (see chapter 4).

The question is could a mechanical stretch or membrane distortion in this area dissociate the coupling between APD and the membrane compartment? It is difficult to explain how, but at the same time this cannot be ruled out.

B. Slowing the time course of recovery as indicated by a larger recovery index

The curve under isovolumic contraction had a greater time of recovery index than free loaded contraction. The calculated recovery indices for both are in the range of those found for the recovery of I_{si} by Trautwein et al (1975) who also found, like Gettes and Reuter (1974) depolarization of the resting membrane potential to slow the recovery of I_{si} . The recovery indices of the free loaded and isovolumic contraction restitution curves were also similar to those obtained for I_k current in cat ventricular muscle at -85 and -70mV respectively (McDonald and Trautwein 1978b). These results suggest the slowing down of the restitution curve under isovolumic contraction in area 6 to be due to either slower recovery of I_{si} or a K^+ current (i.e. the time-dependent current). Reduced I_{si} and its recovery could be favoured because it explains the other features of the isovolumic restitution curve. (see A above). It is difficult

to speculate how such a change in I_{si} and I_k recovery is produced. One possibility may be high stress and strain in this area under isovolumic contraction. Increasing hydrostatic pressure in a chamber containing Grant Squid axon was observed to slow the kinetics and reduce the magnitudes of the fast Na^+ inward current and the K^+ repolarizing current (Conti et al 1982a, b). Therefore, slow recovery of I_{si} and/or I_k in this area (area 6) of the isolated rabbit heart is a possibility that cannot be ruled out. One complication to this suggestion is that the duration

of the steady state AP is almost at the plateau, suggesting a greater degree of membrane recovery at a steady state rate of stimulation under isovolumic contraction compared to that under free loaded contraction. Also, as mentioned in A (above), a reduction in the $Na^+ - K^+$ pump and/or $Na^+ - Ca^{++}$ exchange under isovolumic contraction in this area may contribute to the slowing down of the restitution curve since they are involved in restoring the pre-stimulus conditions.

An analogous situation seems to exist between base and apex restitution curves, when they are compared to each other, under normal conditions of contraction in insitu pig heart (Lab and Yardley unpublished data and this study section 13.5.1.2). The above suggestion, of I_{si} being the determinant of APD at longer intervals, and the activation of K^+ outward current/s is/are more important at short intervals, and the possible involvement of the $Na^+ - K^+$ pump and $Na^+ - Ca^{++}$ exchange are in keeping with this complicated effect of isovolumic contraction on the restitution curve of area 6.

As in area 1, the differences between the restitution curves under the two mechanical conditions for the second beat are also reduced in this area, as shown in figures 8.11 and 8.12. There was greater change in the recovery index of the second beat under isovolumic contraction and thus a shift towards the free loaded curve, i.e. opposite to area 1. If the results in area 1 were to be explained by larger magnitude of $[Ca^{++}]_i$ and/or $[Na^+]_i$ accumulation under free loaded contraction during repetitive activity, the opposite is probably happening in area 6, i.e. larger magnitude of accumulation under isovolumic contraction. Greater accumulation of Ca^{++} could result from larger driving force for inward Ca^{++} .

Greater driving force is possible if the reduction of the inward Ca^{++} carried by I_{si} is greater than the efflux, but even if the driving force is increased, it was suggested above that I_{si} is reduced and has slower recovery which makes the suggestion of larger $[Ca^{++}]_i$ accumulation in the second beat under isovolumic contraction tenuous. More attractive suggestion is greater magnitude of accumulation of Na^+ .

The suggestion is that greater accumulation of $[Na^+]_i$ could result in greater activation of the $Na^+ - K^+$ pump. The activities of $Na^+ - K^+$ pump under both conditions of contraction could therefore be closer in the second beat as compared to that in the first beat. If the pump contributes towards faster membrane recovery, the rate of membrane recovery would move closer until a state is reached where the time course of recovery is independent of the pump activity.

It is not to be forgotten that the $\text{Na}^+ - \text{Ca}^{++}$ exchange could also be activated at the plateau level of the AP which could aid in the process of APD recovery. From this suggestion a second suggestion can be put forward and that is the activation of the $\text{Na}^+ - \text{K}^+$ pump and the $\text{Na}^+ - \text{Ca}^{++}$ exchange may possibly override the effects of reduced I_{Si} and its slower rate of restoration on the course of membrane recovery.

13.4.1.2 Intact Pig heart insitu

It was difficult to construct more complete curves than those obtained because of limitations and lack of control over the preparations as compared to isolated rabbit heart. Replacement of the electrode was inevitable especially when constructing the restitution curve when the aorta was clamped.

i) Base

In 2 out of 3 experiments, the plateau of the curve was displaced downwards when the aorta was clamped. One experiment is shown in figure 8.13 . The displacement downwards was similar to that seen in the base of the isolated rabbit heart. In the third experiment the displacement was small. In the other two experiments the displacement was large including large shortening of the steady state duration, which was too large to be due to replacement of the electrode. It is impossible to say where the steady state position is in relation to the plateau because it was not reached. Such large shortening is possibly produced by multiple factors rather than just intracellular accumulation of $[Ca^{++}]$. Besides a possible accumulation of $[Ca^{++}]_i$, a large membrane depolarization (fig. 7.13) could contribute by shifting the current-voltage relation of, for example the K^+ outward current in an outward direction and/or reducing I_{si} . The shortening and the depolarization seem to be the result of cumulative processes, although both seem to have different time courses (fig. 7.13 'top' compared to fig. 7.16), which might suggest different processes are responsible for each.

The changes in the time course of the restitution curve (as judged by the eye) were less consistent than the changes in APD. In one experiment (fig. 8.13), it slowed down under aortic clamp conditions. In the second experiment with a large change in APD it was not possible to decide because of incomplete restitution curve under the aortic clamp conditions, and because of the presence of a supernormal period. In the third there was very little difference, the curve under aortic clamp conditions was only slightly accelerated. The inconsistency is mainly due to lack of control over the preparation and the small number of points which made it difficult to decide on the exact course of the curve. If the time course change observed in figure 8.13 is considered to be representative of what actually

happens, it can be explained by a similar explanation to that offered for slowing down the restitution curve of area 6 in the rabbit heart, i.e. slower I_{si} and/or I_k recovery attributed to high stress or tension on the epicardium under aortic clamp conditions. The upward shift in the base-line of the AP seemed to be large in comparison to the magnitude of the AP (8.13). If this shift is assumed to represent a change in membrane potential, it might suggest a large depolarization of the resting membrane potential which may slow the recovery of I_{si} as reported by Gettes and Reuter (1974) and Trautwein et al (1975).

