THE EFFECT OF ADRENALINE ON MECHANICAL ACTIVITY AND ELECTRICAL EVENTS IN FROG VENTRICULAR MUSCLE

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by

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INTRODUCTION

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The action of catecholamines on cardiac muscle has long been of interest to research workers. Early papers dealt purely with the visible differences of force and rate of contraction produced by adrenaline, but with the improvement in research techniques it became possible to look for the underlying mechanism of these effects. This problem has been approached in three main ways, (a) the biochemical action, (b) the effect on the contractile element of the cell, and (c) the effect on electrical activity in individual cells.

The adrenaline effect on biochemical processes in the cell has now been well analysed (see later for details and references) and, although there is still some doubt, the correlation between these changes and the effect on contractile activity is becoming clearer. It appears at present that the biochemical events leading to increased glycogenesis in the cell are not, except perhaps at a very early stage in the process, concerned with the increase in contractile force (Øye, 1965; Williamson, 1966).

A.V. Hill showed in his classical series of papers that the mechanical part of a muscle could be described as consisting of a contractile element where the power of the muscle was generated, and passive elastic elements aligned both in series and in parallel with the contractile structure. When the muscle was stimulated the contractile element rapidly developed tension, the so called active state. He showed that the twitch tension developed was a function both of the velocity of onset and of the duration of the active state. Applying this concept to cardiac muscle (cat papillary muscle preparations) both Podolsky (1962) and Sonnenblick (1962) have shown that the positive inotropic effect of catecholamines was associated with a reduction in the time to reach peak tension of the twitch. This meant that the duration of the active state was reduced, and that the increased twitch strength was due to an intensification of the tension developed by the contractile element. Naylor (1960) however, found that adrenaline increased

the time to peak twitch tension in toad ventricular strips.

Rather inconsistent observations have been reported concerning the effects of catecholamines on the duration of the cardiac action potential. Trautwein (1963) reviewed the literature and noted that most workers found a prolongation of the action potential after exposure of cardiac tissue to adrenaline. It is now well known that the duration of the action potential controls the duration of the active state in the contractile process, acting roughly as a switch to start and stop mechanical activity (the evidence is well reviewed by Woodbury, 1962 and Brady, 1964). Thus the adrenaline effect on the action potential would be expected to lead to an increase in the duration of the active state.

As quoted above, however, both Podolsky and Sonnenblick found a reduction in the time to peak twitch tension. Therefore, according to the present evidence, excepting Naylor's work, adrenaline causes an adjustment of the mechanism controlling the active state duration and yet the duration time moves

in the opposite direction to that expected.

The development of tension in contractile tissue after exposure to solutions containing excess amounts of potassium has long been recognised and used as a research tool. Lamb & McGuigan (1965) have developed a superfusion technique which allows more accurate examination of this phenomenon in frog ventricular muscle. In an attempt to throw some light on the complex picture described above, the effect of adrenaline on these potassium contractures was examined. This, however, produced what seemed to be a further complication in that adrenaline, contrary to expectation, caused a reduction in the contracture tension produced by each specific concentration of excess potassium.

This thesis therefore deals with the further examination of the adrenaline effect on potassium contractures and also of the effect on twitch duration and electrical activity, using frog ventricular muscle as the experimental tissue.

HISTORICAL SURVEY

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The action of adrenaline on cardiac contraction

In 1884 Gaskell published the results of his research into the anomalous finding of early workers, that although stimulation of the vagus nerve in frogs usually slowed the heart rate, occasionally the opposite happened. He showed firstly (1884a) that crocodiles had separate sympathetic accelerator cardiac nerves, and then that the frog 'vagus' contained sympathetic fibres. By exposing these sympathetic fibres at their origin at the base of the brain and stimulating them he could consistently produce acceleration of the heart rate. His second paper (1884b) enumerated the actions of the sympathetic nerves on the frog heart; they produced augmentation of the rate, strength of contraction and conduction power and excitability of the cardiac muscle.

Oliver & Schäfer (1894, 1895) were first to systematically examine the effects of 'extracts of the suprarenal capsules' on intact animals and also on isolated heart and skeletal muscle. They

showed that the extract increased the rate of activity and the force of contraction of the frog ventricle, especially if the heart had become hypodynamic. It also increased the number of twitches in each burst of activity (Luciani's groups) and abolished the 'staircase' effect at the beginning of these groups. Incidentally, they found the first evidence of the reflex baroreceptor control of heart rate in intact animals, being able to reverse the inhibition of the heart rate usually produced by adrenaline if they first sectioned the vagi.

Soon after this the active principle of the adrenal extract was isolated and called epinephrin by Abel (1900) in America, suprarenin by Fürth (1900) in Germany, and adrenalin by Takamine (1901) in England. Elliot after a series of experiments with the pure adrenaline (1904, 1905), found that its effect was not only similar to that of suprarenal extract, but also closely mimicked sympathetic activity. He was so struck by this latter finding that he postulated that adrenaline was the substance

liberated at sympathetic nerve endings. This idea was not accepted until 1921 when Loewi produced further evidence in favour of humoral transmission at nerve endings. He collected the fluid perfusing a frog ventricle during sympathetic stimulation and found that if he then exposed another ventricle to this fluid its rate and force of contraction were increased. This he said was due to the presence in the fluid of a substance liberated by the sympathetic nerves which he called 'Acceleransstoff.'

Next Cannon & Bacq (1931) produced increased rate and force of contraction of a denervated heart by stimulating the sympathetic nerves to the hind limb. This response could be reduced but not abolished by clamping the inferior vena cava, and was augmented by giving the animal ergot prior to the experiment. From this they deduced that a hormone was involved in the production of sympathetic effects in an organ. They noted the similarity of these effects to that of adrenaline, but called their hormone 'sympathin.' Later Cannon & Rosenblueth (1933) stated definitely that the hormone was not

adrenaline and proposed that it was present in two types in sympathetic nerves, 'sympathin E' and 'sympathin I' being responsible for the excitatory and inhibitory effects of sympathetic stimulation respectively.

Around this time there was considerable interest in the ability of other amines to mimick sympathetic nervous activity. The findings of various workers have been reviewed by Ahlquist (1948). He himself compared the activity of the six most important amines, including adrenaline, noradrenaline and isoprenaline in producing sympathetic-like responses in various tissues. As a result of his findings he was able to put forward a receptor theory to explain the opposite actions of the same drug in different sites.

Most important, however, from the point of view of this thesis, was that his results showed that qualitatively catecholamines and sympathetic stimulation produced similar responses in cardiac muscle. Despite numerous other investigations using more sophisticated techniques in recent years, these

responses remain essentially as described by Gaskell, Oliver & Schäfer and Elliot.

The biochemical actions of adrenaline

The first indication of an underlying mechanism of adrenaline action came from Barcroft & Dixon (1907) and Evans & Ogawa (1914) who noted that adrenaline increased the rate of oxygen utilisation of cardiac muscle. From this they deduced that adrenaline increased the metabolic rate of the tissues; Evans & Ogawa found this to be the case even when the mechanical work of the heart was unaltered. Recently Sonnenblick, Ross, Covell, Kaiser & Braunwald (1965) showed that in a heart-lung preparation after infusion of noradrenaline the increased myocardial oxygen usage correlated closely with the increased velocity of ventricular contraction, i.e. with the parameter related to the increased activity of the contractile element.

Evidence for a metabolic effect of adrenaline also came from other sources. Drummond & Paton (1904) using histological and chemical techniques found that injection of large doses of adrenaline into

rabbits caused a reduction in the glycogen content of the liver. Patterson & Starling (1913) and Cruikshank (1913) also showed that exposure of a heart-lung preparation to adrenaline increased the utilisation of the available blood glucose and also reduced the tissue glycogen stores quickly. Other investigators noted similar results (reviewed by Cori & Cori, 1928b) but not until the carefully controlled experiments of the Coris (1928a, b and c) was it shown without doubt that adrenaline reduced muscle glycogen and that the hyperglycaemia produced was due to a decreased peripheral utilisation of glucose rather than to increased liver glycogenolysis. This work stimulated intense interest in the problem of tissue glycogenolysis and how adrenaline affected the process.

The literature on this subject is exhaustive, but it has been reviewed thoroughly by Ellis (1959), Sutherland & Rall (1960) and most recently by Haugaard & Hess (1965). It was shown that the breakdown of glycogen to glucose-1-phosphate required the presence of an enzyme, phosphorylase. This

enzyme is present in the cell in active phosphorylase a and in inactive phosphorylase b forms and the rate of glycogenolysis depends on the rate of activation of phosphorylase b. Sutherland and his associates then discovered the substance cyclic 3', 5' AMP which increased phosphorylase b conversion to active phosphorylase a and finally that the formation of 3', 5' AMP from ATP depended on a series of enzymatically controlled reactions occurring in or near the cell membrane. These enzymes have not yet been fully elucidated and are at present collectively called the adenyl cyclase system. It has now been shown that adrenaline increases the activity of this adenyl cyclase system, thus making available more 3', 5' AMP for activation of phosphorylase. This explains the increased glycogenolysis found after exposure to adrenaline.

Although this mechanism has been thus fully worked out, considerable controversy has existed as to the cause of the inotropic effect of adrenaline. It has been postulated that the

inotropic effect is purely secondary to an increased formation of high energy phosphate compounds as a consequence of the increased glycogenolysis. However, many workers have not been able to find any correlation between the inotropic effect of adrenaline and the increased phosphorylase activity, a common finding being that small doses of adrenaline which produced inotropic responses had no measurable effect on the phosphorylase a content of the myocardium, (see Haugaard & Hess, 1965).

A recent study of this problem by Øye (1965) has cast some light on the problem. He showed that an almost immediate effect of adrenaline on the cell was to increase the amount of 3', 5' AMP present. Next, with a time lag of several seconds, he noted firstly an augmentation of the twitch tension and at the same time an increase in phosphorylase b kinase activity (the enzyme catalysing conversion of phosphorylase b to the active a form). Finally, there is an increase in the formation of phosphorylase a but this is definitely a later phenomenon than the

inotropic effect. As a result of these observations Øye believes that both the phosphorylase and the mechanical responses to adrenaline are triggered by the increase in 3', 5' AMP activity, although there is no conclusive evidence in favour of the latter statement. Williamson (1966) has similarly shown that the inotropic effect of adrenaline occurs at the same time as there is a marked increase in 3', 5' AMP content in the cell, both preceding clearly any increase in phosphorylase activity. He also believes that the increase in 3', 5' AMP activity is causing in some way the inotropic effect.

The effect of adrenaline on electrical activity in cardiac muscle

The only consistent findings reported for the effect of sympathetic stimulation or sympathomimetic amines on electrical activity in cardiac muscle have been the actions on pacemaker and conducting tissue. Pacemaker cells whether from frog sinus venosus (del Castillo & Katz, 1955;

Hutter & Trautwein, 1956) or mammalian sino-atrial node (West, Falk & Cervoni, 1956; Trautwein, 1963) show a characteristic response. The slope of the pacemaker potential of these cells is increased, which results in shorter beat intervals as neither maximum diastolic membrane potential nor threshold are appreciably affected. A similar effect is seen in excised Purkinje fibres (Otsuka, 1958; Trautwein, 1963). Addition of adrenaline to fluid bathing quiescent Purkinje tissue leads to a depolarisation of the membrane potential, which if large enough sets up spontaneous pacemaker activity. Gargouil, Tricoche, Fromenty & Coraboeuf (1958) noticed that there was often a slight hyperpolarisation of the resting potential after the initial depolarisation, which increased the amplitude of the action potential, and also the plateau of the action potential was prolonged. This latter finding was most pronounced in a hypodynamic heart.

The results of research into the effect of sympathetic action or catecholamines on atrial and

ventricular membrane potentials have been listed in an excellent review by Trautwein (1963). He, with Dudel (1957) found that adrenaline gave a significant dose-dependent hyperpolarisation of the resting membrane potential of dog auricular fibres. A similar effect was noted by Sin-Ya (1961) in frog ventricular muscle. In opposition to this Webb & Hollander (1956) found a slight depolarisation of the resting potential in rat atrial fibres after adrenaline, although their normal resting potential values were lower than those commonly found by other workers.

The effect of adrenaline on the duration of the action potential has been reported variably. It has been found to be slightly shortened in dog and cat atrial tissue and dog right ventricular fibres (Brooks, Hoffman, Suckling & Orias, 1955; Hoffman & Cranefield, 1960) and in embryonic chicken cardiac muscle (Fingl, Woodbury & Hecht, 1952). Prolongation has been shown in atrial fibres from rat (Webb & Hollander, 1956) guineapig (Furchgott, Sleator & de Gubareff, 1960) and rabbit

(Hoffman & Cranefield, 1960) and also in frog ventricle (Lueken & Schütz, 1938). As stated before Gargouil et al (1958) and also Trautwein & Schmidt (1960) noticed that adrenaline produced prolongation of the action potential most markedly in a hypodynamic heart, where the action potential duration is less than normal. Adrenaline also produces a marked lengthening of the action potential when it has first been reduced and shortened by increasing the external potassium concentration (Antoni & Engstfeld, 1961).

To summarise therefore it can be seen that to date the action of adrenaline on electrical activity in cardiac muscle remains dubious in view of the multiplicity of conflicting results. It seems likely, however, that the effects on the hypodynamic heart, in that they attempt to restore 'normality' to the tissue, may represent the true physiological response of cardiac muscle to sympathetic activity and catecholamines.

The dynamics of muscular activity

Gasser & Hill (1924) carried out the first

fundamental research into the dynamics of skeletal muscular contraction. With their results they were able to formulate a viscous-elastic model of the contractile structures of the muscle cell and they showed a relationship existed between speed of shortening and tension developed. Hill then pursued this topic more closely using both mechanical and thermal experimental procedures (see Bibliography for papers). He showed that a contracting muscle behaved as though it consisted of a contractile element which produced the tension of the muscle, with passive elastic components lying both in series and in parallel to it. When the muscle was stimulated the contractile element rapidly developed tension; this he called the active state. The tension of the active state remained at a plateau level for a certain time then decayed slowly. Because of the series elastic element, the tension developed by the contractile part did not appear wholly as external work, as some of it was taken up in the initial stages of the contraction in lengthening the elastic structures. By the time

this was completed the active state had begun to decay so the observed external force was less than the maximum tension developed. Hill also found that the twitch tension was a function both of the velocity of onset and of the duration of the active state.

In skeletal muscle it was found difficult to alter the velocity of onset of the active state, except by altering the external temperature and therefore inotropic interventions produced their effects mainly by controlling the duration of the active state. Goffart & Ritchie (1952) found that noradrenaline increased twitch tension by prolonging the active state. Hill & Macpherson (1954) showed a similar cause for the increased force of contraction produced by substituting bromide, iodide or nitrate for chloride in the bathing fluid. Edwards, Ritchie & Wilkie (1956) found this also and noted that the order of efficiency of these anions in prolonging the time to peak tension was the same as their ability to extend the duration of the action potential.

Abbot & Mommaerts (1959) were the first to apply Hill's concepts to cardiac muscle. They studied the underlying dynamics of the positive inotropic effects of the 'staircase' phenomenon and of repeated postextrasystolic potentiation and found that the velocity of onset of the active state was increased but that its total duration was reduced. Therefore it was postulated that the increased twitch tension in these cases was due to an intensification of the active state, giving as an integral part of this a more rapid onset of peak tension.

Naylor (1960) studied inotropic responses in toad ventricular strips and found (a) that increasing the external calcium concentration, cardiac glycosides and 'staircase' potentiation were all associated with a reduction in the time to peak tension and (b) that noradrenaline, reserpine and reduced external potassium concentration gave unaltered or increased time to peak tension. The latter findings suggest that the inotropic effect produced by these substances was due to an increased duration

of the active state rather than to increased tension development in the contractile element. However, Podolsky (1962) and Sonnenblick (1962a, b) found in cat papillary muscle preparations that catecholamines reduced the time to peak tension, this suggesting that the duration of the active state might be reduced.

Therefore at present two completely different views are held as to the mechanism of the positive inotropic effect of catecholamines on cardiac contraction. This may be due to a difference in response of heart muscle from cold and warm-blooded species but obviously further investigation of this point is necessary.

Potassium contractures in excitable tissues

As mentioned in the introduction exposure of contractile tissues to high concentrations of potassium will cause development of tension which is sustained much longer than in an ordinary twitch. Burridge (1914) showed that excess potassium concentrations up to 0.2% stopped a perfused frog heart in diastole, but that higher

concentrations (5.0 - 30.0%) produced a contracture which fell off slowly even with continued exposure to the contracture solution. He recognised that the excess potassium was not acting on the contractile mechanism but must be having an effect at the cell surface.

This concept of a membrane effect was given further support by Fischer (1924) who showed that production of a contracture in skeletal muscle was associated with a reduction in the measured injury potential and that this reduction was a function of the potassium concentration. At this point in the historical sequence it should be noted that Okamoto (1924) who examined potassium contractures in skeletal muscle in some detail, found that 1:10,000 solutions of adrenaline reduced the developed contracture tension for a specific potassium solution.

The next important stage in the elucidation of potassium effect on contractile cells came in 1946, when Kuffler, using single muscle fibres, demonstrated depolarisation of the cell membrane by excess potassium. Above a threshold level which he was

unable to fix with certainty, this depolarisation was associated with the development of contracture tension. Finally, Hodgkin & Horowicz (1960) examined the quantitative effect of potassium on membrane potential and mechanical tension in single skeletal muscle fibres and they were able to make the following statements: (a) increasing the external potassium to 100.0 mM caused the development of 2.0 - 4.0 Kg/cm^2 tension in a fraction of a second, (b) contracture tension was related to log. external potassium or membrane potential by a steep S-shaped curve and (c) the membrane potential at which tension started to develop was about -50 mV corresponding to an external potassium concentration between 20.0 - 30.0 mM.

Potassium contractures in frog ventricular strips were examined closely by Niedergerke (1956). He noted the relationship between membrane depolarisation and tension developed over a range of potassium concentrations from 2.5 - 100.0 mM. Using these contractures as an experimental tool he was able to demonstrate the dependence of mechanical tension on

calcium ion, being able to increase the contractile response for a particular depolarisation by increasing the external calcium concentration. In later papers with Lüttgau (1957, 1958) he showed that a similar potentiation of contracture tension could be obtained by reducing the external sodium concentration and that the contracture tension was roughly a function of the ratio between the external calcium concentration and the square of the external sodium concentration, i.e. the $[Ca^{++}] : [Na^{+}]^2$ ratio.

A factor limiting the use of contractures in frog ventricular strips as an experimental tool is the problem of diffusion to the centre of the strip. More recently Lamb has used a new technique for examining events in frog ventricular muscle (Lamb & McGuigan, 1965). This method gives very much smaller diffusion distances than had been previously possible, combined with a high perfusion rate and therefore allows rapid alteration of the ionic environment of all the cells. Lamb has re-examined the relation between excess potassium,

membrane potential and contracture tension in frog ventricle in this way (Lamb & McGuigan, 1965; Lamb, 1966). The relation takes the form of a steep sigmoid curve, with a threshold for development of contracture tension around 40.0 mM excess potassium. Increasing the potassium concentration above 200.0 mM does not produce much increase in contracture tension (for further details see Results section).

METHODS

METHODS

General details

The experimental tissue used in this study was frog ventricle. The frogs were unselected as to species (*Rana temporaria* and *Rana pipiens*) or sex. Experiments were carried out in all months of the year and no readily apparent seasonal variation was noted. The frogs were brought from the cold storage tank immediately before use, killed by decapitation and pithing, and the hearts were quickly exposed and excised. They were then used to measure (i) mechanical activity in a superfused preparation, (ii) electrical events in ventricular strips, or (iii) ion fluxes across the membrane using an ordinary perfused preparation and radioactive isotopes.

Solutions. The composition of the solutions used is shown in Table 1. The following points should be noted:-

(i) glass distilled water and 'Analar' (B.D.H.) reagents were used in all solutions.

(ii) the phosphate buffer, a mixture of Na_2HPO_4 and NaH_2PO_4 was calculated to give a pH of 7.2 (Adrian, 1956).

Composition of Solutions used

Solution	Title	Concentration in mM						Sucrose g/litre	Relative Tonicity
		Na^+	K^+	Cl	HPO_4^{--}	$H_2PO_4^-$	Glucose		
A	Normal Ringer	120.0	2.5	121.1	2.15	0.85	5.55	-	1.00
B	K for Na Ringer	5.0	117.5	120.1	2.15	0.85	5.55	-	1.00
C	Ringer + 200.0 mM KCl	120.0	200.0	317.6	2.15	0.85	5.55	-	2.53
D	Ringer + excess sucrose	120.0	2.5	121.1	2.15	0.85	5.55	123.0	2.53
E	Na free Ringer	5.0	2.5	5.1	2.15	0.85	5.55	73.1	1.00

Table 1

Composition of solutions used. The final column of the table gives the relative tonicity of the solutions, taking the tonicity of normal Ringer to be 1.0.

(iii) the effective osmotic pressure of added sucrose was calculated from the equation of Dydyńska & Wilkie, (1963) :-

$$\theta = \frac{1000x}{342.0 \times 0.997} \left[\frac{0.094x}{342.0 \times 0.097} + 1.0 \right]$$

where,

θ = the effective osmotic strength in m-osmoles/ kg water,

x = the amount of solid sucrose added in grams.