(i) Apex

More consistent results were obtained from this area. In the three experiments the plateau of the curve under isovolumic contraction was displaced downwards as shown for a single experiment in fig. 8.14 (from the same experiment as that shown in fig. 8.13). In two experiments the curves of the normal contraction and that of the aortic clamp conditions crossed over. In this area too, it was not possible to judge the position of the steady state APD with respect to the

plateau of the restitution curve because the latter was not reached. The shift downwards is probably due to multiple factors similar to those described for the base, including the effect of depolarization of the resting membrane potential.

The cross over is similar to that seen in area 6 of the rabbit heart and a possible explanation for this cross over and the shift upward of the isovolumic restitution curve at short TPI is that the activation of the K^+ currents become increasingly more important at this phase of the electrical restitution. The shorter the test interval the less I_{si} and so there would be less activation of the time-independent and possibly decreased activation of the time-dependent, K^+ currents. Thus a decreased degree of shortening, would tend to prolong the AP. In comparison to normal contraction with a larger I_{si} , there would be less shortening under aortic clamp conditions. A possible explanation for the slowing down of the time course of the restitution curve may be slower recovery of I_{si} and I_k as explained above for the base.

It is of great interest to see the curves from the apex under the two mechanical conditions crossing over in a quite similar way to those from the base and apex observed by Lab and Yardley (unpublished data) under normal conditions of contraction, and this study. Superimposition of the curves under normal contractions (figs. 8.13 and 8.14) show an extraordinary similarity between the results from normal and aortic clamp conditions, to those from the base and apex under control contraction as seen in fig. 8.15. Comparing fig. 8.14 and fig. 8.15 suggest slightly lower plateau of the apex curve under aortic clamp conditions compared to the plateau of curve from base under normal conditions.

Unfortunately, the above observation and comparison was valid in one experiment out of the three. If this is found to be the case in future studies, it could suggest that the mechanism/s underlying the regional electrophysiological differences and the effect of changing the mechanics of contraction are similar, if not the same

mechanisms, which favour the suggestion that regional differences in the mechanics of contraction could be partly responsible for the regional electrophysiological differences (Khatib and Lab 1982a).

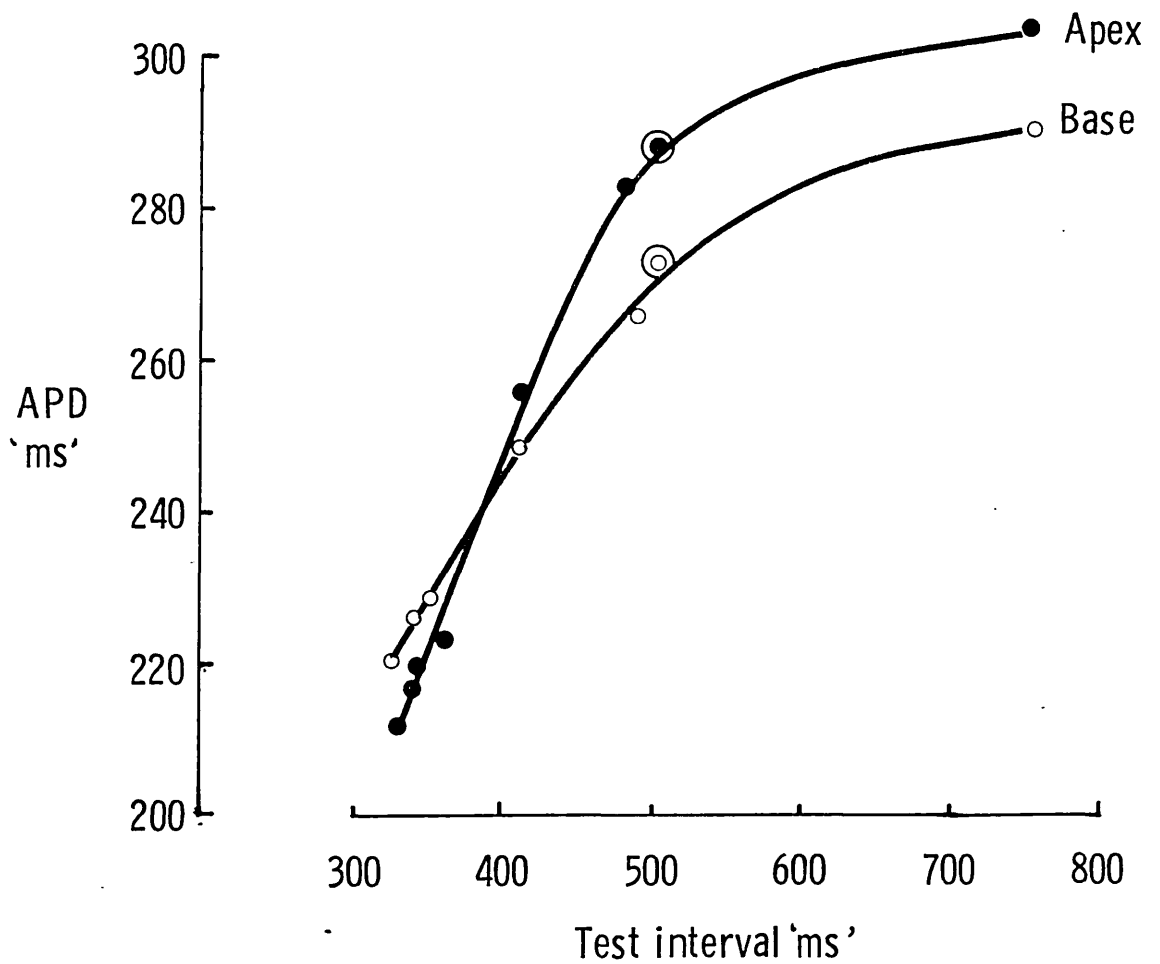


Fig. 8.15 Superimposed restitution curve under normal contraction from base (open circles) and apex (closed circles) of an intact insitu pig heart. Steady state APD's are encircled.

13.4.2 High K^+ perfusion (8mM).

In agreement with other studies on isolated preparations of cardiac muscle, high K^+ transiently increased ventricular pressure and depolarized the resting membrane potential, as indicated by the shift of the base-line of the AP at both areas, fig 9.1 and 9.2 (Weidmann 1956, Lee and Dagostino 1982, Cota and Stefani 1982). The ventricular pressure then gradually fell to a new steady state which was significantly below control steady state, i.e., at 4mM K^+ . The most likely explanation for this transient increase in ventricular pressure is a transient elevation of $[Ca^{++}]_i$. Sheu and Fozzard (1982) often saw a transient $[Ca^{++}]_i$ elevation during the first few minutes of K^+ depolarization in sheep Purkinje fibres. Several mechanisms can be proposed to explain the elevation of $[Ca^{++}]_i$.