(iv) intermediate concentrations of excess potassium were made by mixing the appropriate amounts of solutions C and D. This meant that these solutions were iso-osmotic, with a tonicity equal to that of Ringer + 200.0 mM excess KCl.

(v) the calcium concentration of all the Ringer solutions was 1.0 mM unless otherwise stated. The range of calcium concentrations used was 0.0 - 7.0 mM.

(vi) in a few experiments a Ringer was used where the chloride was replaced by equimolar amounts of sulphate. This was done either by adding the correct amounts of K_2SO_4 and Na_2SO_4 with the addition of solid sucrose to maintain normal tonicity, or by using

the benzene sulphonate salts of Na and K. In the Ringer using the normal sulphate salts it was necessary to add excess calcium to saturation.

Drugs. All drugs were made up as stock solutions and stored at 7° C. The following were used in this study:-

Adrenaline: the stock solution was Evan's Medical 1:1000 multi-dose vial.

Noradrenaline: the stock solution, 1 X 10^{-3} g/ml, was prepared from solid L-noradrenaline bitartrate (Koch-Light Laboratories).

Isoprenaline: a 1 X 10^{-3} g/ml solution was made as the hydrochloride from solid D, L-N isopropyl-noradrenaline (Aldrich Chemical Co., Inc.).

Pronethalol: solid 'Alderlin' (I.C.I.) pronethalol HCl was used to prepare a 1 X 10^{-2} g/ml stock solution.

Ouabain: a 1 X 10^{-3} M stock was prepared from ouabaine glucoside (Laboratory Nativelle, Ltd.).

All stock solutions were discarded after 3-4 weeks and replaced. Final dilutions were made immediately before use and these solutions were not used for longer than 15 min. It is well known that dilute

solutions of adrenaline and the other adrenergic amines are especially prone to oxidation, but no loss of potency due to this was noticed in 15 min.

Mechanical activity

The effect of adrenaline and other drugs on the tension developed by frog ventricular muscle was examined under various conditions. The ventricle was attached to an isometric recording transducer whose output was displayed on a recording unit. The ventricle was driven at a fixed rate and superfused from above by the solution under test.

The superfusion technique. The heart was excised and the ventricle was separated from the rest of the cardiac tissue by a cut through the base of the ventricle at the atrioventricular junction. The ventricle was then placed upright on the cut edge and divided into anterior and posterior halves by a vertical cut through the apex. The half to be used was washed clean of blood, then mounted on a small wax block with its luminal surface uppermost. An entomological pin was pushed through each corner of the base, fixing it to the block and the apex was

connected by Arbrasilk 4/0 braided silk thread to the transducer.

The half ventricle could then be perfused with a jet of Ringer by gravity feed (Fig. 1). The fluid overflowed from the ventricle into a gutter around the wax block and was removed by suction. The Ringer solutions were kept in reservoirs mounted at the same height above the preparation (60-90 cm). Rubber tubing leading from the reservoirs terminated in a length of glass delivery tubing (inside diameter 1.5 - 2.0 mm) which passed through a Perspex block above the preparation bored to fit the glass tubing closely. The flow rate and the position of the tube were adjusted so that the ventricle was perfused adequately with the minimum flow rate, usually 1 ml/min. It has been calculated from the radius of the jet that it strikes the surface of the ventricle at about 760.0 dynes/cm^2.

Detailed analysis of the physics of this preparation has not been made, but the following points appear correct:-

(i) between the point of impact of the jet and

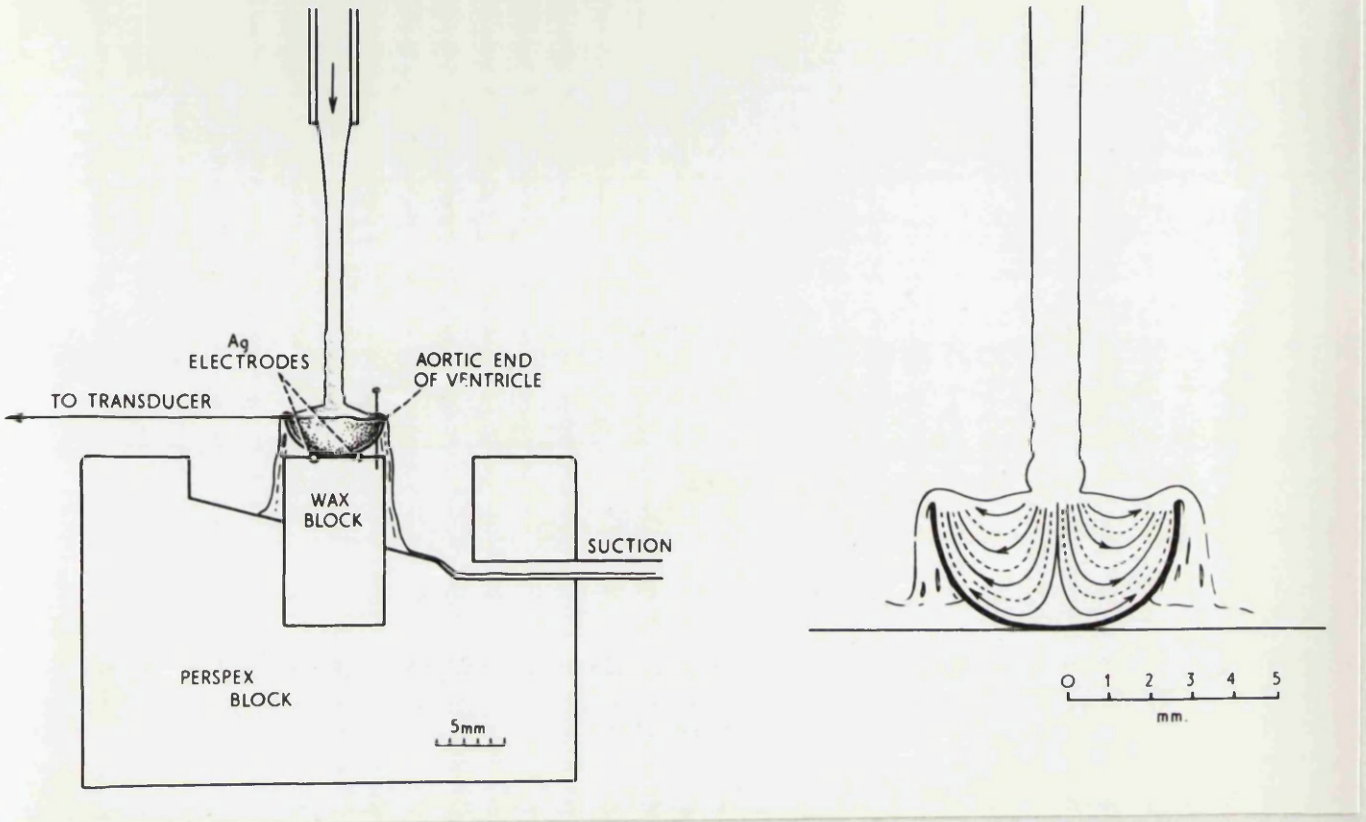

Fig. 1

(a) the left hand diagram shows the experimental arrangement. A half ventricle is pinned down to the wax block by two entomological pins through the base. The apex is then connected to the transducer by a silk thread.

(b) suggested route of fluid through the ventricle. Note the reflected waves on the perfusing fluid.

the exit over the edge of the ventricle potential energy is converted into kinetic energy and so the fluid must accelerate. This explains the constriction of the jet just before it hits the ventricle and also the reflected waves up the jet as seen in Fig. 1a.

(ii) the fluid is thought to pass through the ventricle as shown in Fig. 1b, moving at high velocity between the strands of cells but exerting little pressure on the ventricular walls.

(iii) the pressure across the ventricular walls at any point is simply the hydrostatic pressure at that point, increasing therefore from above downwards. The evidence for the former statement is that if the pool of Ringer outside the ventricle is allowed to gather until its depth is equal to the height of the ventricle then the ventricle collapses; evidence for the latter statement is found in the histological sections of the superfused ventricle (Fig. 2c) which shows that the strands of cells are better separated and thinner at the lower part of the ventricle, a finding consistent with

the pressure gradient. However the maximum hydrostatic pressure is only of the order of 0.5 cm of water.

The conclusions drawn from these facts were that the pressure across the superfused ventricle was about normal, but that the perfusing fluid passed more quickly and in a greater volume per unit time over the cells than in the intact animal.

The perfusing fluid could be quickly changed without disturbing the tissue merely by clipping off the tube in use, removing it from the Perspex block and inserting the delivery tubing leading from the appropriate reservoir. With practice this operation took only a few seconds, during which time the first solution drained away from the ventricle, so there was little mixing of the two solutions.

For consistent results with this technique it was important, (a) to set up the preparation carefully so that the sides of the half ventricle approximated to an isosceles triangle as closely as possible, and (b) when changing the perfusing Ringer the delivery tubing had to be positioned accurately

before starting perfusion, so that the fluid hit the centre of the ventricle, distended it uniformly and so gained rapid access to all of the cells.

Histology. A ventricular strip, a perfused ventricle and a superfused half-ventricle were exposed to Bouin fixative while in their normal state of distension during an experiment. Sections were then made and Fig. 2 shows the typical pattern found. The strand sizes in the ventricular strip were of the order of several hundred microns. The strand sizes in the perfused and superfused preparations were similar, in the superfused tissue the average size being 16.2 $\pm$ 0.6μ ($\pm$ 1 S.E. of the mean, n = 398), and the diffusion distances were therefore much less than in the strip.

Tension recording. In the early part of this work a transducer developed by Dr. J.R. Greer in this department was used to measure the isometric tension. It utilised a photo-electric system in which an opaque vane on the end of a leaf spring was mounted between a bulb and twin photocells in such a way that exactly

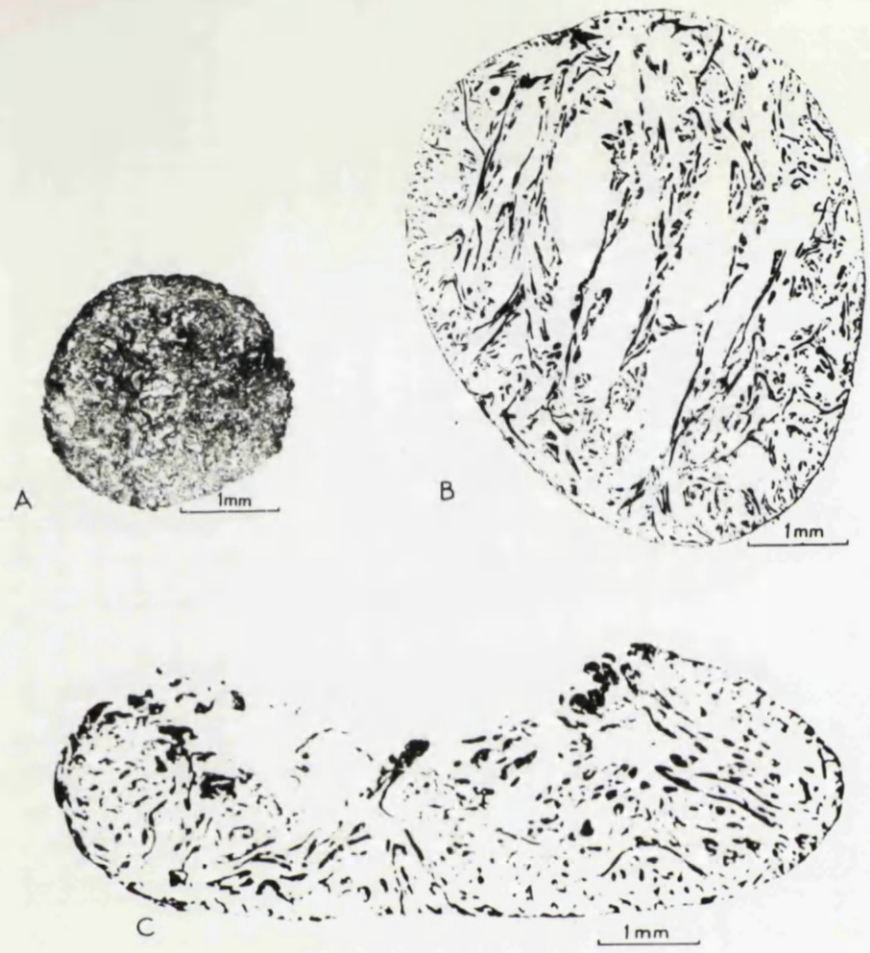

Fig. 2

Histological sections. The ventricle was fixed while being exposed to Ringer in the following ways:-

(a) ventricle in a pool of unstirred Ringer.

(b) ordinary perfused preparation.

(c) superfused preparation.

Preparations fixed with Bouin and stained with haemalum and eosin.

half of each cell was in shadow. The cells were wired in a bridge circuit and small movements of the vane produced relatively large changes in voltage output. The drift was less than 5 mV/hr and hum and noise about 1/10 of this. The natural resonance of the vane assembly was 30 c/s, and the frequency response of the order of 0-20 c/s.

For later experiments a Grass force displacement transducer was used, at its most sensitive range. It had a maximum working range of 0.05 Kg, a minimum reliable force of 2.0 mg, and a self resonance frequency of 85 c/s. This was a more enclosed transducer than the photo-electric one, and so was less likely to get vital parts splashed with Ringer during the experiments.

The output of the transducers was displayed on a Devices Single Channel Recorder which writes with a heated stylus on heat sensitive paper. The Grass instrument was connected to the recorder through a Cannon socket and a Devices S 1 coupling unit. It was easy to adjust the sensitivity of the recorder to produce reproducible results with little distortion

from background hum and vibration. The overall frequency response for the Grass transducer and the recorder together was 0-60 c/s.

Stimulation of the preparation. The base of the ventricle was removed during the dissection to stop spontaneous activity as it was necessary to have the heart rate steady. The tissue was stimulated through two silver wire electrodes fixed in the wax block of the perfusion apparatus. A Devices Digitimer driving a Devices Isolated Stimulator was used to give a supramaximal pulse (10 volts) of 1 msec duration. The rate of stimulation was 30/min unless otherwise stated. Fig. 3 shows the layout of the entire apparatus.

Electrical events in individual cells

This section was concerned with the recording and measurement of electric potentials in individual ventricular cells using micro-electrodes. The potentials were displayed on an oscilloscope screen and could be measured with a voltage calibrator mounted in the circuit. The output of the oscilloscope could be led to a pen recorder to

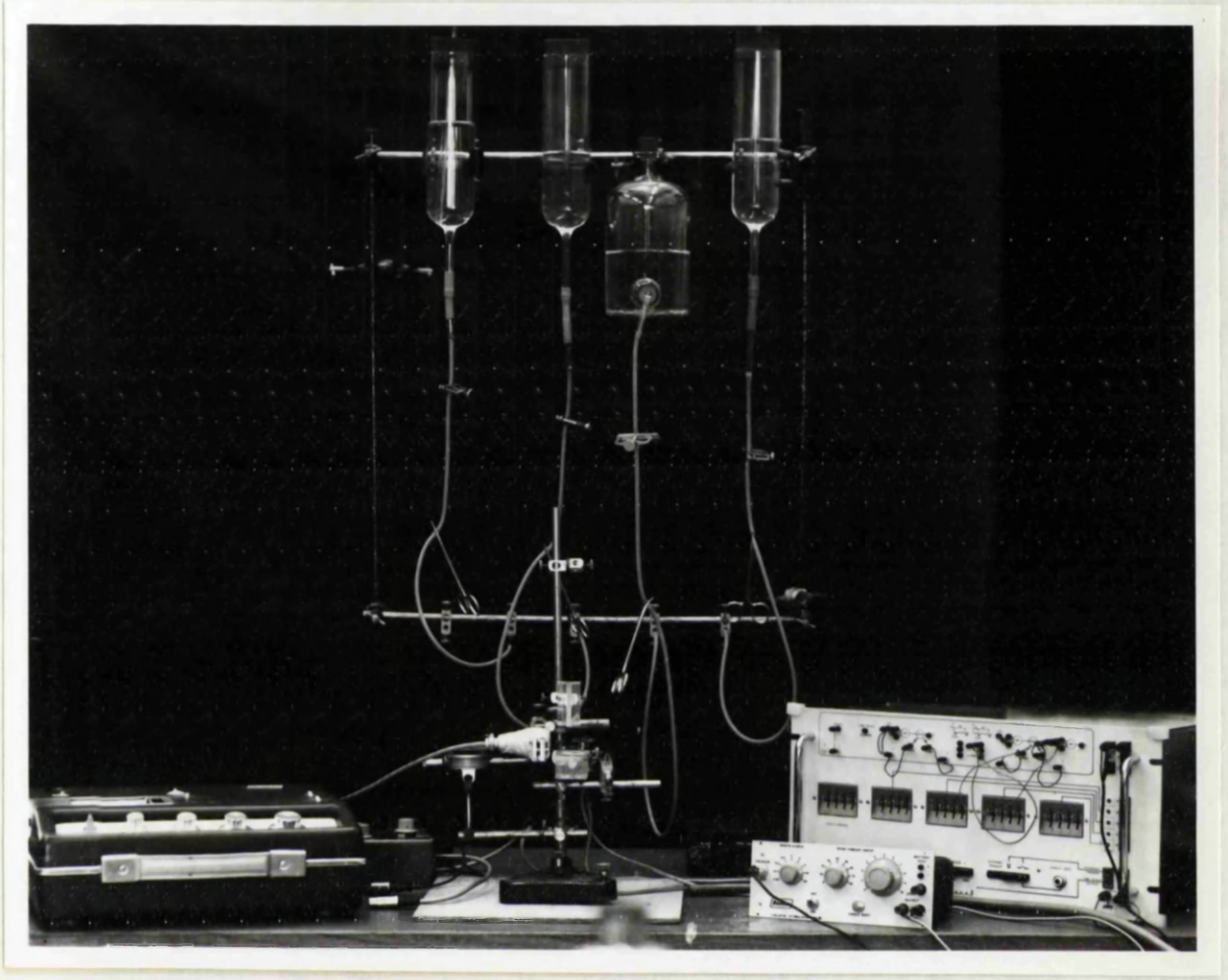

Fig. 3

General layout of the apparatus for the recording, superfusion and stimulation of a half ventricle for the examination of mechanical activity.

obtain permanent records. Usually sample readings of activity were taken before and during exposure of the ventricle to a drug and/or an alteration in ionic environment. These readings were then averaged and tested for significance using Student's t test. Occasionally continuous readings were taken with the micro-electrodes remaining in the cell throughout a change of solution.

The experimental apparatus. The experimental bath was fashioned from dental wax with a Perspex block set into the bottom of the bath to allow illumination of the specimen from below. A pair of stimulating electrodes were fixed into the base beside the Perspex block. They were supplied via terminals at the side of the bath by a Devices Digitimer and Isolated Unit as described before. A spiral Ag/AgCl electrode, the indifferent electrode for the DC amplifier was mounted in the bath from a terminal at the side. Both this electrode and the Ag/AgCl electrode connecting the cathode follower head to the micro-electrode were re-chlorided before each experiment. This abolished some instability of the

apparatus which had been troublesome previously. The bath was supplied from reservoirs above by gravity feed and the fluid level was kept constant by a suction outlet so that the bath contained 5 ml. of Ringer. The Ringer was allowed to flow through the bath, except when recording, when both inflow and suction were stopped. The perfusing fluid could be completely replaced in the bath in approximately 10 sec.

The ventricle was cut in half as described above and was held on the floor of the bath by light glass clips. It was positioned over the stimulating electrodes and the Perspex block with its pericardial surface uppermost. Although the diffusion distances are very much longer in this preparation there was no danger due to this of a reduction in the effect of a solution because (a) the micro-electrode was only penetrating cells on the surface of the tissue, and (b) the new solution was perfused freely through the bath for 3 - 4 min before recording was started.

The micro-electrodes were held in a piece of soft rubber tubing which was connected to a polythene

tube held on a brass rod mounted in a Prior micro-manipulator. An Ag/AgCl electrode from the cathode follower head passed through the tubing and made contact with the 3M KCl inside the micro-electrode.

In order to minimise movement of the micro-electrode tip, the manipulator, the bath and the input cathode follower head were mounted on a piece of 3/8" steel plating, which was set on six rubber door stops and laid on a slate slab on the bench.

Micro-electrodes. The micro-electrodes were pulled from Pyrex glass tubing with an outside diameter of 1.5 - 1.75 mm and wall thickness of 0.3 - 0.4 mm, using the Micro-pipette Puller M 1 (Industrial Science Associates, Inc.). This instrument utilises the principle of the apparatus first described by Alexander & Nastuk (1953). The glass was cleaned by boiling in distilled water before use. The micro-electrodes were placed in specially designed holders, kept in distilled water at 7°C for several hours, then filled with 3M KCl solution by boiling under reduced pressure for 5 - 10 min. Micro-electrodes with resistances in the range 5 - 30 MΩ were selected and

stored in 3M KCl till required; any electrodes not used in 7 - 10 days were discarded.