1. The depolarization produced by elevated $[K^+]_o$ could cause a release of Ca^{++} from the releasing store (probably the SR).
2. Transient increase in Ca^{++} entry via I_{si} channels.
3. Reduction of Ca^{++} efflux via the Na^+-Ca^{++} exchange.

Increased Ca^{++} uptake and decreased efflux, produced by membrane depolarization (by elevating $[K^+]$ in the bathing medium) has been shown in frog heart, goldfish ventricles, isolated sarcolemmal preparations, frog and dog ventricles, isolated rat ventricular cells and in isolated chick embryo ventricular cells (Niedergerke 1963, Busselen 1982, Bartschat et al 1980, Fry et al 1981, Barry and Smith 1982).

The fact that Cota and Stefani (1982) found the rate of tension development in isolated strips of frog ventricle during the initial phase of K^+ contractures to be independent of external Ca^{++} and the fact that it was transient suggest a release of Ca^{++} from an intracellular store was behind the transient increase in ventricular pressure seen in the present study. Other studies on cat papillary muscle and trabeculae, and on human ventricular muscle showed no transient increase in twitch tension but it decreased below the control steady state level (Tritthardt et al 1976, Christie 1982). An obvious explanation for this decline would be a decrease in $[Ca^{++}]_i$ after prolonged perfusion with high K^+ . Such reduction in $[Ca^{++}]_i$ can be attributed to reduced I_{si} because of shorter APD as well as membrane depolarization and to a reduction in $[Na^+]_i$ which would reduce the $Na^+ - Ca^{++}$ exchange at the plateau of the AP. The reduction in $[Na^+]_i$, which has been demonstrated in rabbit papillary, sheep ventricular and purkinje fibres (Lee and Fozzard 1975, Ellis 1977, Eisner et al 1981a, Ruiz-ceretti et al 1981, Sheu and Fozzard 1982) could be the result of a combined effect of reduced I_{Na} at depolarized potentials (Noble 1981) and to a stimulation of the Na^+ -pump by external K^+ , as well as to a reduced Na^+ passive influx as a secondary effect to a reduced electrical gradient (Ellis 1977, Eisner et al 1981a, Lee and Dagostino 1982). Eisner et al (1981a) and Lee and Dagostino (1982) found a steep direct relation between $[Na^+]_i$ and twitch tension in sheep and dog purkinje fibre at normal $[Na^+]_i$ range. These results suggest that a possible small decline in $[Na^+]_i$ at steady state of high K^+ may be partly responsible for a reduction of $[Ca^{++}]_i$, hence the drop in the developed ventricular pressure.

Intracellular buffering systems, like the mitochondria or increased uptake by a sarcolemmal membrane compartment may also play a role in sequestering Ca^{++} . Finally, increased Ca^{++} pumping across the sarcolemma by an ATPase enzyme is a further mechanism that cannot be ruled out.

Another effect of increasing $[\text{K}^+]$ in the perfusing Krebs was shortening APD in both areas. Such shortening has been observed in various preparations of cardiac muscle (see 13.2.1). A possible explanation for this shortening is increased K^+ conductance and a shift in the steady state current-voltage relationship in the outward direction (chapter 7). Another possible mechanism contributing to with an outward current is the activation of the Na^+ -pump, while depolarization of the resting membrane potential may decrease I_{si} , (Gettes and Reuter 1974).

() Area 1 Restitution Curves

Comparing free loaded contraction curves under control and high K^+ (figs. 9.5 & 9.6) show a downward shift of the whole curve at high K^+ which can be explained by the effect of K^+ on APD. Although a reduction in $[\text{Ca}^{++}]_i$ (see above), may tend to prolong APD (see chapter 10) thus shifting the curve upward, the fact that it shifted downward suggests that the process/s that is/are responsible for the downward shift overcame any other process/s tending to change the curve in the opposite direction. The large acceleration of recovery index of the free loaded contraction curve at high as compared to normal K^+ can be explained by a combination of a possible faster activation and faster recovery of K^+ currents and increased activation of the Na^+ -pump

which can contribute towards faster return to control conditions after an AP. McDonald and Trautwein (1978b) found little change in I_K time constant between -80 and -60 mV in cat ventricular fibres. For this reason and because the magnitude of the membrane depolarization at high K^+ is not known in this preparation, the former suggestion seems to be less certain.

When inspecting the results in figure 8 of Gettes and Reuter's study (1974) a greater AP shortening can also be seen at higher $[K^+]_o$ in guinea pig papillary muscle. A faster recovery of I_{si} under depolarized conditions, to explain the faster membrane recovery, maybe unlikely because Gettes and Reuter (1974) showed a slower rather than faster recovery. The faster membrane recovery can also be seen by the fact that the steady state APD fell on the plateau part of the restitution curve under high K^+ . It should be borne in mind, as an alternative to any changes in the kinetics of any currents, that the APD is shorter under high K^+ which means longer diastolic interval between AP's during which the membrane recovery takes place. Therefore, any test AP at shorter intervals will be triggered at a time of greater degree of membrane recovery under high K^+ compared to normal. However, Elharrar and Surawicz (1983a) found the duration of AP per se did not influence the kinetics of APD restitution in dog Purkinje fibres.

At high K^+ the downward shift of the whole of the restitution curve under isovolumic contraction was much greater than that at normal K^+ (figure 9.6 vs figure 9.5), but there was not a large change in the recovery index of curve as that under control K^+ . The large downward shift could suggest greater elevation of $[Ca^{++}]_i$ under isovolumic contraction at high K^+ compared to at control K^+ . Such

a suggestion is supported by the results in table 9.1, which shows the steady state isovolumic ventricular pressure to be higher at high K^+ as compared to normal K^+ . In each of 4 experiments the difference was significant ($p < 0.05$) but no difference in experiment 1 and the opposite change in experiment 6. The % increases in ventricular pressure under isovolumic to free loaded contraction of all experiments were 147 and 167% for normal and high K^+ respectively. Whether this further increase in $[Ca^{++}]_i$ at high K^+ was due to increased Ca^{++} influx or reduced sequestration by or increased release from an intracellular compartment is not clear, but any one of these mechanisms can explain a greater elevation of $[Ca^{++}]_i$. The fact that the downward shift produced by isovolumic contraction is independent of that produced by elevating K^+_o suggests the mechanisms underlying the shift is/are different under the two conditions. However, the results do not eliminate the possibility that K^+_e accumulation may partly play a role in the changes.

ii) Area 6 Restitution Curves

The effect of high K^+ on the restitution curve of this area can be explained in a similar way as for area 1 above, viz. the downward shift is a consequence of AP shortening which in turn might be due to increased K^+ outward currents as a result of increased K^+ conductance. The acceleration of the curve could emphasise the possible importance of the Na^+ -pump activation in affecting the time course of membrane recovery under the present experimental condition.