Tip potentials were not measured directly but the electrical activity recorded by each electrode was assessed according to the criteria laid down by Adrian, (1956). Like Adrian, it was found that if an electrode was giving aberrant readings indicative of tip potential interference, breaking the tip immediately abolished the tip potential and, if the resistance was still satisfactory, it could be used and gave steady results.

Electrical equipment. This was of conventional design consisting of a cathode follower feeding a balanced DC amplifier. The input grid current of the cathode follower valve was 8.0×10^{-13} amps. The output was displayed on one beam of a Tektronix 502A Dual Beam oscilloscope. The vertical output of the oscilloscope was connected to an audiomonitor which gave an audible indication of the sweep position. It could also be led to a Siemens Oscillominx pen recorder which was used to obtain permanent records of action potentials. The frequency

response of this recorder was 0 - 500 c/s with a frequency range up to approximately 1000 c/s.

A voltage calibrator was inserted in the circuit between the indifferent bath electrode and earth and could be used to back off and measure changes in potential of the circuit when the micro-electrode penetrated a cell. It also supplied a one volt signal which could be passed through a 1×10^9 Ω resistor attached to the cathode follower grid and used to measure the resistance of the micro-electrodes.

As the experiments were carried out in a screened room there was only negligible mains interference so that a bench screen was not required.

Fig. 4 shows the apparatus used in this section.

Isotope tracer studies

This was only a small part of the experimental work and was concerned only with the movement of chloride across the cell membrane under the influence of adrenaline, using the radio-isotope of chloride, ^{36}Cl. The isotope was obtained from The Radiochemical Centre, Amersham, as a sterile, isotonic solution of $Na^{36}Cl$.

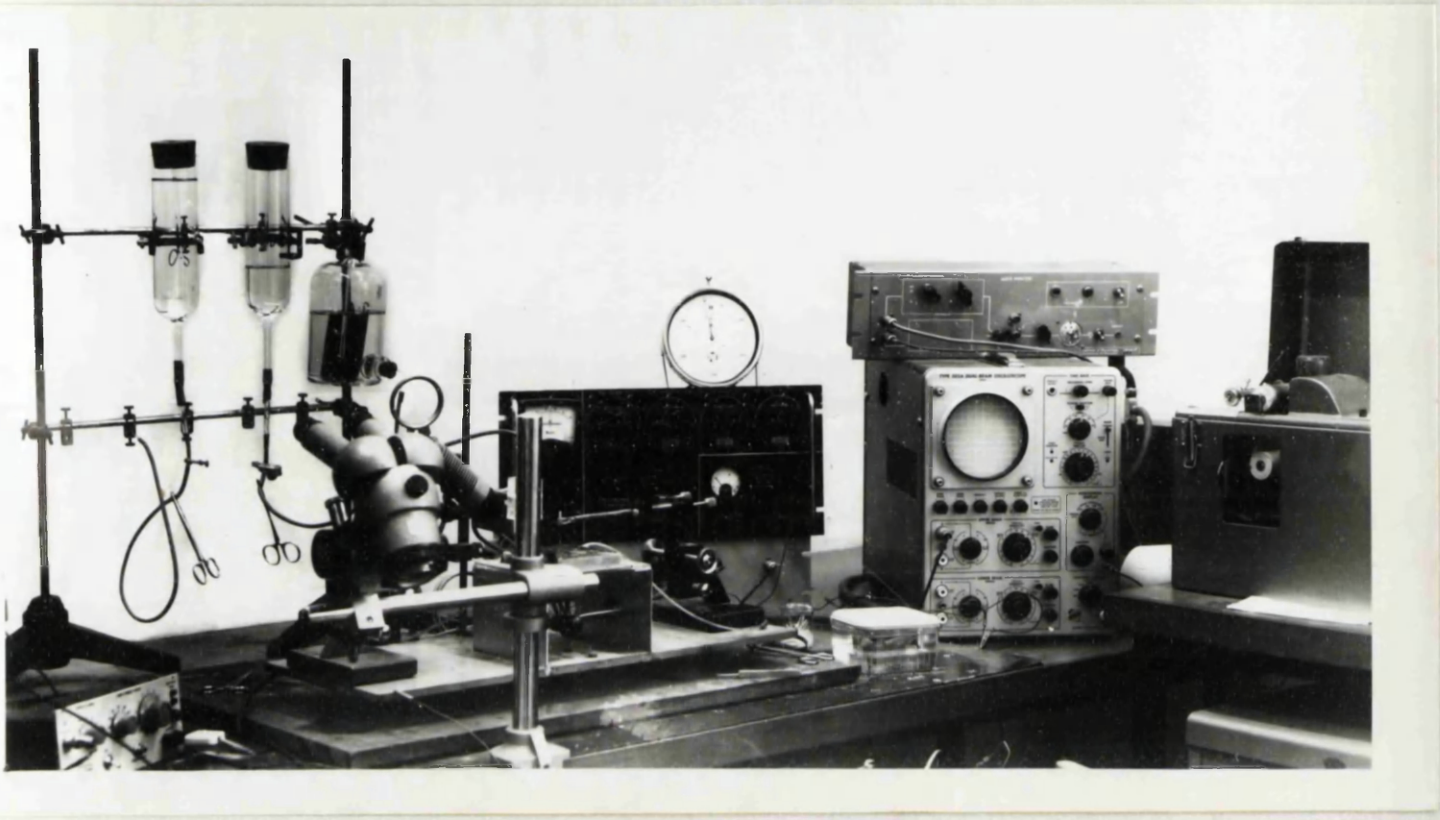

Fig. 4

General layout of the apparatus for the examination of electrical activity in frog ventricle.

Technique. For these experiments a ventricle was cannulated and perfused in the classical fashion except that a small hole was made in the base of the ventricle to increase the flow rate. This did not affect the perfusion of the tissue and it increased the effective washing of the cells, the perfusion rate being of the order of 60 ml./min.

Soak solutions were made by substituting appropriate amounts of the $Na^{36}Cl$ solution for the normal NaCl of the Ringer.

The cannulated ventricle was suspended freely from a clamp inside a siliconised glass funnel. It was perfused with Ringer by gravity feed and the effluent passed through the funnel into a series of boiling tubes mounted in a rotating rack. The effluent was collected in each boiling tube for a fixed time, usually 30 sec, giving a volume of approximately 30 - 35 ml. in each tube.

Efflux experiments: the ventricle was placed in the ^{36}Cl soak solution for 30 - 60 min. This allowed time for the exchange of much of the intracellular chloride for the isotope. The ventricle was then

perfused, firstly with normal Ringer, then Ringer containing adrenaline, and finally with normal Ringer again, and the effluent collected as described above. Influx experiments: the ventricle was soaked for only a short time in the ^{36}Cl. In order to expose all the cells to the soak solution it was perfused through the cannulated ventricle by a Watson-Marlow H.R. Flow Inducer pump using a recirculation method as the volume of the soak was usually only 10 ml. The ventricle was then perfused with normal Ringer and the effluent collected. After perfusing the ventricle for some time to ensure that all the isotope had been washed away, the above procedure was repeated exactly with the same ventricle except that now adrenaline was added to the ^{36}Cl soak.

Counting the activity. Five ml. aliquots of the content of each tube were evaporated to dryness on 2" stainless steel ribbed planchettes. The activity was then counted on a Tracerlab Gas Flow low background counter. At the end of the experiment the ventricle was extracted in 5% NHO_3 and the residual activity found by counting an aliquot of

this extract in a similar manner. Typical samples had a count rate of 200 - 800/min, compared to the background rate of 1 count/min. As the counts of the extracts were more than ten times the background the error of the counts could be taken to be the square root of the total count (Loevinger & Berman, 1951).

Analysis of the results. The total activity leaving the ventricle in each time interval was calculated. This was plotted as the efflux in counts/min/$\frac{1}{3}$ min against time on semi-log graph paper. As the residual activity left in the ventricle at the end of the experiment was known, it was possible by adding up the efflux activities to calculate the activity left in the ventricle at any time. These results were plotted as content of radioactivity (in counts/min) against time on semi-log graph paper. Extrapolation of the linear part of this graph gave the initial content of activity in the ventricle in counts/min. From this graph it was usually possible to see if there was any alteration in flux with changing conditions, but for greater accuracy rate

constants for each time interval on this graph were obtained using the equation,

$$k = \frac{\log_e A - \log_e B}{t_{A-B}}$$

where

k = the rate constant

A, B = adjacent points on the graph

t_{A-B} = time interval between points

The rate constants were then plotted as a histogram against time. No attempt was made to estimate quantitative movements of ^{36}Cl across the cell membrane.

RESULTS

RESULTS

This section of the thesis has been divided into three parts, as follows:-

(a) the effect of adrenaline on potassium contractures and membrane depolarisation,

(b) the effect of adrenaline on the twitch and action potential, and

(c) the results of experiments on ionic fluxes.

THE EFFECT OF ADRENALINE ON POTASSIUM CONTRACTURES AND MEMBRANE DEPOLARISATION

Potassium contractures in a superfused preparation

Potassium contractures in a superfused preparation differ in several respects from those previously described in other cardiac muscle preparations (Niedergerke, 1956; Lüttgau & Niedergerke, 1957, 1958). A full examination of the contracture in superfused tissue has been carried out by Lamb & McGuigan (1966). Fig. 5 is a recording of the isometric tension developed in a superfused frog half ventricle during a typical experiment. The first part of the trace shows the twitch tension developed when the ventricle is perfused with normal Ringer and stimulated electrically at 30/min. At the

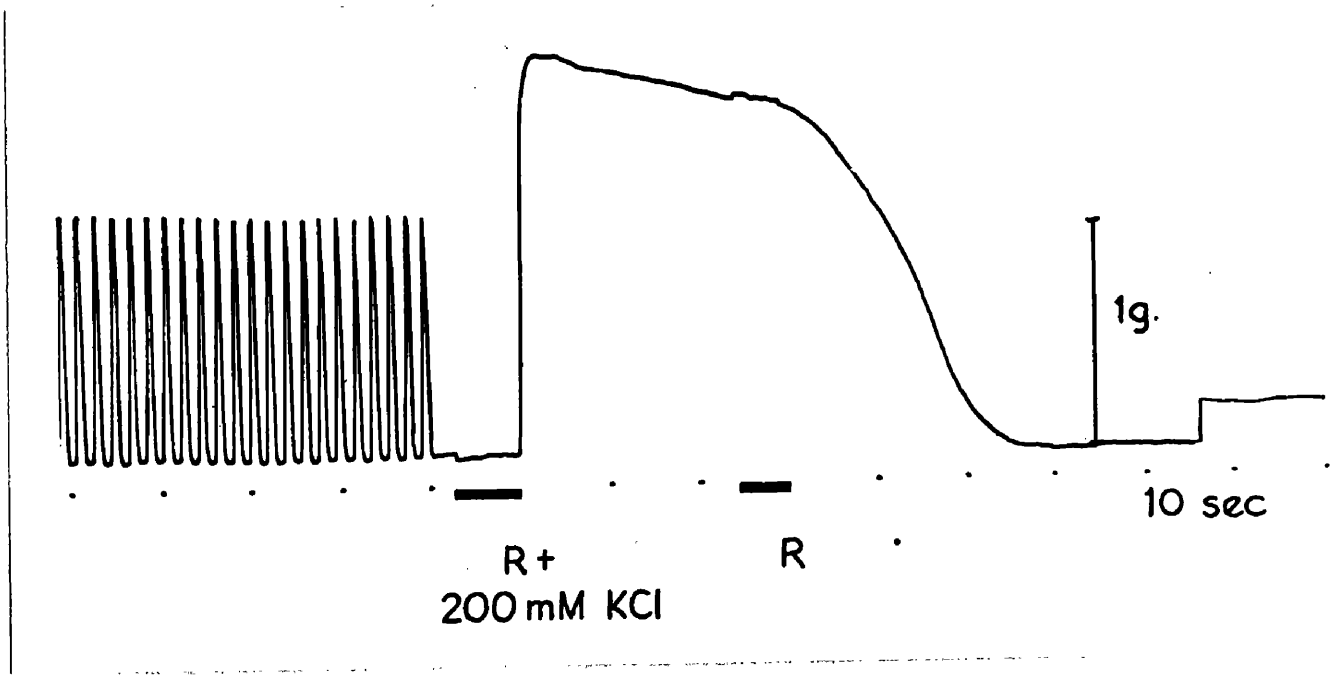

Fig. 5

Record of a typical potassium contracture in a superfused frog half ventricle. The first part of the record shows the isometric tension developed when the ventricle is perfused with normal Ringer and driven electrically at 30 beats/min. At the first horizontal bar the ventricle is perfused with Ringer containing 200.0 mM excess KCl and there is rapid development of contracture tension. At the second horizontal bar the high KCl Ringer is exchanged for normal Ringer and the contracture tension falls off rapidly. In this, and all subsequent records of contractures, the black bars represent periods of change of the perfusing fluids and the time marks represent 10 sec intervals.

External Ca^{++} 1.8 mM. Stimulated at 30/min during recovery periods.

point represented by the horizontal bar the stimulator is switched off and the perfusing fluid is replaced by Ringer containing 200.0 mM excess KCl. This causes a sustained depolarisation of the cells leading to rapid development of contracture tension, which rises to a peak then falls off slowly. At the second horizontal bar (also shown by artefacts on the record) the high potassium Ringer is replaced by normal Ringer once more, and the contracture tension falls off rapidly. After production of a contracture the ventricle is stimulated in normal Ringer for five minutes before the next contracture is initiated. This has been shown to be an adequate time for recovery.

Relation between excess potassium and tension. The tension developed in the ventricle is related to the logarithm of the external potassium concentration, or to the membrane potential, by a steep curve (Fig. 6). It should be noted here that the tensions in this figure, and in all subsequent figures and tables, are expressed as g tension/g wet weight tissue in an attempt to equate tension development in ventricles

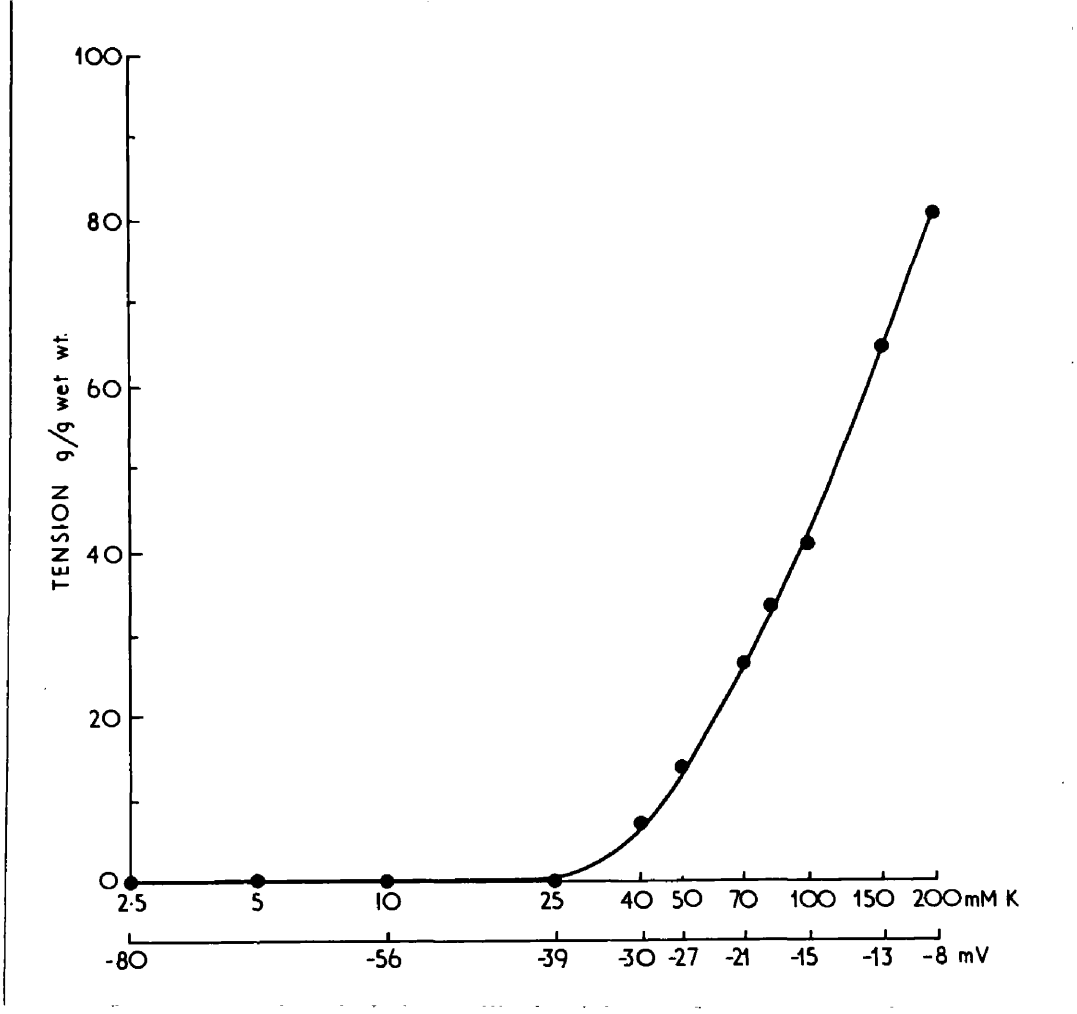

Fig. 6

Relationship between external potassium concentration or membrane potential and contracture tension. The points are the mean values from six tension measuring experiments and five potential measuring experiments in which the potassium was varied at constant osmolarity (corresponding to that of Ringer + 200.0 mM excess KCl).

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

of different sizes. Although not entirely satisfactory this method allows for fairly good correlation between tension values for the same potassium concentration in different experiments. The threshold at which excess potassium will produce contracture tension is of the order of 40.0 mM excess KCl, corresponding to a membrane potential around 30.0 mV. Smaller concentrations of potassium do not produce enough depolarisation of the cells to initiate a contracture. Exposing the ventricle to 200.0 mM excess potassium gives the maximal depolarisation possible by this method and it is assumed that the tension developed is very close to the maximum tension that the contractile element of the cell is capable of producing. The average ratio between maximum contracture tension and maximum twitch tension is 0.82; this is considerably greater than the contracture: twitch ratio in skeletal muscle (see Hodgkin & Horowicz, 1960).

Effect of time on contracture tension. The contracture tension for a particular concentration of excess potassium remains relatively constant throughout the

course of an experiment, unlike the twitch tension which falls fairly quickly with time in the superfused preparation as the ventricle becomes hypodynamic. Fig. 7 shows a typical experiment. At the start of the experiment the contracture : twitch ratio was 0.95, at the end it was 0.32. The initial contracture tension was 61.0 g/g wet weight, the final one 57.0 g/g wet weight (it should be noted that the contractures in this experiment were induced with 100.0 mM KCl, therefore the tension developed was probably not maximal).

The effect of adrenaline on potassium contractures

In these experiments the ventricle was exposed to the high potassium solution under test, then after the recovery period and while still being stimulated was perfused with Ringer containing adrenaline. Once the maximal inotropic effect of adrenaline on the twitch had been obtained, another contracture was induced using the same high potassium solution. Fig. 8 shows the effect of 1×10^{-6} g/ml adrenaline on a contracture produced by 200.0 mM excess potassium, and although the twitch tension has increased as

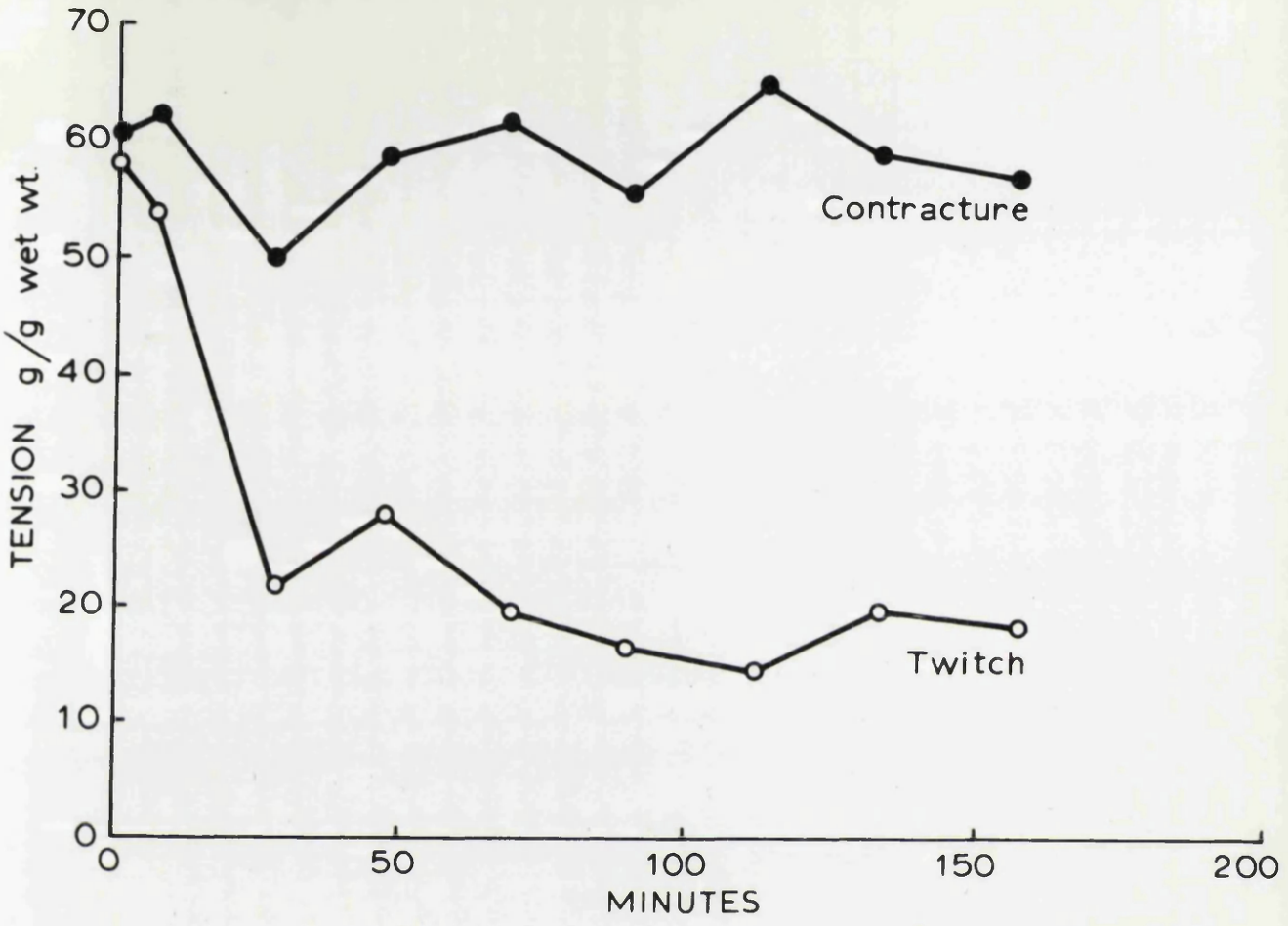

Fig. 7

Contracture and twitch tensions in a ventricle superfused for three hours and stimulated at 30/min. External Ca^{++} concentration 1.0 mM. Contractures induced with Ringer + 100.0 mM excess KCl. Contracture tension remains relatively constant, twitch tension declines quickly at first, then more slowly.