The downward shift of the curve under isovolumic contraction is much

larger at high K^+ compared to normal K^+ . This could be related to the suggested greater accumulation of $[Ca^{++}]_i$ under isovolumic contraction at high K^+ . If the curve acceleration of recovery index in area 1 under isovolumic contraction was due to faster recovery of I_{si} , (13.5.1.1) which in turn could be due to elevated $[Ca^{++}]_i$ the question that can be asked is why the recovery index in area 6 was slowed under isovolumic contraction instead of being accelerated even at high K^+ ? In fact the curves seem also to cross over at a shorter test interval than the shortest possible in the present study (figs. 9.8 and 9.10). Such results suggest that the mechanism/s responsible for the downward shift and those responsible for the changes in the recovery index of the curve are different at least in this area.

The effect of isovolumic contraction on the recovery index of curve seems to be opposed and/or reduced by elevating extracellular K^+ which suggests a possible depression of the Na^+ -pump in this area under isovolumic contraction being partly responsible for the slow of AP recovery. Other possible effects of isovolumic contraction on K^+ current kinetics (13.5.1.1) being opposed by elevated K^+ is another possibility. A slight depression of the Na^+ -pump under isovolumic contraction at high K^+ can explain the decrease in the extent of shortening and the smaller magnitude of the restitution curve. Notice also the differences between free loaded and isovolumic contraction curves at high K^+ are much less than the respective differences at control K^+ . One way of explaining this is that greater depression of the Na^+ -pump has taken place under isovolumic contraction at control K^+ but this is partially corrected at high K^+ by increased activation of the Na^+ -pump. It might be said

that a reduction in $[\text{Na}^+]_i$, for the reason explained at the beginning of this section, would reduce the pump activity at high K^+ under both mechanical conditions. This could be so, but maintaining high K^+ perfusion may maintain high pump activity until a balance is reached between Na^+ efflux and influx at which the $[\text{Na}^+]_i$ might be less than control and the pump activity is higher. Finally, since the results suggest high $[\text{K}^+]_o$ can reduce the effect of isovolumic contraction in slowing the curve time course in this area, a suggestion could follow which is a lack of extracellular accumulation of K^+ in this area under isovolumic contraction. This is still in agreement with the suggestion in 13.3.1, that the Na^+ pump is probably not activated in this area under isovolumic contract at normal conditions of perfusion.

13.4.3 Low Ca^{++} perfusion (0.5 mM)

In agreement with results from dog ventricular and Purkinje fibres, isolated rabbit heart, cat papillary muscle, sheep and calf ventricular muscle and frog atrial and ventricular muscle (Hoffmann and Suckling 1956, Surawicz et al 1959, Johannsson and Wohlfart 1980, Bass 1975, Allen 1977, Boyett and Jewell 1978, Siegal and Hoffmann 1980, Iinuma and Kato 1979 and Lab and Yardley unpublished data), lowering $[\text{Ca}^{++}]_o$ prolonged APD of both area 1 and area 6 as shown by fig. 10.1. From the results of Bassingthwaite et al (1976), Kass and Tsien (1976), Isenberg (1977c, d) and Siegal and Hoffmann (1980) the prolongation can be explained by reduced time-independent K^+ outward current (I_{K1}). Urata and Goto (1982), by means of a double sucrose gap found I_{K1} was depressed and the activation of the delayed outward current (I_x) in frog atrial muscle was profoundly delayed in Ca^{++} free perfusion. Lowering $[\text{Ca}^{++}]_o$ results in increased $[\text{Na}^+]_i$ in sheep ventricular and Purkinje fibre (Ellis 1977, Sheu & Fozzard 1982). This increase in $[\text{Na}^+]_i$ has been suggested to be partly due to the $\text{Na}^+ - \text{Ca}^{++}$ exchange and/or to Ca^{++} effects on passive membrane conductance of leak of Na^+ . This might suggest increased activity of the Na^+ -pump under low Ca^{++} condition, thus contributing by an outward current. Also a reduced I_{si} because of the low $[\text{Ca}^{++}]_o$ means lower plateau level of AP and in ventricular muscle of sheep and calf, a lower plateau of AP is accompanied by shorter AP (Bassingthwaite et al 1976, Reuter and Scholz 1977). Other processes take place when decreasing $[\text{Ca}^{++}]_o$ which theoretically should shorten the AP. For example, I_{si} has been shown to be sensitive to $[\text{Ca}^{++}]$ in numerous studies (see Kohlhardt 1981

for references). Increasing $[Ca^{++}]_o$ produces an increase in I_{si} whilst drastic decline can be seen after lowering $[Ca^{++}]_o$ in mammalian as well as amphibian cardiac muscle. The current-voltage relationship of I_{si} shifts upward in an outward direction over the range of -70 to +20mV indicating less I_{si} being activated particularly at the plateau potential (Mascher and Peper 1969, Beeler and Reuter 1970a, Reuter and Scholz 1977, Kohlhardt 1981, Urata and Goto 1982). When I_{si} is reduced it is expected to shorten the AP as predicted from the mathematical model proposed by Beeler and Reuter (1977). Bassingthwaite et al 1976, observed prolongation at low $[Ca^{++}]_o$ only at stimulation rate higher than 5/min. At lower rate, the AP in fact shortened at low $[Ca^{++}]_o$.

(i) Area 1 Restitution Curves

If the downward shift of the isovolumic contraction restitution curve was due to intracellular accumulation of Ca^{++} we would expect to see reduced or no differences between free loaded and isovolumic curves if Ca^{++} accumulation is reduced or prevented. One way of doing this is to reduce $[Ca^{++}]_o$. The results of such experiments indeed showed reduced differences between free loaded and isovolumic contraction, as shown in figure 10.3 and 10.5. The plateau of the curve under isovolumic contraction shifts downward somewhat but in fact this is due to downward displacement in two experiments only. Non-parametric tests showed no significant differences for experiments of figure 10.5. The explanation for the reduced differences between the free loaded and isovolumic contraction curves may be due to reduced or absence of $[Ca^{++}]_i$ accumulation under isovolumic contraction which was suggested to be responsible for the downward displacement at normal $[Ca^{++}]_o$. The isovolumic contraction curve seems to accelerate (recovery index = 30ms) compared to free loaded contraction (recovery index = 43ms). In

5 experiments the two curves were virtually superimposed as shown in fig. 10.3 of a single experiment. These results, therefore, support the above suggested accumulation of $[Ca^{++}]_i$ being responsible for the downward displacement of the isovolumic contraction curve.