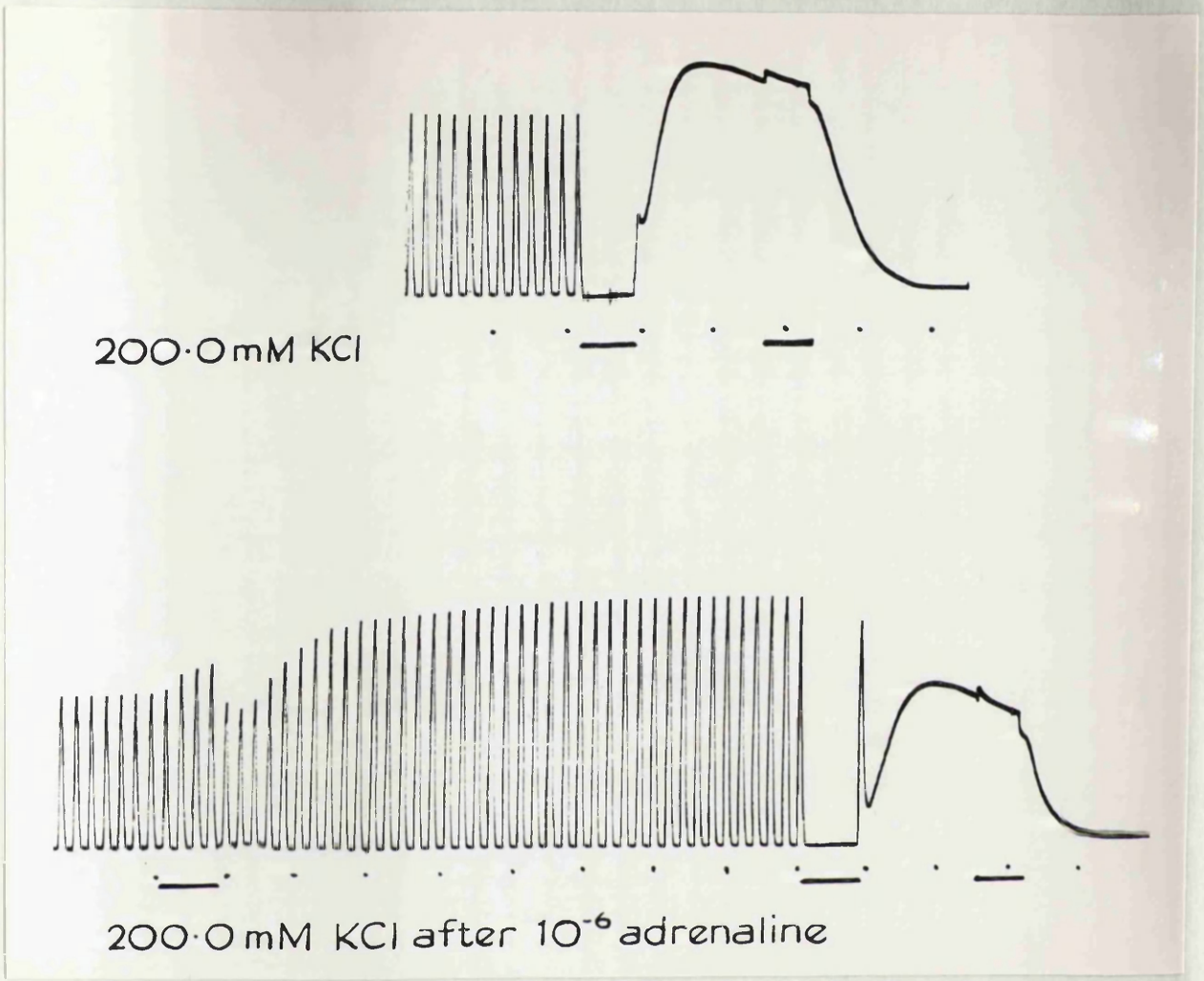

Fig. 8

The effect of adrenaline on potassium contractures induced with 200.0 mM excess potassium. The upper record shows the normal contracture. After a five minute recovery period with the ventricle perfused with normal Ringer and stimulated at 30/min, the ventricle was then exposed to Ringer + adrenaline (1×10^{-6} g/ml). This is shown at the first bar of the lower record. Once the maximum inotropic response on the twitch had been obtained, the ventricle was again exposed to the high potassium Ringer, with the production of another contracture.

External Ca^{++} 1.0 mM.

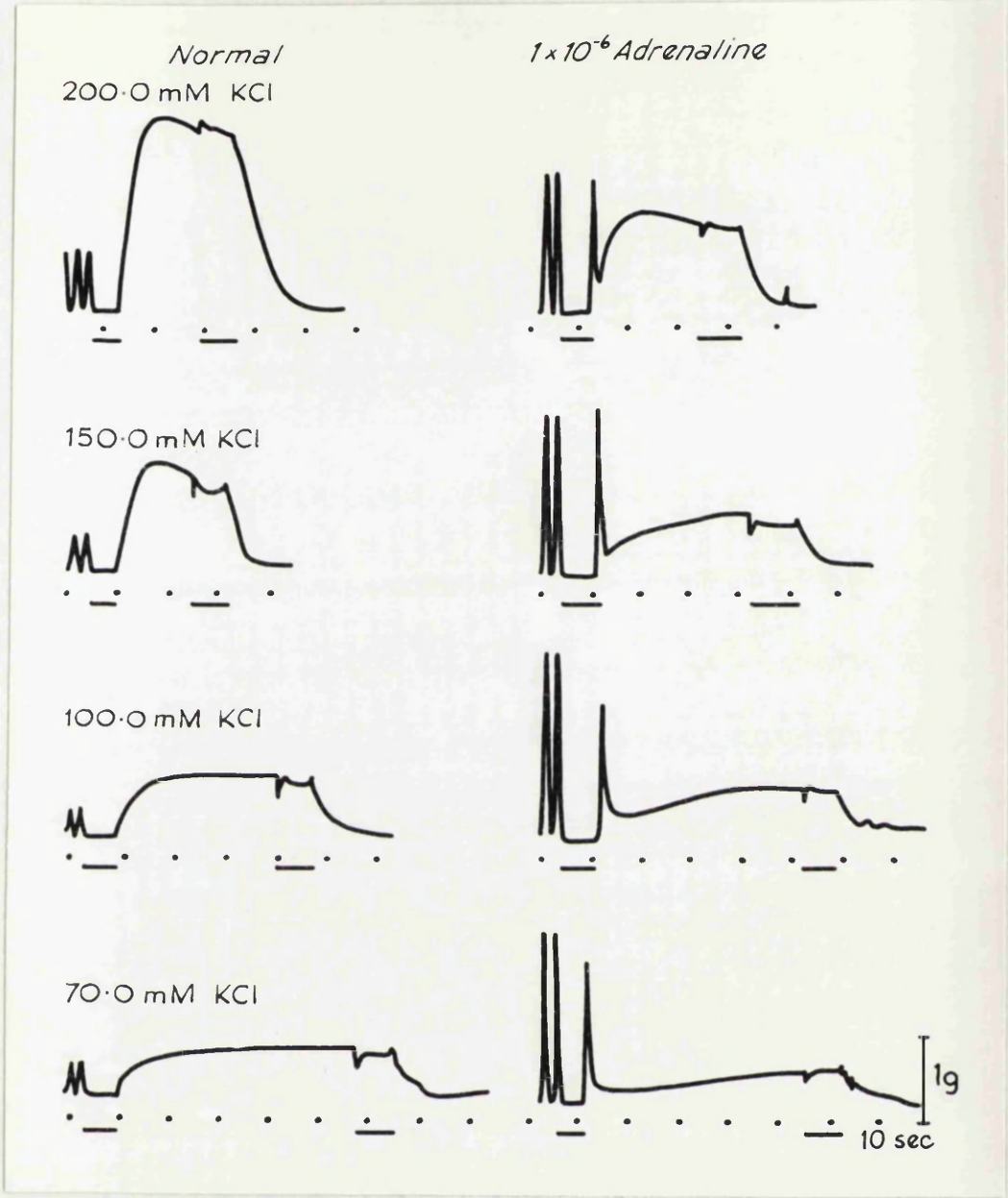

Fig. 9

The effect of adrenaline on potassium contractures induced with varying concentrations of excess KCl. The left hand records show the contracture tension normally produced by the appropriate potassium solution and the right hand records are the tension developed after prior exposure of the ventricle to adrenaline, (1 X 10^{-6} g/ml) using the same high potassium concentrations.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

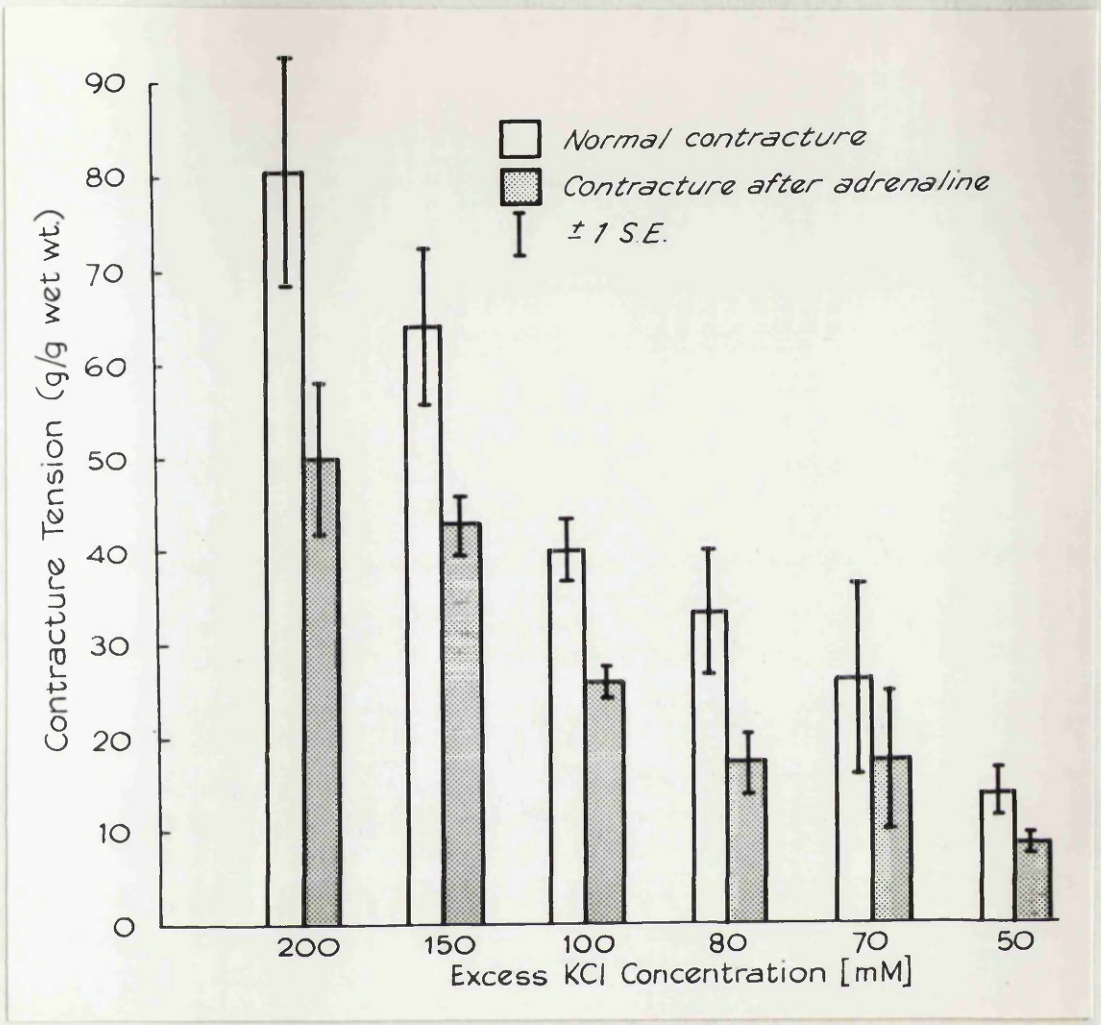

Fig. 10

Histogram showing average contracture tensions (expressed as g/g wet weight tissue) for all the high potassium solutions tested, before and during exposure of the ventricle to 1 X 10^{-6} g/ml adrenaline.

The number of observations for each plot varies and can be found in Table 2.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

External KCl concn. (mM)	No. of observations	Normal contracture tension (g/g wet wt)	Contracture tension after adrenaline (g/g wet wt)	Reduction in tension after adrenaline (g/g wet wt)	% reduction in tension after adrenaline
50.0	4	13.6 ± 2.2	8.0 ± 0.9	5.6 ± 1.5	37.8 ± 8.5
70.0	3	25.4 ± 6.1	17.0 ± 4.3	8.4 ± 1.9	33.4 ± 1.4
80.0	4	33.2 ± 6.5	16.9 ± 3.3	16.2 ± 3.6	48.1 ± 4.7
100.0	8	40.1 ± 3.3	25.8 ± 2.6	14.3 ± 2.1	35.7 ± 4.0
150.0	4	64.1 ± 7.6	43.1 ± 2.4	21.0 ± 5.7	30.8 ± 5.6
200.0	7	80.5 ± 12.8	49.8 ± 7.9	30.7 ± 6.1	38.5 ± 3.1

± denotes 1 S.E. of the mean

All differences are significant ($P < 0.001$).

Table 2

Effect of 1 X 10^{-6} g/ml adrenaline on contracture tension induced with various concentrations of excess potassium. External Ca^{++} concentration 1.0 mM. Ventricle stimulated at 30/min during recovery periods.

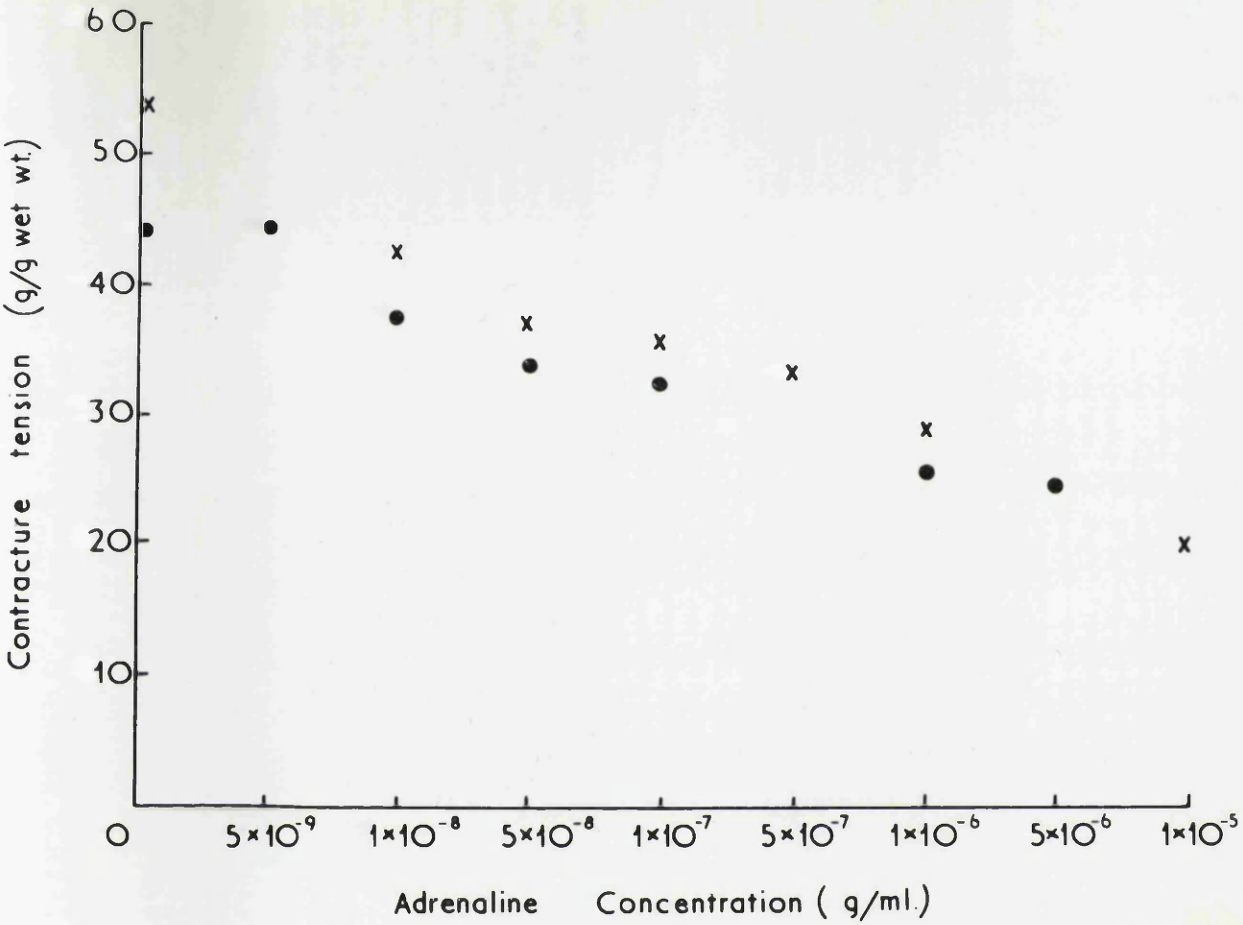

Fig. 11

Dose response curve of adrenaline action on potassium contractures. Contractures induced with Ringer + 200.0 mM excess KCl. Results of two experiments.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

Another marked difference in the contracture after adrenaline shown well in Figs. 8 and 9 is the spike of tension which precedes full development of contracture tension. A similar spike is often seen under normal circumstances using excess potassium solutions only just able to produce a contracture, but this never happens at higher concentrations. This spike is of the same order of magnitude as the preceding twitches and it seems likely that it is in fact a simple twitch which develops before the contracture. This was assumed to be due to the increased excitability of the tissue under the influence of adrenaline and that the stimulus of the high potassium solution striking the cell produced an action potential, and therefore a twitch, before the slower steady depolarisation was complete. One effect of this initial twitch is to prolong the time to peak contracture tension by a variable amount compared to the time to peak tension of a contracture induced with the same potassium solution in the absence of adrenaline. As the contracture tension normally declines with time ($T\frac{1}{2}$ 40 - 60 secs,

Lamb & McGuigan, 1966) this delayed development of peak tension might in itself be expected to diminish the tension. However when the tension before and during exposure to adrenaline was measured at a constant time after application of the high potassium solution, rather than comparing the peak tensions as had been done previously, there was no appreciable difference in the reduction of tension.

It might be supposed, however, that the presence of this spike could be reducing the contracture tension in some other way and experiments were therefore carried out to attempt to show the action of adrenaline without the complication of this twitch. The ventricles were perfused with solutions which markedly reduced the twitch tension and then contractures were induced before and during exposure of the ventricle to adrenaline.

Firstly perfusing the ventricle with Ringer made hypertonic by addition of sucrose caused a marked reduction in twitch tension and allowed the effect of adrenaline on the contracture to be examined easily.

This Ringer had 123.0 g/litre of solid sucrose added (solution D, Table 1), this giving an osmotic strength equivalent to the contracture fluid used, Ringer + 200.0 mM excess KCl. Fig. 12 shows the result of one experiment and it can be seen that in the hypertonic Ringer the twitch was reduced and the contracture tension after adrenaline was reduced by an approximately similar amount to that normally found, but there was no complicating twitch at the beginning of the contracture. The average values for all the experiments done in hypertonic Ringer and using 200.0 mM excess KCl and 1 X 10^{-6} g/ml adrenaline were: (a) normal contracture tension 85.1 g/g wet weight, (b) tension after adrenaline 61.9 g/g wet weight, and (c) percentage reduction in contracture tension 31.2%. The reduction in tension in these experiments, although less than the average percentage reduction in normal Ringer, was well within the range of values obtained in normal Ringer.

Also the effect of reducing the twitch by increasing the potassium concentration in the Ringer was tested. It was found that increasing the external

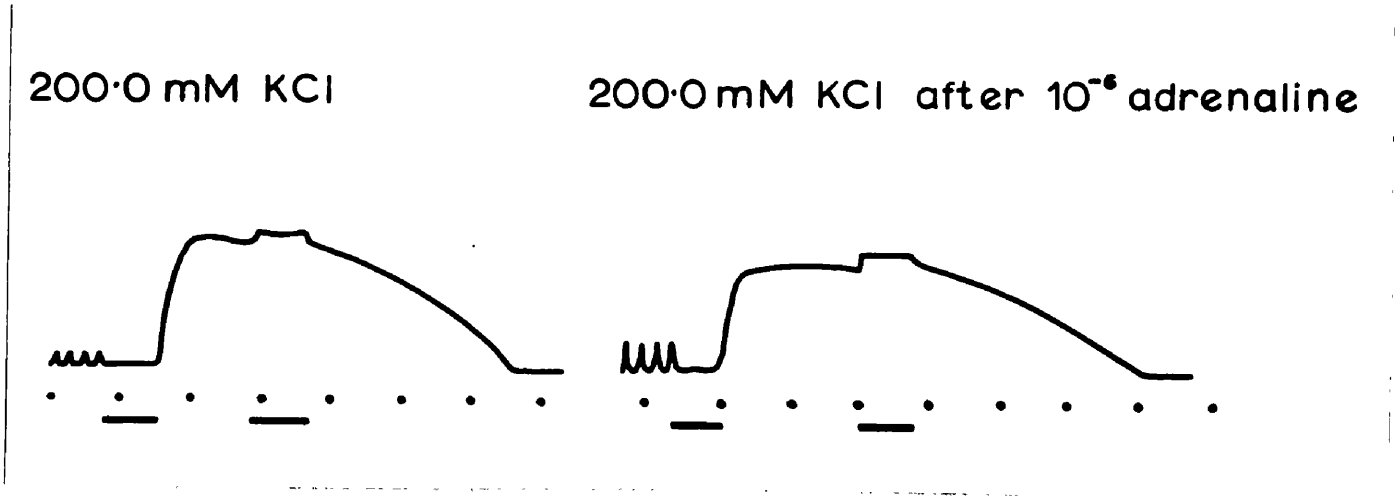

Fig. 12

The effect of adrenaline on potassium contractures when the ventricle is perfused with hypertonic Ringer. It can be seen that the twitch is much reduced, and that the contracture after adrenaline is not complicated by a preceding spike. Contracture tension is still reduced however. Ringer was made hypertonic by addition of 123.0 g solid sucrose/litre.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

potassium to 10.0 and 25.0 mM caused complete loss of contractility in response to electrical stimulation, whereas the contracture tension was not reduced. After exposure to adrenaline (1 X 10^{-6} g/ml) there was partial recovery of the twitch in the 10.0 mM KCl solution, but none in the 25.0 mM solution. The contracture after adrenaline in the first solution was preceded by a very small twitch and in the second there was no twitch. The percentage reductions in contracture tension were: (a) normal Ringer, 42.1% (b) 10.0 mM KCL Ringer, 44.8% and (c) 25.0 mM KCl Ringer, 50.2%.

The results of these experiments therefore suggest that the preceding spike seen in contractures induced after adrenaline does not play a significant part in the reduction of the contracture tension.

The effect of adrenaline on contractures induced in chloride-free solutions In an attempt to show whether this effect of adrenaline on potassium contractures might be due to an action on the movement of chloride across the membrane, similar

experiments to those described above were carried out using Ringer solutions where the chloride had been replaced by sulphate (see Methods). The contractures produced in these Ringers were usually small and liable to distortion from perfusion and other artefacts, but as shown in Fig. 13, it was still possible to see that adrenaline reduced the contracture tension by similar amounts in normal and chloride-free Ringer.

Time course of adrenaline action The full effect of adrenaline on the contracture is not seen until it has produced its maximum inotropic effect on the twitch. If the adrenaline under test is added to the contracture fluid, so that the ventricle is not exposed to it until the contracture is induced, there is no reduction in contracture tension. This suggests that the adrenaline action is not an immediate effect at the membrane, but that it is dependent on events taking place at a slower rate within the cell.

Effect of adrenaline on sodium-free contractures

It is possible to induce a contracture in frog

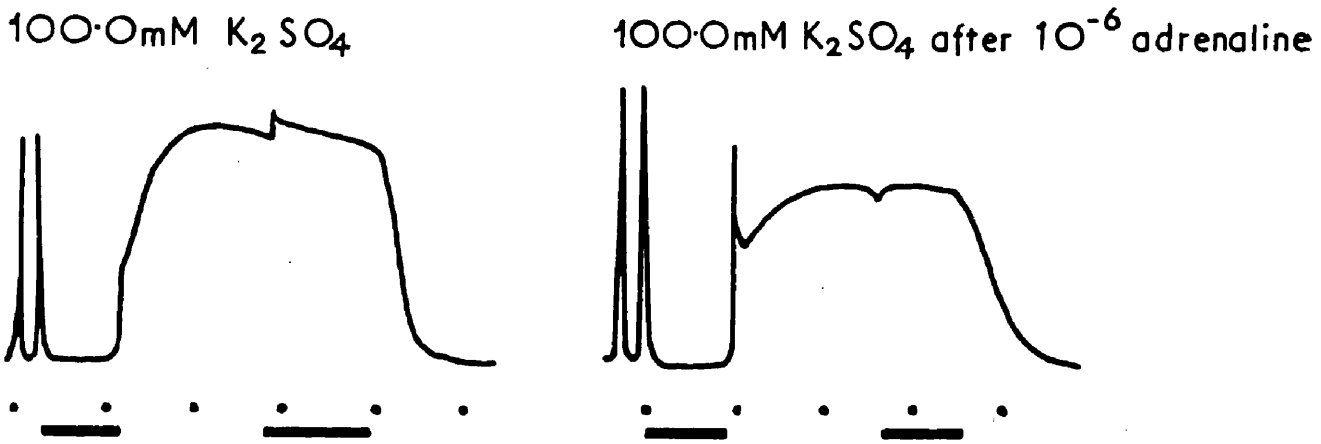

Fig. 13

Effect of adrenaline on potassium contractures in sulphate Ringer. The Ringer was prepared with appropriate concentrations of Na_2SO_4 and $CaSO_4$, with added sucrose to maintain the osmotic pressure similar to that of normal Ringer. The presence of sulphate ion reduces the amount of ionised calcium present in the solution. In order to increase the ionised calcium as much as possible therefore, the solution was saturated with $CaSO_4$ (approximately 9.0 mM). This makes estimation of the external Ca^{++} concentration impossible.

Stimulated at 30/min during recovery periods.

ventricle by perfusing it with sodium-free Ringer (Lüttgau & Niedergerke, 1958; Niedergerke, 1963a). Using the superfusion technique the development of tension is rapid and the contracture so produced is similar in shape to a potassium contracture. The NaCl of normal Ringer was replaced by an iso-osmotic amount of solid sucrose (solution E, Table 1) and the external potassium concentrations used were 2.5, 1.0 and 0.0 mM. The effect of 1 X 10^{-6} g/ml adrenaline on contractures induced with these solutions is shown in Table 3. There does not seem to be much effect on the contractures of varying the potassium concentration, except that the average tension developed in 1.0 mM KCl is slightly smaller than that found at the other two concentrations. However, it can be seen that adrenaline causes a reduction in contracture tension in all cases, the average percentage reduction of the series being 26.7%.

In a few instances the sodium of the Ringer was replaced with iso-osmotic concentrations of KCl,

Conditions	No. of observations	Normal contracture tension (g/g wet wt)	Contracture tension after adrenaline (g/g wet wt)	Reduction in tension after adrenaline (g/g wet wt)	% reduction in tension after adrenaline
Na^+ free Ringer + 2.5 mM KCl	6	72.0 ± 7.2	58.0 ± 6.3	14.0 ± 2.8	19.8 ± 3.9
Na^+ free Ringer + 1.0 mM KCl	5	64.8 ± 11.2	44.1 ± 8.5	20.7 ± 3.3	33.1 ± 3.3
Na^+ free Ringer + 0.0 mM KCl	7	69.8 ± 6.0	50.6 ± 5.1	19.2 ± 3.6	27.3 ± 4.5

± denotes 1 S.E. of the mean

All differences are significant ($P < 0.001$)

Table 3

Effect of 1 X 10^{-6} g/ml adrenaline on contractures induced by exposing the ventricle to sodium-free solutions. The concentration of potassium in these Ringers was altered as shown in the table. External Ca^{++} concentration 1.0 mM. Ventricle stimulated at 30/min during recovery periods.

(solution B, Table 1). Adrenaline also caused a reduction in the contracture tension produced by this solution, the average percentage reduction being 28.2%.

The effect of adrenaline on membrane potential

It has been shown by Dudel & Trautwein (1957) that adrenaline causes a hyperpolarisation of the resting potential of dog auricular fibres. A similar hyperpolarisation of the resting potential of skeletal muscle fibres was noted by Brown, Goffart & Vianna Dias (1950) after adrenaline.

In the light of these results it seemed that possibly one of the reasons for the reduction in contracture tension after adrenaline might be a hyperpolarisation of the membrane potential usually produced by the high potassium Ringer under test. As shown in Fig. 6 the relationship between contracture tension and membrane potential forms a steep curve, so that a small reduction in potential will produce a relatively large reduction in tension.

A series of experiments was therefore carried out in the following way to measure the depolarisation

of the membrane potential produced by various concentrations of excess potassium before and during exposure of the ventricle to 1 X 10^{-6} g/ml adrenaline. Sample readings of the membrane potential of cells bathed in a high potassium solution (usually ten penetrations) were taken, then the tissue was allowed to recover in normal Ringer. The ventricle was then exposed to adrenaline, firstly in normal Ringer, then in the high potassium Ringer under test. Sample potential values were again recorded. Between all these stages of the experiment the resting potential in normal Ringer was checked to ensure that the tissue remained electrically stable. The procedure given above was sometimes reversed and the ventricle was exposed to adrenaline first and then the normal potential in the potassium solution was taken.

The results are shown graphically in Fig. 14 and the actual values are given in Table 4. Table 4 also shows the effect of the adrenaline solution on the resting potential in normal Ringer (2.5 mM KCl). There is a slight increase in membrane potential in all cases, which was significant both for individual

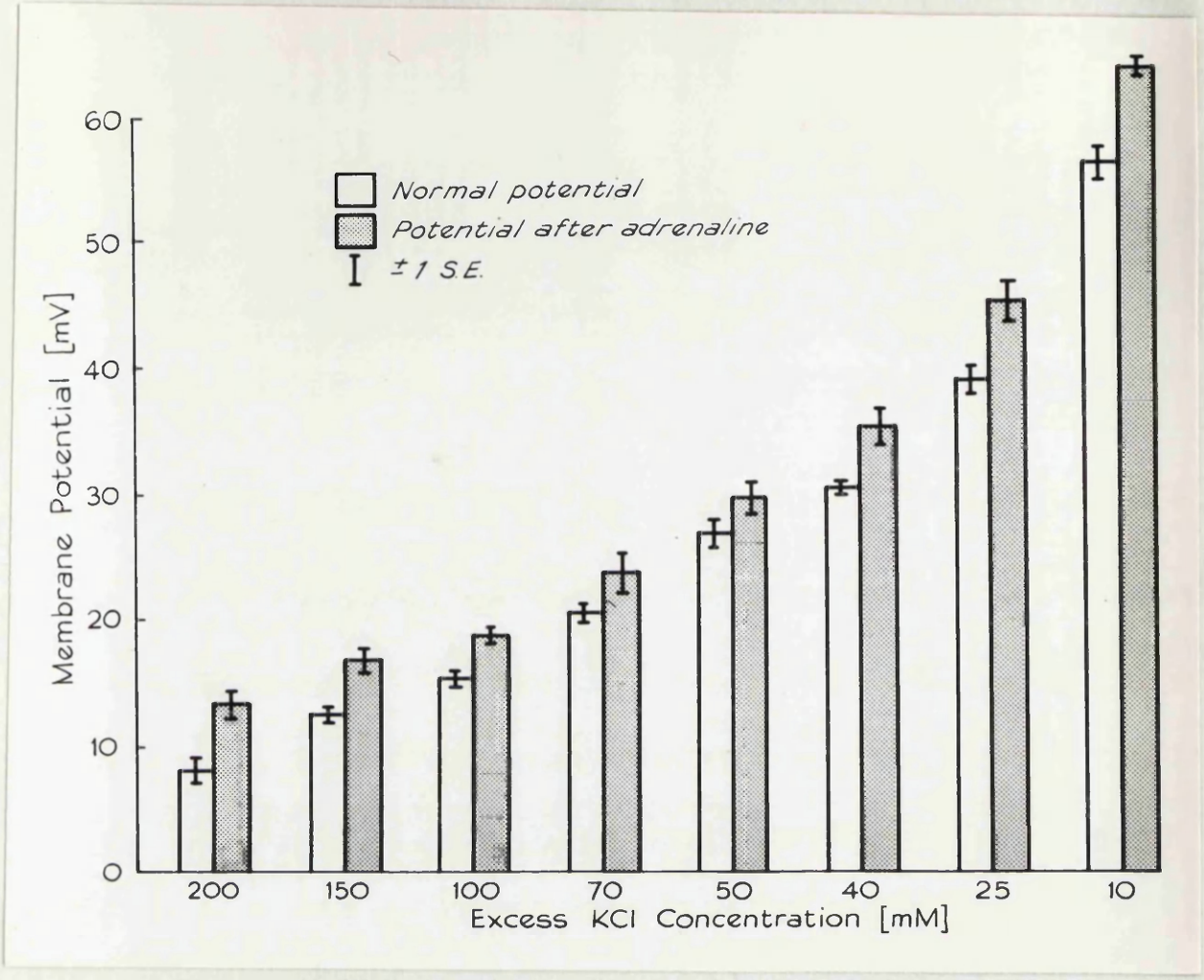

Fig. 14

Histogram showing the membrane potential of frog ventricle cells after exposure to various concentrations of excess potassium. The hyperpolarisation of the membrane in these high potassium solutions in the presence of 1×10^{-6} g/ml adrenaline is shown.

It should be noted that for the purposes of clarity of the record the change in potential in normal Ringer (2.5 mM K^+) is not shown. The average value of this hyperpolarisation is given in Table 4.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods. Each plot is the average of 4 experiments with 10 penetrations in each experiment.

External KCl concn. (mM)	No. of experiments	Membrane potential (mV)	Membrane potential after adrenaline (mV)	Increase in potential after adrenaline (mV)	% increase in potential after adrenaline
2.5	26	79.5 ± 1.7	86.5 ± 1.6	7.3 ± 0.8	8.2 ± 0.8
10.0	4	55.8 ± 1.0	63.2 ± 0.5	7.4 ± 0.6	11.7 ± 1.0
25.0	4	38.9 ± 0.9	43.9 ± 1.8	5.0 ± 1.2	11.0 ± 2.4
40.0	4	30.4 ± 0.5	35.2 ± 1.3	4.8 ± 0.7	13.5 ± 1.7
50.0	5	26.8 ± 0.9	29.7 ± 1.0	2.5 ± 0.3	8.4 ± 1.0
70.0	4	20.6 ± 0.6	23.8 ± 1.3	3.2 ± 0.8	13.0 ± 2.5
100.0	4	15.4 ± 0.5	18.9 ± 0.4	3.5 ± 0.3	18.7 ± 1.4
150.0	4	12.6 ± 0.3	17.0 ± 0.6	4.4 ± 0.4	25.8 ± 1.5
200.0	4	8.4 ± 0.8	13.5 ± 1.1	5.1 ± 0.7	37.6 ± 3.9

Each experiment is the average of ten penetrations before and ten penetrations during exposure of the ventricle to adrenaline.

± denotes 1 S.E. of the mean. All differences are significant ($P < 0.001$).

Table 4

Effect of 1 X 10^{-6} g/ml adrenaline on the membrane potential of frog ventricular cells in normal Ringer (2.5 mM KCl, line 1 in the table) and on exposure to the excess potassium concentrations shown.

External Ca^{++} concentration 1.0 mM, stimulated at 15/min.

experiments and for groups of experiments with the same external potassium (Student's t test, ($P < 0.001$). Although this increase in membrane potential after adrenaline was always present it was still small and as with all measurements of potential it was necessary to exclude the possibility of personal selection giving a bias to the results. Therefore several experiments were carried out as a blind trial with unknown solutions, one of which contained adrenaline. In every case it was possible to say definitely which solution had adrenaline present.

Adrenaline action on membrane potential in sodium-free solutions The hyperpolarisation of the membrane potential by adrenaline is increased if the ventricle is exposed to sodium-free Ringer (solution E, Table 1). Table 5 gives the average values for the potential of frog ventricular cells before and during exposure to 1×10^{-6} g/ml adrenaline in sodium-free Ringer and varying external potassium concentrations. The table also compares the effect of adrenaline on the membrane potential in

Conditions	Membrane potential change (mV)*								
	2.5 mM KCl			1.0 mM KCl			0.0 mM KCl		
	Normal	Adrenaline	Difference	Normal	Adrenaline	Difference	Normal	Adrenaline	Difference
Normal Ringer	79.5 ± 1.7	86.5 ± 1.6	7.3 ± 0.8						
Sodium-free Ringer	84.6 ± 2.2	94.7 ± 2.2	10.2 ± 1.5	91.1 ± 3.7	102.3 ± 4.0	11.2 ± 0.7	97.6 ± 1.5	113.1 ± 3.2	15.6 ± 2.1

The figures for Normal Ringer are the average of 26 experiments, with 10 penetrations in each
The figures for sodium-free Ringer are the averages of 4 experiments, with 10 penetrations in each
± denotes 1 S.E. of the mean. All differences are significant ($P < 0.001$).

Table 5

Effect of 1 X 10^{-6} g/ml adrenaline on the membrane potential of frog ventricular fibres in normal and sodium-free Ringer, with varying external K^+ concentrations.

External Ca^{++} concentration 1.0 mM.
Stimulated at 30/min.

normal Ringer against the effect in sodium-free Ringer with normal K+ concentration (2.5 mM). It can be seen firstly that the average membrane potential in the sodium-free solutions is greater than that in normal Ringer, the hyperpolarisation increasing as the external potassium is reduced. This is in contradiction to the results of Draper & Weidmann (1951) who found no constant change in membrane potential in sodium-free solutions using dog and kid Purkinje fibres as their experimental tissue. After exposure of the ventricle to adrenaline there is a further hyperpolarisation of the membrane, again increasing as the external potassium is reduced. It can also be seen that the hyperpolarisation of the membrane in sodium-free Ringer with normal K^+ concentration is greater than the hyperpolarisation produced by the same strength of adrenaline in normal Ringer.

<u>The relation between the mechanical and electrical actions of adrenaline</u>

The results of the action of adrenaline on contracture tension and membrane potential are brought

together in Fig. 15. In this graph tension and potential for each concentration of potassium are plotted before and during exposure of the ventricle to 1 X 10^{-6} g/ml adrenaline. The arrows link the plots for each potassium solution. The effect of adrenaline is to reduce both along the slope of the curve connecting the pre-adrenaline plots (see also Fig. 6). This would suggest that the effect of adrenaline on the contracture tension is in part at least mediated through the action on the membrane potential, as the new increased potential after adrenaline is associated with development of the contracture tension which would be normally expected according to the well defined relationship between tension and potential.