Comparing the restitution curves at low $[Ca^{++}]_o$ with those at normal $[Ca^{++}]_o$ for both conditions of contraction shows the curve at low Ca^{++} to be shifted upward, which is still in agreement with the above proposal and has been seen in cat and rabbit papillary muscle (Bass 1975, Boyett and Jewell 1978, Johansson and Wohlfart 1980) and in isolated rabbit (Lab and Yardley unpublished data). But instead of a slower time course as predicted from the explanation put forward in 13.5.1.1i to explain the acceleration under isovolumic conditions, the curve was very much faster. This faster time course is unlikely to be due to experimental errors because it was observed previously in this laboratory by Lab & Yardley in the isolated rabbit heart, and by Johansson and Wohlfart (1980), as shown by the early rising phases of the curves at short test intervals of their curves (figure 3a in Johansson and Wohlfart (1980)). But the results of Beeler and Reuter (1970a), Gettes and Reuter (1974), Reuter and Scholz (1977) showed slower activation, inactivation and recovery of I_{si} at low $[Ca^{++}]_o$, which would predict slower time course at low Ca^{++} . At low rates of stimulation, there was no difference between the time courses at 0.56 and 2.25mM of Ca^{++} in cat papillary muscle (Boyett and Jewell 1978) but acceleration was seen at 9mM Ca^{++} . These results suggest that the mechanism/s responsible for the time course of curve is/are more complicated than just being determined by I_{si} in these experiments, e.g. the acceleration of curve at low $[Ca^{++}]_o$ and under isovolumic contraction could be due to reduced mechanical shortening which is shared between the two conditions. Alternatively, an increase in the activity of the $Na^+ - K^+$ pump at low Ca^{++} may play a role in determining the time course of membrane recovery.

Another feature of the restitution curve at low compared to normal $[Ca^{++}]_O$ is the large amplitude which may be explained as follows;

Since the K^+ repolarizing current remains activated even after full repolarization, and because of the long APD the diastolic interval in between beats would be very short, any premature beat will be therefore accompanied by an increased magnitude of K^+ outward current and reduced I_{si} . The earlier the AP the larger the K^+ current and the smaller the I_{si} . Thus greater shortening of the AP will result in greater magnitude of curve. But notice that the steady state durations are very close to, and in some experiments they fell on, the plateau of curve suggesting near complete membrane recovery between steady state beats.

ii) Area 6 Restitution Curves

The differences between free loaded and isovolumic restitution curves seen at control $[Ca^{++}]_O$ (figure 10.8) were very much reduced at low $[Ca^{++}]_O$ in this area too (figure 10.7 and 10.9). Similar to area 1, these results suggest the above effect of isovolumic contraction at control $[Ca^{++}]_O$ could have been mediated through $[Ca^{++}]_i$ modulation which in turn could modulate I_{si} or other mechanisms.

The simplest explanation that can be suggested to explain these results at low $[Ca^{++}]_O$ would be the possibility that I_{si} is already reduced under free loaded contraction and there would be little change under isovolumic contraction to produce any further effect.

The problem as in area 1 is if changes in the kinetics of I_{si} were responsible for the changes under isovolumic contraction, the curve at low Ca^{++} should have been slower than at normal Ca^{++} , because I_{si} would be smaller and its recovery slower as described above.

Similar arguments to those described for area 1, may also apply here and one more explanation could be applied for both areas is that the reduction in I_{si} under low Ca^{++} could have decreased activation of K^+ currents, which in turn could mean less inactivation hence less need for time for recovery, that is to say less inactivation. This would entail faster recovery of K^+ currents thus membrane recovery becomes less dependent on time.

13.4.4, Low temperature perfusion (27°C).

Coraboeuf and Weidmann (1954) found that lowering the temperature depolarized the resting membrane potential in calf and sheep Purkinje fibres. Evidence, presented in chapter 7, shows that the $\text{Na}^+ - \text{K}^+$ pump to be electrogenic generating an outward current. Therefore, inhibition of the pump at low temperature in Coraboeuf and Weidmann's study could be the cause of the resting membrane depolarization. Glitsch (1972), Deleze (1960) and Page and Storm (1965) were reported by Glitsch (1982b) to have demonstrated membrane hyperpolarization (repolarization) upon rewarming in cat papillary and in guinea pig atrial muscle. The membrane potential increased rapidly and this was dihydroouabain sensitive, which suggested the activation of the $\text{Na}^+ - \text{K}^+$ pump to be responsible for it. Isenberg and Trautwein (1975) found when the temperature of the bathing solution was lowered quickly from 37° to 20°C for a period of two minutes the outward current was markedly reduced. This effect was reversed on rewarming, but absent in the presence of dihydroouabain. They suggested that part of the outward current is generated by the $\text{Na}^+ - \text{K}^+$ pump which was inhibited by cooling. Further evidence was demonstrated by Eisner and Lederer (1980a). Lowering the temperature from 36° to 26°C significantly reduced an outward current which was reactivated by the admission of Rubidium (Rb) to the K^+ - free bathing medium of sheep Purkinje fibres. Since Rb^+ was demonstrated to activate only the $\text{Na}^+ - \text{K}^+$ pump (Eisner et al 1981b), the above results suggest the pump to be depressed at lower temperature and this is responsible for the depolarization of the resting membrane potential. Lowering the temperature to around 20°-25°C has also been shown to affect the kinetics of the slow inward Ca^{++} current and the K^+ outward repolarizing currents.

The activation, inactivation and recovery of these currents were slowed at lower temperature in guinea pig papillary muscle and cat trabeculae and papillary muscle (Gettes and Reuter 1974, Kohlhardt et al 1975, McDonald and Trautwein 1978a). The fast Na^+ current was not much affected nor was the amplitudes of all the other currents. Lowering the temperature of the perfusing Krebs to 24°C prolonged APD in both area 1 and 6 as shown by the steady state durations in figure 11.2 and 11.4 as compared to those in any of the restitution curves at 34°C . This has also been observed in sheep and calf Purkinje fibre (Coraboeuf and Weidmann 1954) guinea pig atrial muscle (Thyrum 1974) sheep ventricular muscle (Cohen et al 1976) in cat papillary muscle (Boyett and Jewell 1978) and in rabbit ventricular muscle (Benndorf and Nilius 1982). But the increase in APD in the present study was very large, from about 170ms at 34°C to about 300ms at 24°C , in area 1, with a similar order of magnitude in area 6. This was due to a combined effect of lowering the temperature and decreasing the steady state rate of stimulation to 100/min. It was difficult to stimulate at 150/min because of alternans in the APD. But in all experiments increasing the rate gradually to 150/min showed shorter APD at the higher rate of stimulation, and the APD at 24°C remained longer by about 50ms than that at 34°C . The most likely explanation for this prolongation is the effect of temperature on the kinetics of the currents outlined above. The ventricular pressure seemed to be higher at lower temperature, but this was complicated by the difference in the steady state rate of stimulation, which made the explanation difficult.