The interaction between ouabain and adrenaline

Haas & Trautwein, (1963) have published a paper giving evidence that adrenaline increased the sodium efflux from cardiac muscle. Also, as mentioned above, the hyperpolarisation of the membrane produced by adrenaline is increased if the ventricle is perfused with sodium-free Ringer, this having the effect of

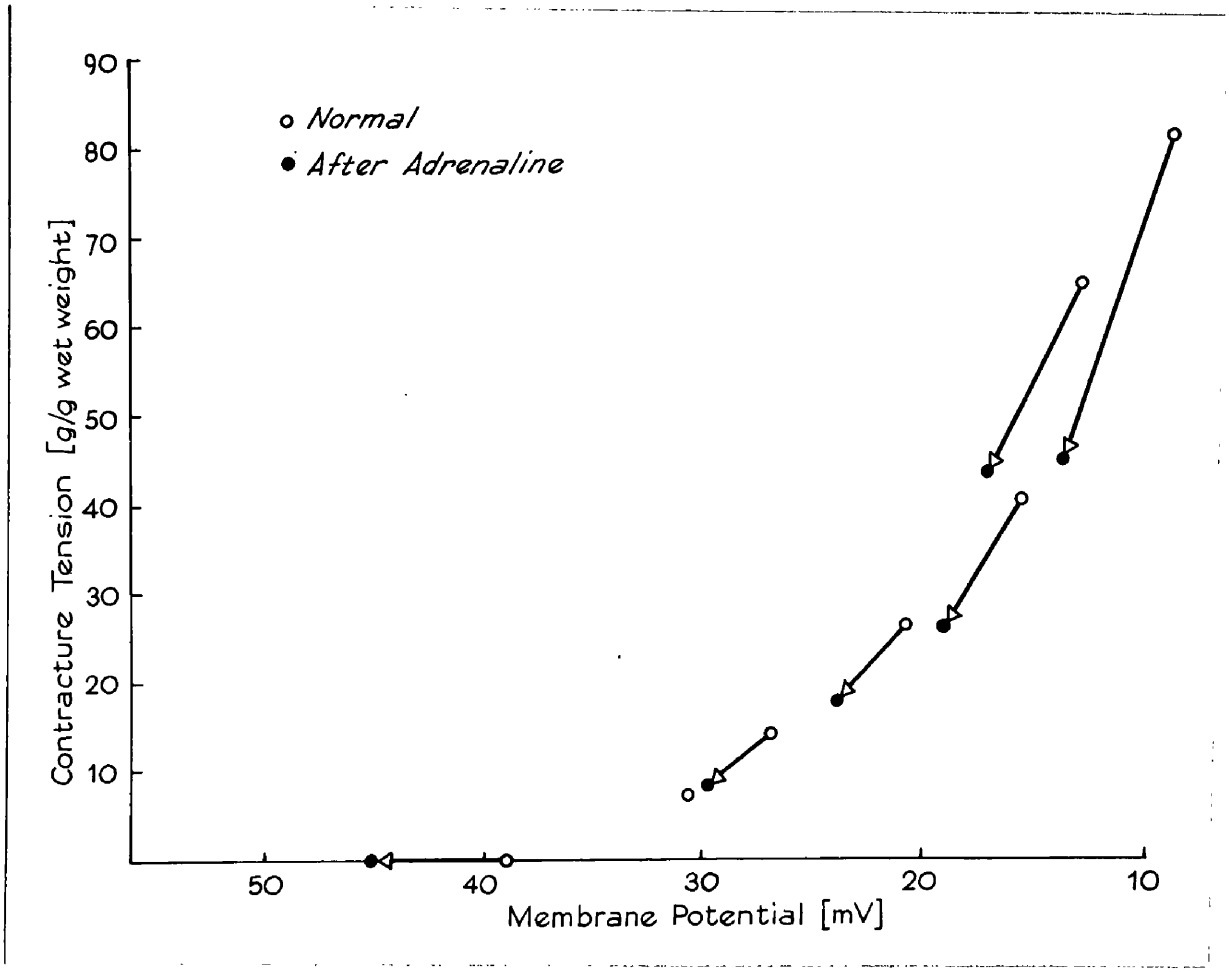

Fig. 15

The relation between contracture tension and membrane potential before and during exposure of the ventricle to 1 X 10^{-6} g/ml adrenaline. The arrows link the plots for a particular concentration of potassium. It can be seen that the effect of adrenaline is to move the relationship for each concentration of potassium along the curve of the pre-adrenaline plots. In effect this means that at the new potential after adrenaline there is development of the tension which would be expected according to the normal relationship between contracture tension and membrane potential.

changing the concentration gradient for sodium to an outward direction which would be likely to increase any efflux. As adrenaline is increasing the metabolic activity in the cell (see Haugaard & Hess, 1965), a possible explanation for this efflux of sodium is an increased working of the sodium pump. There is evidence accumulating in the literature that the sodium pump is in part at least working in an 'electrogenic' manner, i.e. that it is not electrically neutral (Kernan, 1962; Keynes & Rybová, 1963; Mullins & Noda, 1963). It is believed that the sodium pump is making a direct contribution to the membrane potential by moving positively charged ions out of the cell, and not replacing them, thus causing the interior of the cell to become more negative with respect to the exterior and therefore increasing the magnitude of the potential across the membrane. It appears possible therefore that if the activity of the sodium pump is increased, it will increase this 'electrogenic' sodium efflux and produce a hyperpolarisation of the membrane.

Experiments were therefore carried out to test

this hypothesis. Ouabain in toxic doses is known to block the sodium pump mechanism (Schatzmann, 1953; Glynn, 1957). Therefore in several experiments the ventricles were perfused with Ringer containing 1×10^{-5} M ouabain, and then the effect of adrenaline on the membrane potential was examined. Table 6 gives the results of these experiments both on the resting potential and on the potential in Ringer + 100.0 mM excess KCl. The hyperpolarising action of adrenaline was completely blocked by ouabain. In normal Ringer it can be seen that ouabain itself depolarised the membrane and when adrenaline was then added to the Ringer not only was the hyperpolarising effect blocked but there was a further depolarisation of the membrane. Adrenaline also caused a very slight, not significant, depolarisation of the membrane after ouabain when the ventricle was exposed to the high potassium solution.

At this time the ability of ouabain to block the action of adrenaline on the contracture tension was examined. When a toxic dose of ouabain had

Potential	Normal	After 1 X 10^{-6} g/ml adrenaline	After 1 X 10^{-5} M ouabain	After adrenaline and ouabain
Resting membrane potential	84.5 ± 5.3	94.0 ± 2.1	75.5 ± 2.1	67.3 ± 3.0
Potential in Ringer + 100.0 mM excess KCl	14.6 ± 0.3	17.8 ± 0.3	15.2 ± 0.6	14.6 ± 0.5

± denotes 1 S.E. of the mean (n = 40)

Table 6

The interaction between adrenaline and ouabain on the membrane potential of frog ventricular cells in normal Ringer and in Ringer + 100.0 mM excess KCl. It can be seen that in the presence of ouabain the hyperpolarising action of adrenaline is blocked, and in the normal Ringer there is a well marked further depolarisation of the cell when it is exposed to adrenaline.

External Ca^{++} concentration 1.0 mM.
Stimulated at 15/min.

produced a marked reduction of the twitch tension adrenaline was added to the Ringer and then a contracture was induced with a high potassium solution. It was found that even after a marked diminution of twitch tension by ouabain the action of adrenaline on the twitch was unchanged. It also appeared that the action of adrenaline on the contracture had been blocked, as the contracture tension after ouabain and adrenaline was similar to the normal tension developed. However, it was later discovered that ouabain produces considerable alteration of the tension: potential relationship for the contracture, and this will affect the result of these experiments. For this reason there is now considerable doubt as to the validity of the interpretation of these experiments as a test of this hypothesis and no definite conclusion can be made at present.

Inhibition of adrenaline action by pronethalol

Pronethalol is known to block the beta-receptor (Ahlquist, 1948) actions of the adrenergic amines,

such as their effect on cardiac muscle (Black & Stevenson, 1962). Fig. 16 shows the action of 1×10^{-6} g/ml adrenaline on the twitch and contracture tension in a superfused ventricle before and during exposure of the ventricle to 1×10^{-5} g/ml pronethalol. Pronethalol itself produced a reduction of the twitch tension but had no effect on the contracture. It was, however, equally effective in blocking the action of adrenaline on both twitch and contracture.

The effect of adrenaline on the membrane potential after exposure to pronethalol (using the same concentrations as above) was also tested in one experiment, in Ringer + 100.0 mM excess KCl. The potential in the high potassium solution averaged 14.6 ± 0.3 mV which was increased by adrenaline to 17.3 ± 0.3 mV (P 0.001). When the ventricle was exposed to the high potassium solution and 1×10^{-5} g/ml pronethalol the potential was virtually unchanged from normal, being 15.2 ± 0.2 mV (not significant, P 0.05), and when adrenaline was now added to the bath the potential was unchanged at

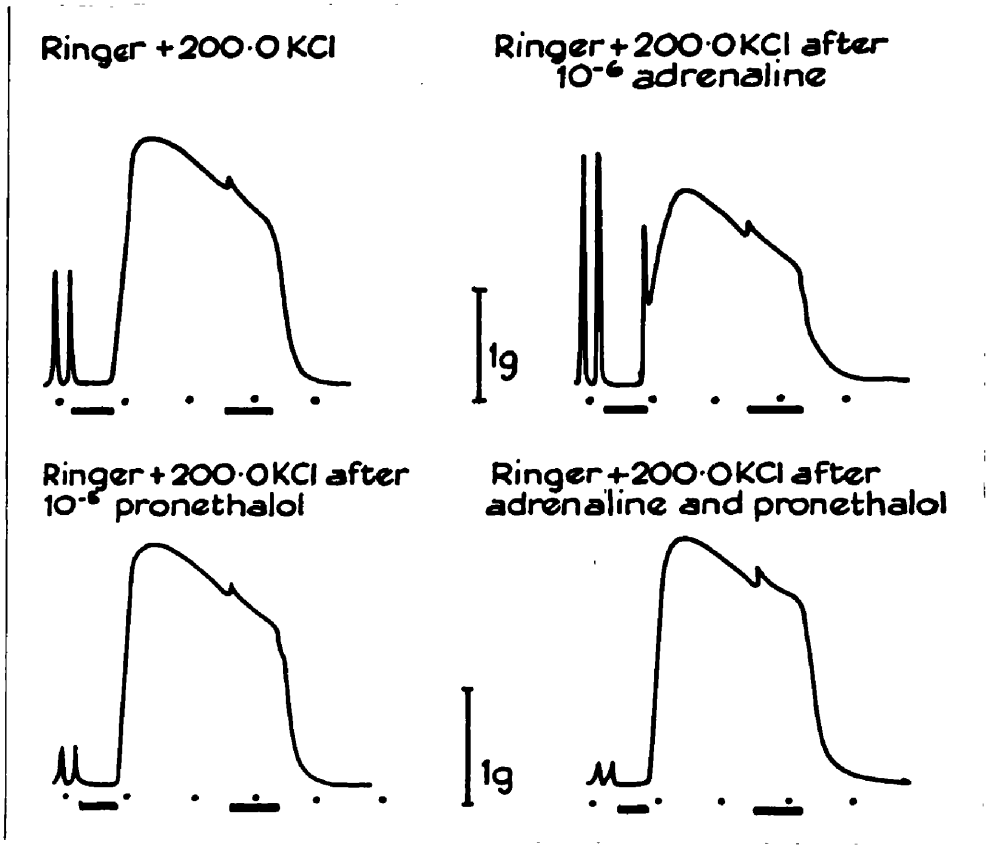

Fig. 16

Interaction between pronethalol and adrenaline on twitch and contracture tension in a superfused frog ventricle

Top left: normal twitch and contracture.

Top right: twitch and contracture after 1 X 10^{-6} g/ml adrenaline.

Bottom left: twitch and contracture after 1 X 10^{-5} g/ml pronethalol, which has diminished the twitch tension, but has had no effect on the contracture tension.

Bottom right: twitch and contracture in the presence of both adrenaline and pronethalol. There is a complete block of adrenaline action on both.

External Ca^{++} 1.0 mM. Stimulated at 30/min during recovery periods.

15.1 ± 0.3 mV (± one S.E. of the mean, n = 10).

Pronethalol therefore could block completely both the effect of adrenaline on twitch and contracture tension and also the effect on the membrane potential.

THE EFFECT OF ADRENALINE ON THE ACTION POTENTIAL AND THE TWITCH

As mentioned in the Introduction there is still considerable doubt concerning specific points of the action of adrenaline on action potential and twitch, especially the effect on the duration of the action potential and also on the time to peak twitch tension. These factors are both very important as both Woodbury (1962) and Brady (1964) have reviewed the literature on heart muscle not exposed to adrenaline and they both come to the conclusion that the duration of the action potential controls the time to peak tension of the twitch, taken to be a measure of the active state in the contractile element of the cell. Woodbury states - "Roughly speaking, depolarisation turns on the contractile process and repolarisation turns it off. The time from the moment

of stimulation to the peak of the contractile tension is proportional to the duration of the action potential." Brady reviews the more recent literature and comes to the conclusion that, in ventricular tissue at least, the action potential duration definitely controls the time to peak tension of the twitch. Kaufmann & Fleckenstein (1965) have examined the relation between tension and action potential duration in cat atrial and papillary muscle with cooling and they found a striking parallelism between the increase in action potential duration and the increase in twitch tension. Although they did not look specifically at the effect of cooling on the time to peak twitch tension, it can be seen from their records that the time to peak did increase as the action potential became prolonged.

However, a study of the literature on the action of adrenaline on twitch and action potential reveals a situation which appears to be against this simple relationship. The usual finding is that adrenaline increases the duration of the action potential (Lueken & Schütz, 1938; Webb & Hollander, 1956;

Gargouil et al, 1958; Furchgott et al, 1960; Hoffman & Cranefield, 1960; Trautwein & Schmidt, 1960; Antoni & Engstfeld, 1961), but Sonnenblick (1962) and Podolsky (1962) both find that adrenaline reduces the time to peak tension of the twitch. Naylor (1960), however, found that noradrenaline increased the time to peak twitch tension of toad ventricular muscle twitches. Therefore he alone has found the change in the time to peak tension which would be expected knowing the alteration in the action potential after adrenaline, namely that it should be increased. The purpose of the next section of this thesis was therefore to examine the relationship between tension and action potential in frog ventricle after exposure to adrenaline. In the course of the experiments several other effects of adrenaline were noted, some of which were examined in greater detail.

Alteration of the action potential by adrenaline

Fig. 17 shows the effect of 1 X 10^{-6} g/ml adrenaline on the action potential of a frog ventricular fibre, perfused with Ringer containing 1.0 mM Ca^{++}

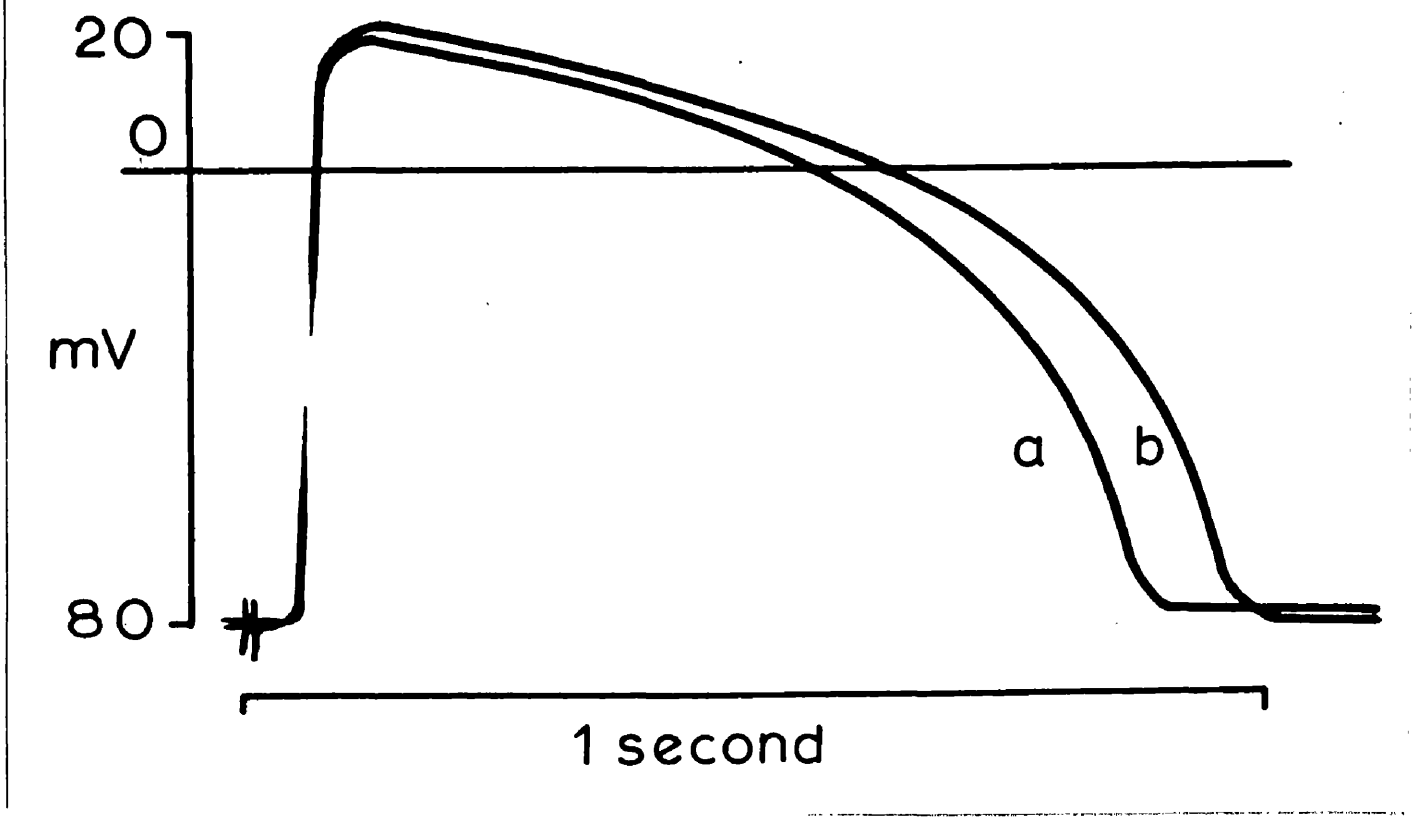

Fig. 17

Effect of 1 X 10^{-6} g/ml adrenaline on the action potential of a frog ventricular fibre. Record (a) is the normal action potential, and (b) is the action potential after exposure to adrenaline. The micro-electrode remained in the cell throughout.

External Ca^{++} 1.0 mM. Stimulated at 15/min.

It should be noted here that in this, and all subsequent figures showing action potentials, that the upstrokes of the action potentials are superimposed, rather than the stimulus artefact. This shows changes in the duration of the action potential and of the overshoot more clearly, and also, as shown here that the latent period of stimulation has changed. In most cases it is difficult to see which stimulus artefact is associated with which action potential record. In all cases however the latent period was reduced after adrenaline (see text and Table 9)

and stimulated at 15/min. Adrenaline has (i) hyperpolarised the resting membrane potential, as found in the previous section of this work, (ii) increased the magnitude of the overshoot of the action potential and (iii) has prolonged the duration of the action potential, the effect being especially marked on the first part of the repolarisation phase. The average values for the resting potential, overshoot and time to half repolarisation of the action potential before and during exposure to adrenaline are given in Table 7 and also for a larger series in the second row of Table 8 (both 1.0 mM Ca^{++} and stimulated at 15/min).

Dose-dependence of adrenaline effect This is shown in Fig. 18, which gives recordings of action potentials from the same cell under the influence of increasing concentrations of adrenaline. It can be seen that the effect of adrenaline on all three factors, but especially on the duration of the action potential is increased with increasing concentrations of adrenaline.

Effect of 1×10^{-6} g/ml. adrenaline on the characteristics of frog ventricular cell action potentials.

Conditions	No. of observations	Resting potential (mV)	Overshoot (mV)	Duration of action potential to 50% repolarisation (msec)
Normal	16	82.0 ± 1.4	25.1 ± 1.5	696.0 ± 29.0
After 1×10^{-6} g/ml. adrenaline	10	88.3 ± 2.2	35.1 ± 1.4	873.0 ± 47.5

± denote 1 S.E. of the mean.

All these differences are significant ($P < 0.05$)

Table 7

Effect of 1×10^{-6} g/ml adrenaline on the characteristics of frog ventricular cell action potentials in normal Ringer. It can be seen that adrenaline has hyperpolarised the resting potential (increased by 7.1%), has increased the overshoot value (by 30.0%) and has increased the duration of the action potential (by 20.3%).

External Ca^{++} concentration 1.0 mM.
Stimulated at 15/min.