(c) Area 1 Restitution Curves

From the above discussion it is conceivable that the Na^+-K^+ pump activity is depressed in this study at low temperature. It could not have been completely inhibited because the temperature was not low enough (Glitsch 1982b). The calculated recovery indices at low were much faster than those at 34°C under both mechanical conditions, at a time they were expected to be slower because of the temperature effect on the kinetics of I_{si} and the I_{k} currents as well as the depolarization of the resting potential. There are two possible explanations for the acceleration:

1. The acceleration may be due to the decrease of steady state rate of stimulation. Boyett and Jewell (1978) found large acceleration of restitution curve on changing the steady state rate from 160 to 80/min. But El-Harrar and Surawicz (1983a) have shown a small change in the kinetics of the AP restitution at 120/min compared to 40/min in dog Purkinje fibers when the APD was corrected for the downward shift by expressing the test APD as % of the steady state APD. However, they did not go to as high rate of stimulation as in my study and in that of Boyett and Jewell (1978).
2. Because of the supernormal periods, only values on the rising phase were used to calculate the recovery indices and this could have exaggerated the true time course in the faster direction.

It was interesting to see very fast restitution curves at normal and high Ca^{++} in Bass's study (1975) on cat papillary muscle under the same condition of low temperature.

The two restitution curves under free loaded and isovolumic contraction (figure 11.2) have similar recovery indices, thus the acceleration of the curve under isovolumic contraction seen at 34°C seems to be temperature dependent which is in keeping with suggestion that

the $\text{Na}^+ - \text{K}^+$ pump activity being at least partly responsible for the acceleration under isovolumic contraction. Also there was very little difference, if any at all, between the recovery indices of the electrical restitution curves at normal and 7.2mM Ca^{++} in cat papillary muscle at 23°-25°C as shown in figure 11 of Bass's study (1975) , but in rabbit heart at 34°C, Ouabain slowed the electrical restitution curves (Lab and Yardley unpublished data).

Contrary to the recovery index changes, the plateau of the isovolumic curve shifted down just as it did at 34°C, which may suggest that the mechanism responsible for the downward shift is not affected by the temperature.

The plateau of cat papillary restitution curve at 24°C (Bass 1975) shifted down at high $[\text{Ca}^{++}]_0$ in a similar way to that shown in figure 11.2 and 11.4 under isovolumic contraction. This supports the suggested accumulation of intracellular Ca^{++} being responsible, at least in part, for the downward shift. Cohen et al (1975), Noble & Cohen (1978) showed ouabain to shift the plateau of the interval - duration curve downward in sheep Purkinje and ventricular fibres with little effect on the time course, but they did not specify the temperature of the preparation.

However, their shift downward is also in favour of intracellular Ca^{++} accumulation, because Ouabain has been shown to increase intracellular Ca^{++} in various cardiac tissues, probably through an indirect effect on the $\text{Na}^+ - \text{Ca}^{++}$ exchange as a result of intracellular Na^+ accumulation after $\text{Na}^+ - \text{K}^+$ pump inhibition (Brody 1981, Langer 1981, Kass et al 1978, Burt and Langer 1982, Lee and Dagostino 1982) or through some other effect on the Ca^{++} movement (Bremner et al 1977). A feature of the restitution curves not frequently seen at 34°C but observed in all experiments under free loaded and isovolumic contraction in both areas at low temperature is the supernormal period at 400/500ms TPI's. Supernormal AP's have also been seen in rabbit papillary muscle (Gibbs and Johnson 1961, Wohlfart 1979) in cat papillary muscle (Bass 1975) sheep ventricular and Purkinje fibres (Noble and Cohen 1978) and in dog ventricular muscle (Iinuma and Kato 1978, 1979). A number of mechanisms have been put forward to explain this phenomena, and there is still limited evidence in support of each one;

1. Bass (1975) suggested an inverse relation between membrane Ca^{++} content and the area of the AP. He suggested that a transient post AP decrease in membrane Ca^{++} content, associated with elevation of intracellular Ca^{++} , is responsible for the supernormal period. If an extrasystolic AP is then interpolated shortly after repolarization of the control AP and before the released Ca^{++} had returned to the membrane and restored the pre-AP level of Ca^{++} , the extra AP will have a more positive and more prolonged plateau and greater area because of prolonged Ca^{++} current. Exaggeration of the changes in low Ca^{++} and their attenuation in high Ca^{++} in the same study supported his explanation. Contradictory to this Iinuma and Kato (1978, 1979) reported opposite effects, to the above, of low and high

$[Ca^{++}]_o$ on the supernormal AP. They found verapamil, caffeine and isoproterenol to reverse the prolongation of the premature supernormal AP, and it shortened. But they concluded, as Bass did, that augmented Ca^{++} - influx during the premature AP was responsible for the prolongation, in other words, increased I_{si} is responsible for the prolongation which was also endorsed by Hiraoka and Sano (1977).

2. Another, different, mechanism was proposed by Cohen et al (1976). They proposed that an increase in $Na^+ - K^+$ pump activity by the premature AP (either through elevated $[K^+]_e$ or $[Na^+]_i$) could lower $[K^+]_e$ below resting values transiently and the AP would be lengthened through decreasing membrane conductance to K^+ as a result. In support of their claim they found Ouabain and low temperature to abolish or reduce the prolongation. But the results in the present study and in Bass's study (1975) at $24^\circ C$ does not agree with Cohen's et al results at low temperature. The fact that Ouabain reduced the prolongation in Cohen's et al study could be attributed to intracellular Ca^{++} elevation as described above, which is in agreement with Bass's study at high Ca^{++} .

The results of curves in area 1 (also in area 6), figure 11.2, suggest that whatever the mechanism responsible for AP prolongation is, it is enhanced under isovolumic contraction. The explanation of Cohen's et al (1976) is attractive here, because all along it was suggested that the $Na^+ - K^+$ pump activity is enhanced under isovolumic contraction, but it is difficult to explain why the AP did not shorten because of increased outward current generated by the pump; instead it prolonged, and why the plateau of the supernormal AP was also elevated. The latter suggests a possible decrease in K^+ outward current or an increase in I_{si} .

It is possible that a decrease in $[K^+]_e$ overcame any other effect in producing prolongation through a decrease of K^+ conductance.

The decreased extent of shortening under isovolumic contraction may be due to lowering $[K^+]_e$ which would reduce the extent of shortening at lowering $[K^+]_e$ which would reduce the extent of shortening at short TPI. At 34°C this effect may be insignificant or absent because of different kinetics of other currents or some other unknown mechanism/s, which produce greater shortening under isovolumic contraction.