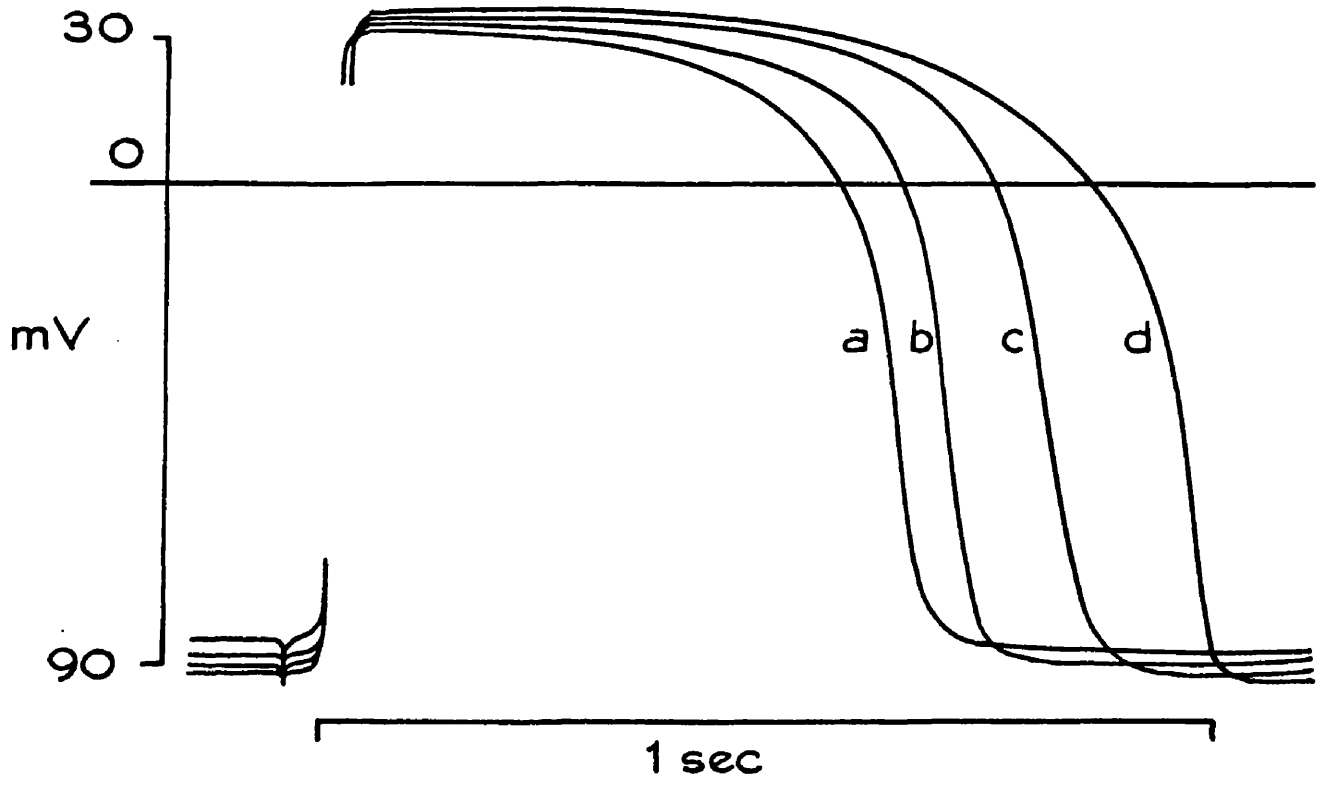

Fig. 18

The effect of varying strengths of adrenaline on the characteristics of the action potential. The micro-electrode remained in the cell throughout, as it was exposed to higher concentrations of adrenaline.

(a) normal action potential

(b) A.P. after 1 X 10^{-6} g/ml adrenaline

(c) A.P. after 5 X 10^{-6} g/ml adrenaline

(d) A.P. after 1 X 10^{-5} g/ml adrenaline.

External Ca^{++} 1.0 mM. Stimulated at 15/min.

Interaction between adrenaline and increased external potassium

Antoni & Engstfeld (1961) showed that adrenaline could restore contractility to a heart rendered inexcitable by exposure to high external potassium concentrations. This observation was repeated and the result of one experiment is shown in Fig. 19. The ventricle was perfused with Ringer containing 10.0 mM excess KCl; this caused the membrane depolarisation, reduction in overshoot and shortening of the action potential duration as shown. Then 1 X 10^{-6} g/ml adrenaline was added to the bath and produced a partial reversal of these effects. The change in the action potential, although not restoring complete normality was sufficient to allow complete redevelopment of twitch tension.

The effect of the external Ca^{++} concentration on the action potential

Niedergerke (1956) and Niedergerke & Orkand (1966a) found that altering the external Ca^{++} concentration produced changes in the shape and duration of the action potential. The effect of a

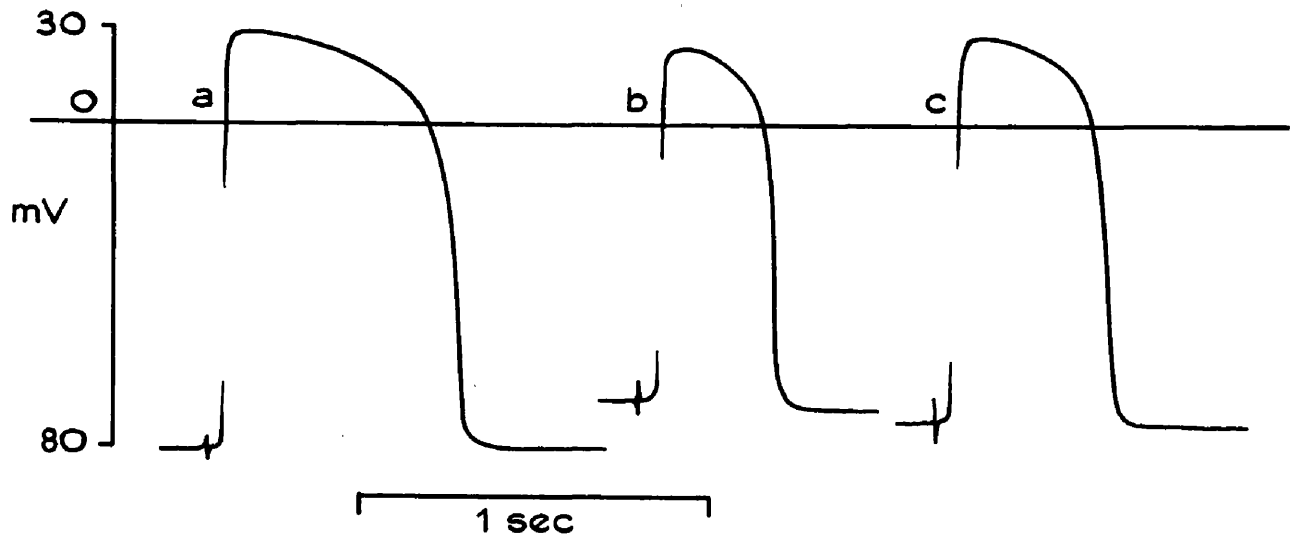

Fig. 19

Effect of increasing the external K^+ concentration on the action potential and partial restoration of normality by 1 X 10^{-6} g/ml adrenaline.

(a) potential in normal Ringer (2.5 mM KCl).

(b) potential in Ringer + 10.0 mM excess KCl.

(c) potential in the high KCl Ringer after exposure to 1 X 10^{-6} g/ml adrenaline.

The values of the parameters of the action potentials were:-

Conditions	Membrane potential	Overshoot	Time to 50% repo
(a)	81.0 mV	28.0 mV	620 msec
(b)	59.0 mV	20.0 mV	320 msec
(c)	66.0 mV	28.0 mV	410 msec

External Ca^{++} 1.0 mM. Stimulated at 15/min.

small range of concentrations on frog ventricular cell action potentials is shown in Fig. 20 (stimulated at 15/min). Increasing the external Ca^{++} gives a progressive reduction in overshoot and in the duration of the action potential. This is similar to the results found by Niedergerke & Orkand at this rate of stimulation. Also the shape of the action potential is altered in the high calcium Ringer, it is now more like the action potential normally recorded from mammalian cardiac tissue, in that the repolarisation phase can now be divided into three parts, (i) an initial fast repolarisation, then (ii) the 'plateau' or period of relatively slow recovery, and finally, (iii) another fast repolarisation to the membrane potential. The average values of the membrane potential, overshoot and time to half repolarisation of the action potential in 0.5, 1.0 and 2.0 mM Ca^{++} are given in Table 8. It should be noted that although in individual experiments the duration of the action potential was increased on going from 1.0 to 0.5 mM Ca^{++} Ringer, the average value for the time to half repolarisation given in

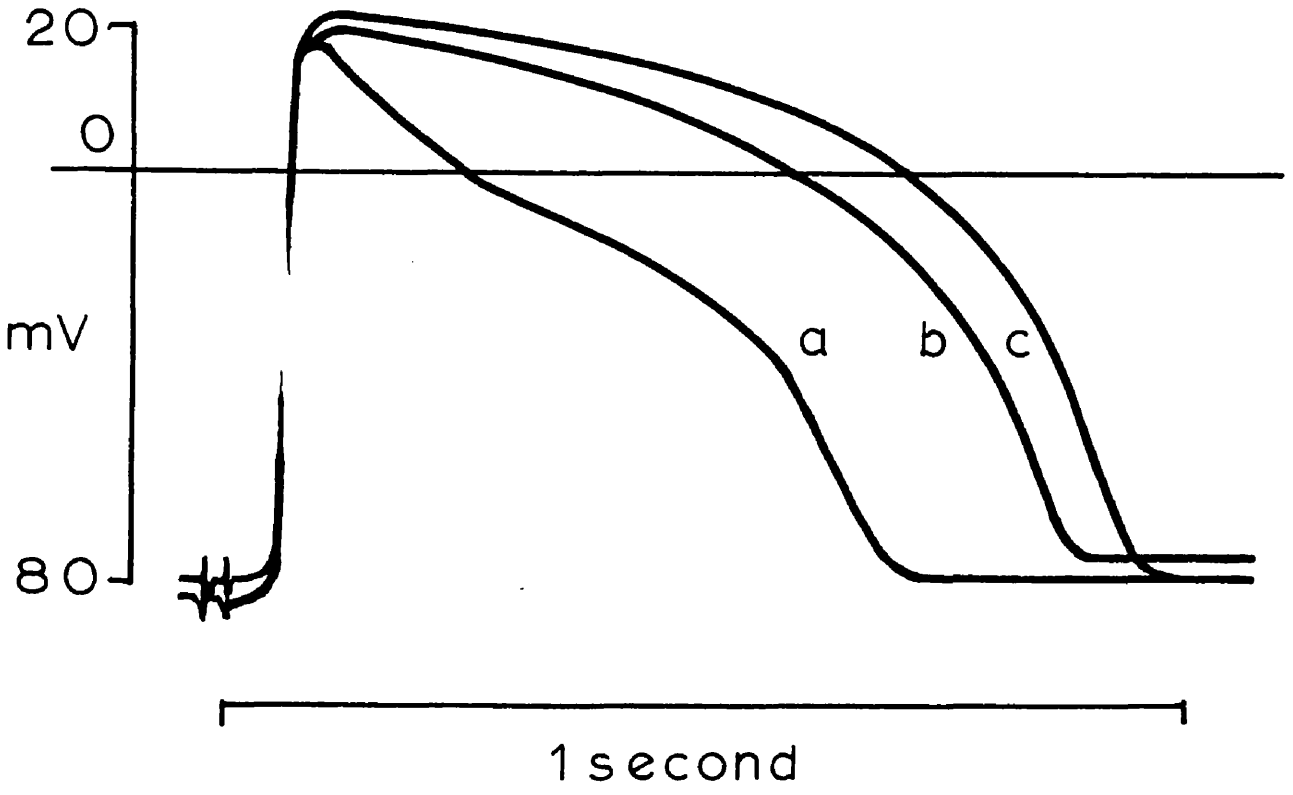

Fig. 20

Effect of external Ca^{++} concentration on action potential characteristics.

(a) potential in 2.0 mM Ca^{++} Ringer.

(b) potential in 1.0 mM Ca^{++} Ringer.

(c) potential in 0.5 mM Ca^{++} Ringer.

Stimulated at 15/min.

the table for 0.5 mM Ca^{++} Ringer is less than that for 1.0 mM Ca^{++}. This is probably due to the fact that the number of observations in 0.5 mM Ca^{++} Ringer is much smaller.

Adrenaline action at varying external Ca^{++} concentrations

The effect of 1 X 10^{-6} g/ml adrenaline on action potentials taken from one ventricle at the three calcium concentrations given above and stimulated at 15/min are shown in Fig. 21 and Table 8 gives the average values for the parameters after adrenaline for all the experiments done. It can be seen that the prolongation of the repolarisation time by adrenaline is highest in the low calcium solution and becomes smaller as the Ca^{++} concentration is increased.

Niedergerke & Orkand (1966b) have also shown that alterations in the external Na^{+} concentration affect the action potential. They were attempting to show that the alteration of the action potential was dependent on the ratio $[Ca^{++}]/[Na^{+}]^2$ of the external fluid, but the results were more complicated in the case of varying the external Na^{+} concentration,

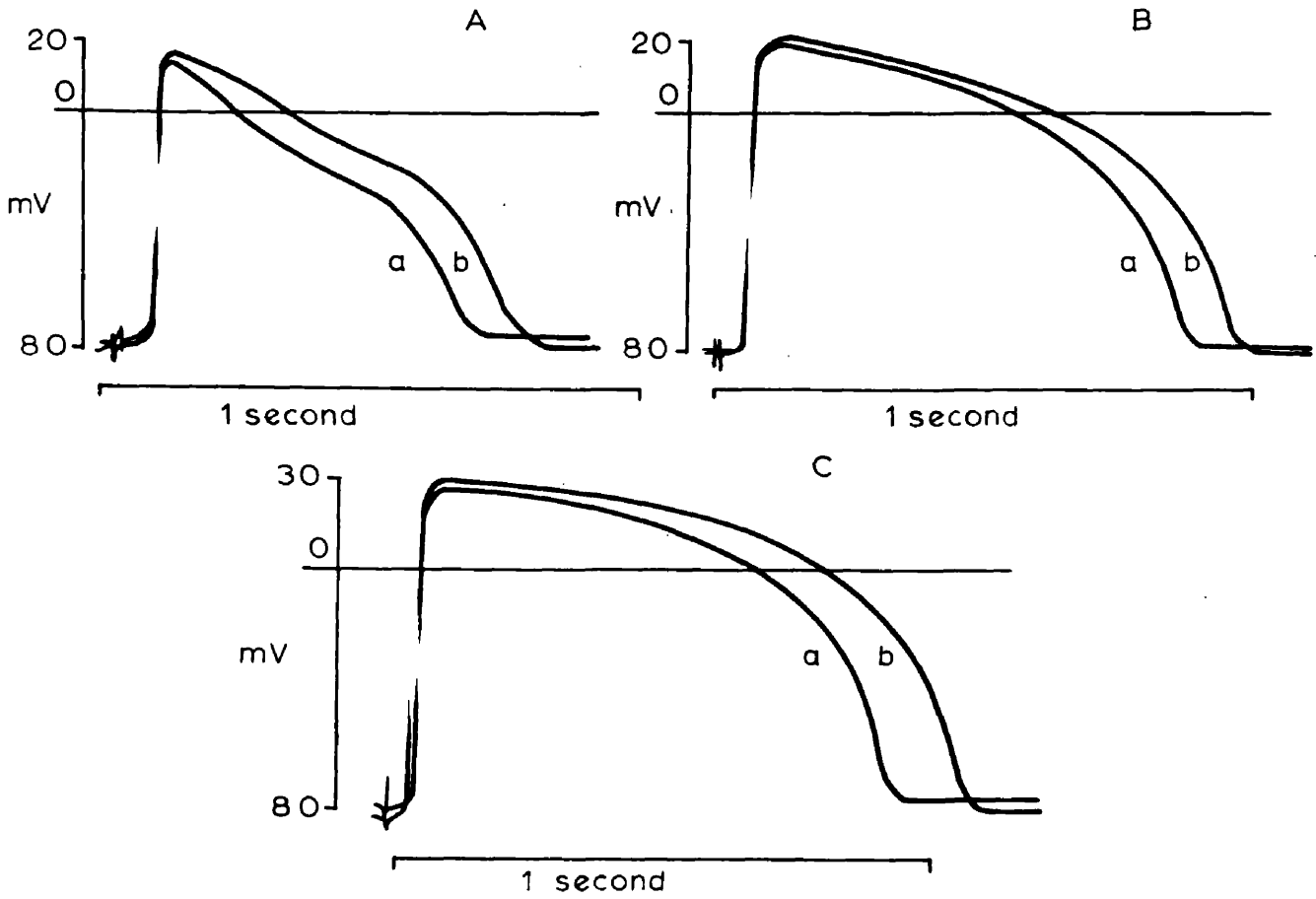

Fig. 21

Effect of adrenaline on characteristics of the action potential at varying external Ca^{++} concentrations.

(a) Ringer 2.0 mM Ca^{++}.

(b) Ringer 1.0 mM Ca^{++}.

(c) Ringer 0.5 mM Ca^{++}.

(a) before, and (b) after exposure to 1 X 10^{-6} g/ml adrenaline.

Stimulated at 15/min.

External Ca^{++} concn. (mM)	Resting potential (mV)		Overshoot (mV)		Duration of action potential to 50 % repolarisation (msec)			
	Normal	Adrenaline	Normal	Adrenaline	Normal	Adrenaline	Increase after adrenaline	% increase after adrenaline
0.5	75.2 ± 2.1	83.3 ± 1.8	19.5 ± 1.2	30.0 ± 1.8	618 ± 55	832 ± 33	214	25.7
1.0	82.7 ± 0.9	85.2 ± 0.4	23.6 ± 1.0	31.1 ± 1.7	699 ± 18	830 ± 17	131	15.8
2.0	75.3 ± 2.1	78.8 ± 1.1	14.0 ± 0.5	20.2 ± 0.4	483 ± 10	550 ± 7	67	12.2

The figures are the averages of observations taken from five experiments
± denotes 1 S.E. of the mean
All differences are significant (P at least < 0.05)

Table 8

Effect of 1 X 10^{-6} g/ml adrenaline on the characteristics of frog ventricular cell action potentials with varying external Ca^{++} concentrations as shown.

Stimulated at 15/min.

as the action potential depended not only on the ratio $[Ca^{++}]/[Na^{+}]^2$ but also on the absolute amount of sodium ions present in the Ringer. Evidence will be presented later to show that the action of adrenaline on mechanical activity in the ventricle is dependent on the ratio $[Ca^{++}]/[Na^{+}]^2$, but unfortunately time did not permit an examination of the effect of adrenaline on the action potential under conditions where the ratio was changed by altering the external Na^{+} concentration.

The effect of rate of stimulation on adrenaline action

Niedergerke (1956) and Niedergerke & Orkand (1966a) have shown that calcium in concentrations above approximately 3.0 mM has a progressively depressant effect on the size of the overshoot and the duration of the action potential at normal rates of stimulation. However, stimulating the heart at very low rates (around 1/min) 'protected' the action potential from this depressant effect until the external Ca^{++} concentration was raised to much higher values. At this rate of stimulation increasing the Ca^{++} concentration tended to increase the overshoot value and produced a variable effect on the duration of

the action potential.

Therefore the effect of adrenaline on the action potential at various Ca^{++} concentrations was examined when the ventricle was stimulated at 2/min. The recorded action potentials of one experiment are shown in Fig. 22. It can be seen that at this rate the effect of adrenaline on the duration of the action potential is increased at all concentrations of calcium, but most markedly at the low value. One interesting point which should be noted is that at this rate the normal action potential in 2.0 mM Ca^{++} Ringer is similar in shape to the action potential in normal Ringer (1.0 mM Ca^{++}); it has not developed the alteration in the repolarisation phase seen at higher rates of stimulation. However, the size of the overshoot is still smaller in 2.0 mM Ca^{++} Ringer than in normal, so it would appear that even at this rate of stimulation there is still some depression of the action potential by calcium, which was not found in the experiments of Niedergerke & Orkand who used a rate of 1/min.

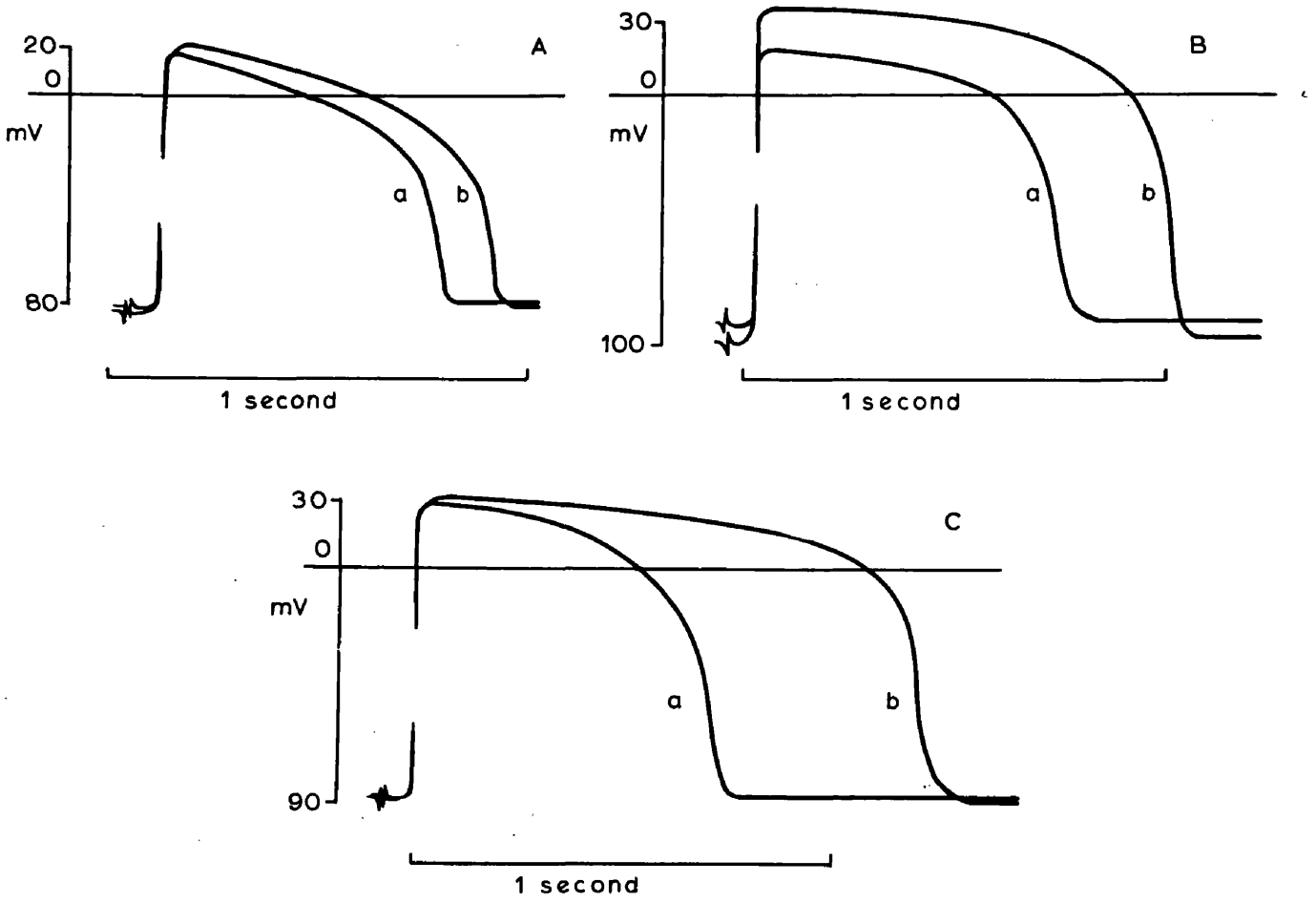

Fig. 22

Effect of adrenaline on characteristics of the action potential at varying external Ca^{++} concentrations.