(i) Area 6 Restitution Curves

The restitution curves of this area behave in a similar way to those of Area 1. The downward displacement of the curve under isovolumic contraction may be related to an intracellular accumulation of Ca^{++} . The difference between the recovery indices does not seem to be large enough to be considered to be significant, which suggests the slowing down of isovolumic curve to be abolished at low temperature and may be $Na^+ - K^+$ pump dependent.

Also similar to area 1, the prolongation of the supernormal AP is greater under isovolumic contraction, and if Cohen's et al (1976) explanation for this turns to be right the results suggest increased activity of the $Na^+ - K^+$ pump under isovolumic contraction and during the supernormal AP at 24°C.

13.5 The T-wave of the ecg

It is well accepted that the Q-T interval of the ecg is representative of the APD and that the T-wave coincides with the repolarization phase and arises from regional differences in APD (Hoffmann and Suckling 1954, Lipeschkin 1976a). However, the polarity of the T-wave is still disputed, and two suggestions have been put forward to explain its positive direction in mammals;

1. The first was suggested by Bayliss and Starling in 1882 as quoted by Noble and Cohen (1978). It was proposed that the longer AP at the base in comparison to that at the apex is responsible for the positive T-wave.

2. The second interpretation by Spach and Barr (1975) suggests a gradient of repolarization between endocardium and epicardium APD to be responsible for the positive polarity which implies recovery direction from epicardium to endocardium i.e. opposite the depolarization sequence.

In the present study the ecg changes were used as a correlative study to corroborate the changes observed in APD under isovolumic in comparison to under free loaded contraction. Changes in Q-T intervals are considered to represent changes in APD while changes in the amplitude of the T-wave are considered to describe regional differences in APD behaviour. It must be borne in mind that the areas from which the ecg was recorded were not the same areas as those for the AP's, therefore, the results must be interpreted with caution.

If the AP is shorter under isovolumic contraction we would expect the Q-T interval to be shorter, such shortening of the Q-T can indeed be seen in figure 12.6 and figure 12.8, unfortunately in those experiments from which ecg was recorded the change in the Q-T interval with control Krebs was not large enough at 34°C to be seen, but because the change is much greater with high K^+ it can be clearly seen as in figure 12.6. However, the change in APD under isovolumic contraction in insitu pig heart was large enough to reflect shortening of the Q-T interval as seen in figure 12.10a, b. But the no difference in APD between free loaded and isovolumic contraction at low Ca^{++} is supported by the similarity of the Q-T intervals under the two mechanical conditions in figure 12.3 and figure 12.7.

The regional differences in AP shortening produced by changing to isovolumic contraction in the isolated rabbit heart is reflected by the T-wave depression in figure 12.1 with control Krebs and by its enlargement with high K^+ in figure 12.2 and figure 12.6. The T-wave also became smaller in the epicardial ecg of the intact insitu pig heart as shown in figure 12.9. Similar T-wave depression of the epicardial ecg has been observed in intact frog and intact insitu pig heart (Lab 1982). The change in the T-wave is gradual in the present study and in Lab's (1982) which endorsed the slow change in APD shortening after changing to isovolumic contraction. In those experiments in which the reversibility of the T-wave change was examined showed a reversible changes when going back to free loaded contraction, also the changes in the T-wave and in the Q-T intervals were reproducible in the same preparation as mentioned in the results section.

Changes in the mechanics of contraction produced by increased after load or preload in intact hearts have shown to be accompanied by changes in the T-wave. In rats made hypertensive, the T-wave was seen to be depressed (Keung and Aronson 1981). Tuvemo (1982) observed T-wave inversion in the precordial leads in seven to eight year old children under valsalva manoeuvre. Although no attempt was made to explain these observations a mechanical factor contributing to these changes is a real possibility. More important observation was made by Goldberger (1980). He described deep none-infarctional T-wave inversions occurring after aortic valve replacement for severe aortic regurgitation in human patients which were suggested to reflect the effect of converting pre-operative diastolic overload state to a post-operative systolic overload, thus implicating some types of mechanical changes. Close inspection of his results also showed a shortening of the Q-T intervals in all leads in most cases (figure 1 and 4 of Goldberger study 1980). These changes were most marked in the immediate post-operative period, but regress in the period following the valve replacement. If the above changes were indeed due to mechanical effect they support my results on the isolated rabbit heart and insitu pig heart in that changing the mechanics of contraction could have an effect on APD which seem to vary according to the region on the heart.

Finally, the well known effect of increasing the rate of beating on APD shortening must be accompanied by shorter Q-T interval if there is a close relation between APD and the Q-T interval.

This is indeed seen in figure 12.6, 12.7, 12.8 and 12.10 under free loaded and isovolumic contraction at all perfusion conditions in the isolated rabbit and under both conditions of contraction in the pig heart. Furthermore, the regional differences in AP shortening after a sudden change in rate under free loaded from that under isovolumic contraction is illustrated by the different changes in the T-wave of the first beat with respect to control T-wave under the two mechanical conditions in isolated rabbit as well as in insitu pig heart. At low Ca^{++} there was no difference between free loaded and isovolumic contraction in AP behaviour as seen by the restitution curves(chapter 10). This is supported by the identical behaviour of the Q-Tinterval and the T-wave as shown in figure 12.3 and 12.7. In this preparation a sudden change in rate after a long AP produced T-wave inversion but after a short AP (not shown) the T-wave did not invert but behaved identically under both mechanical conditions. Notice that the shortening of the steady state APD at high K^+ was accompanied as expected by shorter Q-Tinterval (compare the steady state Q-Tinterval under free loaded contraction in figure 12.5 with that in figure 12.6, taking into consideration the difference in the scale). This is in agreement with results reported by Surawicz et al (1959) on isolated rabbit and by Savigny et al (1981) on isolated guinea pig heart. It is also known to happen that during hyperkalema in human (Katz 1977). Also low Ca^{++} and low temperature are accompanied by longer Q-T intervals just as the AP was longer (compare figures 12.3 and 12.8 with figure 12.5 noting the difference in the scale).

This too has been seen in isolated rabbit (Surawicz et al 1959) in turtle and frog ventricles (Ashmen et al 1945 and Mines 1913 as quoted in Noble and Cohen 1978) in isolated guinea pig heart (Savigny et al 1981) as well as in human during hypocalcemia (Katz 1977).

Chapter 14

Summary and conclusions (see table 14.1)

The effects of changing the mechanics of contraction on APD and its behaviour in response to rate changes have been studied in different regions on the intact left ventricle of the isolated rabbit heart and intact left ventricle of pig heart in situ. An attempt was made by constructing the restitution curves to characterise the possible mechanisms through which the mechanics of contraction can produce the electrophysiological changes. Also the effects on the T-wave and Q-T interval of the ecg have been briefly investigated and used to support the changes obtained in APD and its regional difference of behaviour under free loaded and isovolumic contraction.

The results show that under isovolumic contraction the duration of AP at 70% repolarization shortens in most areas and the plateau of the restitution curve is shifted downwards. Depending on the area studied, the recovery index, which is a reflection of the time course of the rising phase of the restitution curve, could either be speeded up or slowed. The downward displacement of the restitution curve is suggested to be brought about by intracellular accumulation of Ca^{++} possibly produced by a change in the sarcolemma permeability to Ca^{++} , hence Ca^{++} flows down its concentration gradient into myocardial cells. Elevated $[\text{Ca}^{++}]_i$ can modulate APD via several ways:-

1. Increasing a Ca^{++} dependent K^+ outward current (rectifying current).
2. Modulation of I_{si} .

	Recovery index 'ms'			Effect of isovolumic contraction	Downward shift of curve plateau under isovolumic contraction	Shortening of steady state APD under isovolumic contraction
	Perfusion conditions	Area	Free-loaded contraction	isovolumic contraction		
Rabbit heart	Normal Krebs	A1	670	416	Acceleration	Yes
		A6	144	247	Slowing	No
	High K ⁺	A1	48	59	-	Yes
		A6	50	80	Slowing	Yes
	Low Ca ⁺⁺	A1	43	30	-	No
		A6	77	58	Acceleration	No
	Low temperature 24°C	A1	35	33	No difference	Yes
		A6	77	65	Small acceleration	Yes
	in situ pig heart	Base	-	-	Slowing	Yes
		Apex	-	-	Slowing	Yes

TABLE 14.1 Summary of all the results

3. Modulation of $\text{Na}^+ - \text{Ca}^{++}$ exchange and $\text{Na}^+ - \text{K}^+$ pump in the right direction. Both processes seem to generate outward current during activity.

The acceleration of the membrane recovery could be brought about by increased activity of the $\text{Na}^+ - \text{K}^+$ pump and probably also the consequent $\text{Na}^+ - \text{Ca}^{++}$ exchange. Both could contribute towards faster restoration of the steady state condition. Failure or reduction of the above process in some areas of the epicardium may allow the effects of other mechanisms that may slow I_{si} and I_{k} recovery to prevail thus slow the membrane recovery. The above suggestions are supported by results obtained at high K^+ , at low Ca^{++} and at low temperature. At high K^+ the $\text{Na}^+ - \text{K}^+$ pump activity can be expected to increase (Ellis 1977, Martin and Morad 1982). The results showed the differences between the plateaux of curves under both conditions were exaggerated in both areas at high K^+ , but the differences in the membrane recovery were reduced. The fact that the isovolumic ventricular pressure was higher at high K^+ compared to normal K^+ suggests greater increase in $[\text{Ca}^{++}]_{\text{i}}$, hence greater downward shift of the curve's plateau.

The disappearance of the downward shift in both areas at low Ca^{++} is in support of the above proposed increased $[\text{Ca}^{++}]_{\text{i}}$ to be responsible for the downward shift. Also the reduced differences in the recovery indices could suggest that the acceleration of

membrane recovery under isovolumic contraction is also Ca^{++} dependent: perhaps via its effect on the $\text{Na}^+ - \text{Ca}^{++}$ exchange and the $\text{Na}^+ - \text{K}^+$ pump.

It was possible at low temperature to dissociate those mechanism/s responsible for the downward shift from those responsible for the changes in membrane recovery. The fact that there was no difference between the two areas in the recovery index when comparing the two mechanical situations, suggests the curve acceleration to be temperature dependent, which could be due to the activation of the $\text{Na}^+ - \text{K}^+$ pump.

From these results I, therefore suggest that the mechanism/s responsible for the downward shift of the restitution curve plateau is/are different from those responsible for changes in the recovery index. Very recently El Harrar et al (1983a) described the restitution of action potential as the sum of two exponentials - a fast ($T_1 = 23.8\text{ms}$) and slow ($T_2 = 132\text{ms}$). They found that inhibition of I_{Na} by TTx or Lidocaine significantly and selectively increased T_2 . In other words to slow the restitution of action potential recovery. Furthermore, they dissociated between changes in steady state APD and the kinetics of the AP restitution, as concluded above in this study. However, in this study it is not possible to rule out any other effects that may involve the production of a metabolic or any other substance that may change the membrane permeability to ions under isovolumic contraction. It is further suggested that the regional differences in the steady state AP shortening could be attributed to

It is further suggested that the regional differences in the steady state AP shortening could be attributed to different degrees of stretch under isovolumic contraction thus producing regional differences in intracellular Ca^{++} accumulation. The regional differences in behaviour in response to a sudden change in rate may be related to regional differences in the pattern of mechanical shortening under isovolumic contraction. Changing the mechanics of contraction to isovolumic in insitu dog heart has been seen to result in regional differences in stretch and in mechanical shortening (chapter 5). Regional differences of pure mechanical distortion of the sarcolemmal membrane and

conformational changes of membrane bound enzymes due to stretch or increased wall stress is possible. Young (1973) demonstrated a change in acetylcholinesterase activity of frog heart produced by increasing the hydrostatic pressure in the chamber containing the heart, and the regional differences in Ca^{++} -ATPase activity between left and right ventricle and between endo-and epicardium have been attributed to stress and strain differences, thus implicating mechanical factors (Schiaffino et al in the press, Gorza et al 1981). Goldberg (1977) in a review described a number of mechanical factors including changes in afterload and preload that can effect the surface ecg especially the T-wave in humans. Changes in the ecg are most likely to reflect changes in AP of the myocardial cells.

It may also be the case that different Ca^{++} stores are functional under different conditions of contractions. Wendt-Gallitelli and Jacob (1982) obtained evidence in guinea pig papillary muscle to suggest different Ca^{++} stores releasing Ca^{++} in a different mode to be responsible for the different pattern of contraction under

different rhythms and rate of stimulation. In the context of these findings, Jones and Cala (1981) obtained the first evidence for functional and structural heterogeneity of cardiac sarcoplasmic reticulum SR).

In conclusion, mechanics of contraction can affect the electrophysiology. There seem to be no single mechanism that can adequately explain the mechanical influence on the AP in different regions of the heart when changing from free loaded contraction to isovolumic contraction and back. It is possible that several mechanisms operate to different degrees in different regions namely:-

1. Changes in the currents responsible for the AP repolarization e.g. I_{si} and a Ca^{++} dependent K^+ current.
2. $Na^+ - K^+$ pump.
3. $Na^+ - Ca^{++}$ exchange.

This is not strange in the light of the recent evidence showing the complexity of the electrophysiological (chapter 1), biochemical and mechanical (chapter 5) regional differences in the left ventricle as well as the complexity of the action potential duration restitution.

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