(a) Ringer 2.0 mM Ca^{++}.

(b) Ringer 1.0 mM Ca^{++}.

(c) Ringer 0.5 mM Ca^{++}.

(a) before, and (b) during exposure to 1 X 10^{-6} g/ml adrenaline.

Stimulated at 2/min.

The effect of calcium and adrenaline on the latent period

It is well known that increasing the external Ca^{++} concentration reduces the excitability of conducting tissues and blocks the passage of impulses (see Leonard & Hadju, 1962; Garb, 1951; Weidmann, 1955). It has also been shown that adrenaline increases the conduction velocity in cardiac muscle (Siebens, Hoffman, Enson, Farrell & Brooks, 1953; Shapiro, 1961).

The latent period values for frog ventricle before and during exposure to 1×10^{-6} g/ml adrenaline with external Ca^{++} concentrations of 0.5, 1.0 and 2.0 mM are given in Table 9. The latent period is prolonged as the Ca^{++} concentration is increased and it can be seen that the reduction in latent period after adrenaline becomes relatively greater as the external calcium is increased.

The effect of adrenaline on mechanical activity

It is proposed firstly to deal with certain aspects of the action of adrenaline arising during the experimental work carried out for this thesis

External Ca^{++} concn. (mM)	Normal latent period (msec)	Latent period after adrenaline (msec)	Reduction in latency after adrenaline (msec)	% reduction in latency after adrenaline
0.5	37.8 ± 1.4	32.5 ± 1.7	5.3 ± 0.5	14.0
1.0	47.5 ± 1.3	35.3 ± 1.7	12.2 ± 0.9	25.7
2.0	58.1 ± 2.5	41.3 ± 2.5	16.8 ± 1.0	28.9

The figures are the averages of observations taken from six experiments
± denotes 1 S.E. of the mean
All differences are significant (P at least < 0.05)

Table 9

Effect of 1 X 10^{-6} g/ml adrenaline on the latent period of frog ventricle stimulated electrically at 15/min. The external Ca^{++} concentrations were varied as shown. All the recordings were taken at the same distance from the stimulating electrodes, approximately 0.5 cm. Taking this distance, the calculated conduction velocities of the tissue in the various Ca^{++} concentrations before and during exposure to adrenaline are:-

(a) 0.5 mM Ca^{++}: 0.13 m/sec and 0.15 m/sec respectively.

(b) 1.0 mM Ca^{++}: 0.11 m/sec and 0.14 m/sec.

(c) 2.0 mM Ca^{++}: 0.09 m/sec and 0.12 m/sec.

and then to discuss fully the effect of adrenaline and other factors on the time to peak twitch tension.

The effect of hypodynamia on adrenaline action As noted previously, the twitch tension of this superfused preparation decreases fairly rapidly with time, whereas the contracture tension remains constant. It was also found that the maximum twitch tension after exposure to adrenaline remained steady or even increased slightly with time. This means that the relative effect of adrenaline on the twitch tension increased with the development of the hypodynamic state. This is shown in Fig. 23.

The effect of altering the Ca^{++} and Na^{+} concentrations on adrenaline action Wilbrandt & Koller (1948) found that the strength of the cardiac contraction was dependent on the ratio $[Ca^{++}]/[Na^{+}]^2$ of the extracellular fluid and that the twitch tension remained steady if the ratio was kept constant. Lüttgau & Niedergerke (1958) confirmed these findings both for twitches and contractures in frog ventricular strips and explained the results as being due to a competition between calcium and sodium ions for a

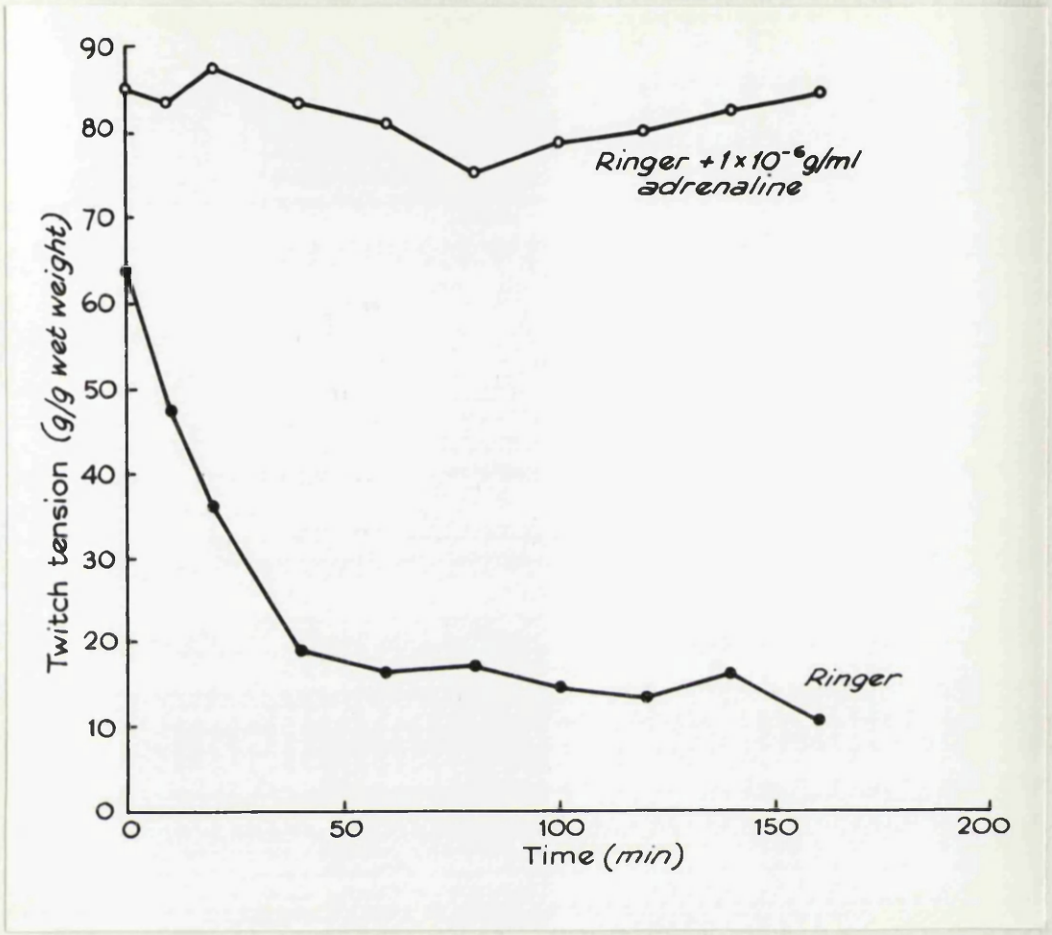

Fig. 23

Twitch tensions in a superfused frog ventricle before and during exposure to 1 X 10^{-6} g/ml adrenaline. Stimulated at 30/min. The twitch in normal Ringer becomes smaller fairly quickly with time, this is the development of hypodynamia in the ventricle, whereas the twitch after adrenaline remains at roughly the same tension throughout.

External Ca^{++} 1.0 mM.

hypothetical receptor site, R, a process which they represented as a bimolecular reversible reaction, thus:-

$$Ca + Na_2R \rightleftharpoons CaR + 2Na$$

According to this hypothesis the compound CaR activates the contractile mechanism whereas the compound Na_2R is inactive. Further evidence in favour of this hypothesis was later produced by Niedergerke (1963a, b).

Therefore it was of interest to examine the action of adrenaline at various values of $[Ca^{++}] / [Na^{+}]^2$.

(a) <u>effect of the ratio on the twitch tension after adrenaline</u>: Fig. 24 shows the percentage increase in twitch tension caused by exposure of the ventricle to 1 X 10^{-6} g/ml adrenaline when the ratio was altered by varying the external Ca^{++} concentration. It can be seen that there is a larger effect of adrenaline at low calcium levels, but that above 1.0 mM Ca^{++} there is no significant change, the percentage increase remaining relatively constant.

Only two experiments have been done to compare

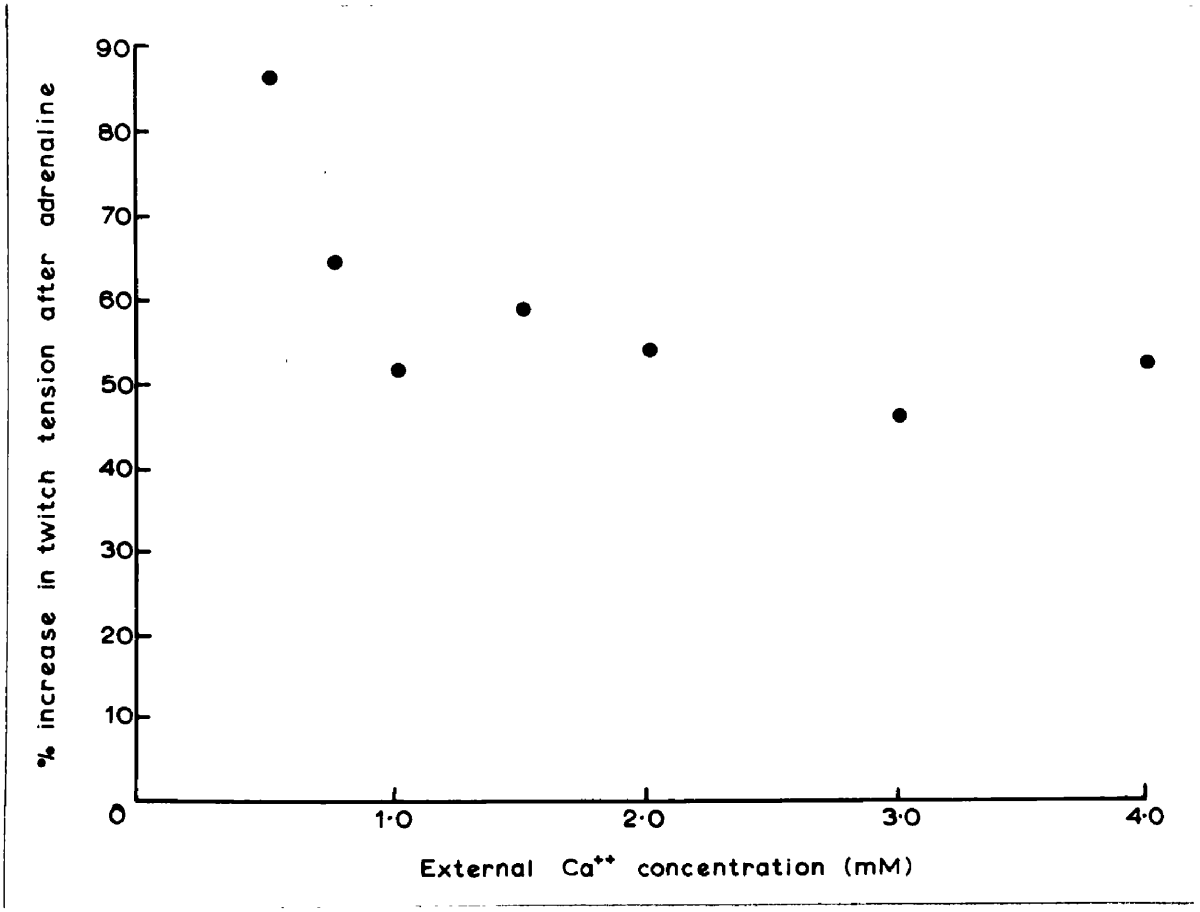

Fig. 24

Percentage increase in twitch tension of a superfused frog half ventricle after exposure to 1 X 10^{-6} g/ml adrenaline at various external Ca^{++} concentrations. The increase is greatest at low concentrations of calcium and remains relatively constant above roughly 1.0 mM external Ca^{++}.

Each point is the average of the results from the eleven experiments carried out. Stimulated at 30/min.

the effect on the action of adrenaline of altering the external Na^+ concentration and in this way changing the $[Ca^{++}] / [Na^+]^2$ ratio. The average values for the percentage increase in tension after 1×10^{-6} g/ml adrenaline under various conditions are given in Table 10.

(b) <u>effect of the ratio on the time for full development of adrenaline action</u>: it is shown in Fig. 25 that the time taken for adrenaline to produce the maximum increase in twitch tension is shortened by increasing the external Ca^{++} concentration (thereby increasing the $[Ca^{++}] / [Na^+]^2$ ratio) up to approximately 2.0 mM, and again there is little change thereafter.

The second row of Table 10 compares the time for development of maximum effect in the two experiments where the ratio was kept constant or doubled by altering either the external Ca^{++} or the external Na^+ concentrations, or both. There is again fairly good correlation of the time taken, when the ratio is at the same value.

	Ratio $[Ca^{++}] / [Na^{+}]^2$			
	Normal		Doubled	
Conditions	1.0 Ca, 100% Na	0.5 Ca, 75% Na	2.0 Ca, 100% Na	1.0 Ca, 75% Na
% increase in tension (g/g wet wt)	53.3	52.8	36.2	25.8
Time taken to give peak effect (sec)	40.0	45.0	19.0	22.0
Alteration of time to peak twitch tension (msec)	+ 13.0	+ 15.0	- 45.0	- 35.0

Figures are the averages of only two experiments

+ denotes a prolongation of time, - a reduction

Table 10

Effect of alteration of the $[Ca^{++}] / [Na^{+}]^2$ ratio of the external fluid on the action of 1×10^{-6} g/ml adrenaline on (i) the twitch tension, (ii) the time the adrenaline takes to produce its maximum effect and (iii) the time to peak twitch tension. The ratio is altered as shown either by changing the external Ca^{++} or the external Na^{+} concentrations, or both.

100% Na^{+} in the table corresponds to 120.0 mM.

The ratio $[Ca^{++}] / [Na^{+}]^2$ values in each case have not been accurately calculated, but they are similar in each case for all practical purposes.

Stimulated at 15/min.

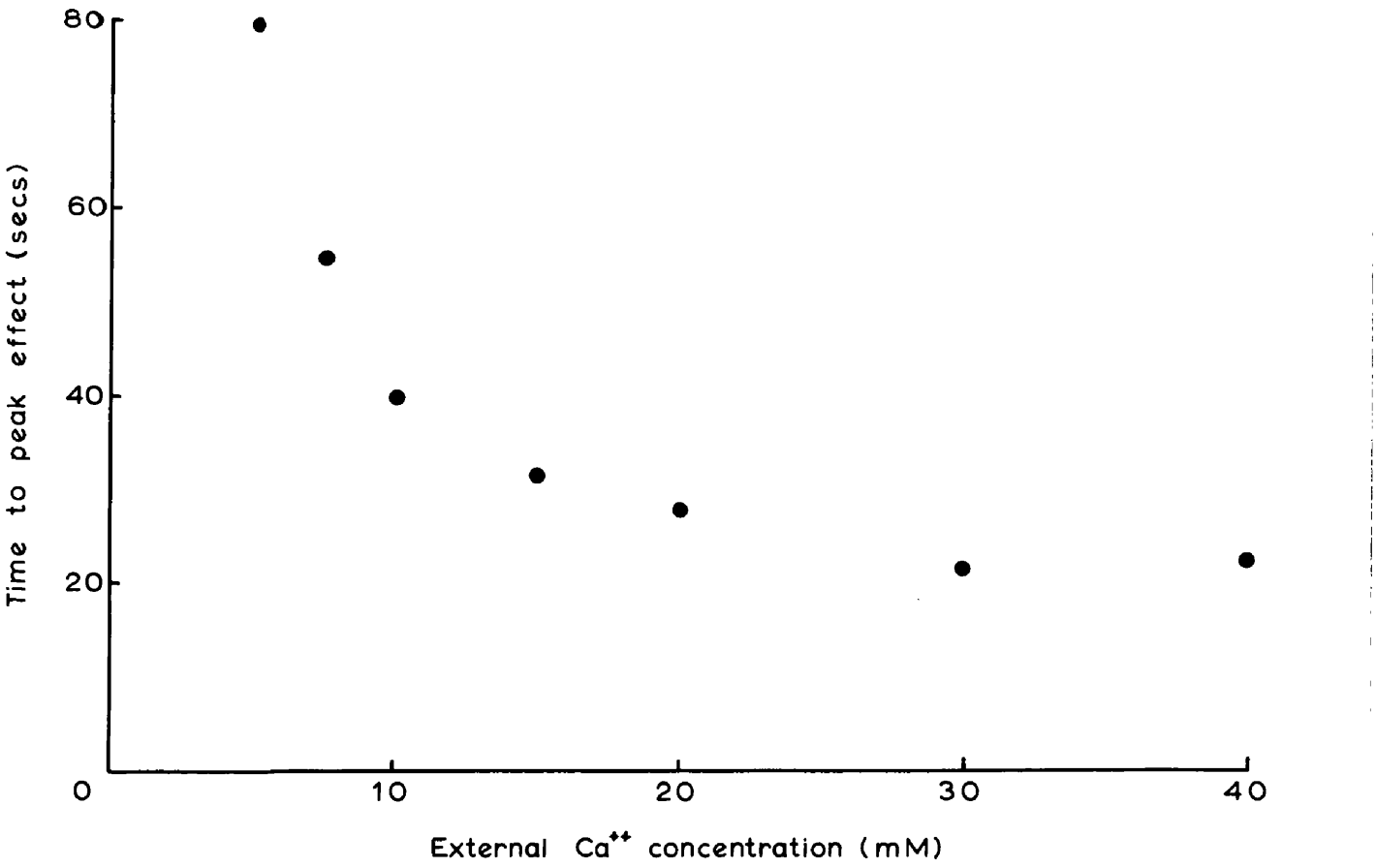

Fig. 25

Time taken for 1 X 10^{-6} g/ml adrenaline to produce the maximal effect on the twitch tension in a superfused frog half ventricle with varying external Ca^{++} concentrations. Above roughly 2.0 mM Ca^{++} the time remains relatively constant at about 20 seconds.

Each point is the average of the results from the eleven experiments carried out. Stimulated at 30/min.

The effect of adrenaline on the time to peak twitch tension

As stated already, the importance of examining the action of adrenaline on the time to peak twitch tension is that it appears on examination of the previous literature to cast considerable doubt on the validity of the belief that the duration of the action potential and the time to peak tension bear a relation to each other. However, the results of previous workers have differed, so the action of adrenaline on the time to peak tension was examined using the superfusion technique and also a search was made to find a possible cause for the variable results so far quoted.

In a carefully controlled series of experiments under similar conditions using normal Ringer, it was found that adrenaline gave, apparently at random, either an increase or a decrease in the time to peak tension. In each experiment, however, if adrenaline produced, for instance, a prolongation of the time to peak at the beginning of an experiment, it continued to do so throughout the experiment.

Therefore the effect of varying the conditions of the experiments on this action of adrenaline was tried. It was found that changing the stimulus frequency (15, 30 and 60/min), the initial resting tension, or perfusing the ventricle under anoxic conditions all produced no alteration of the action of adrenaline. Fig. 26, A, C and D respectively, shows the effect of these interventions on the time to peak tension in the absence of adrenaline; the results found are in agreement with those of earlier workers (see Niedergerke, 1956; Sonnenblick, 1962).

The effect on adrenaline action of changing the ratio $[Ca^{++}] / [Na^{+}]^2$. This was tried in view of the results quoted above showing the effect of altering the ratio on mechanical tension both normally and in the presence of adrenaline and also on the action potential. Fig. 26, B shows the effect of varying the Ca^{++} concentration over a small range, before exposing the ventricle to adrenaline. In agreement with the findings of Sonnenblick (1962) there is little alteration in the

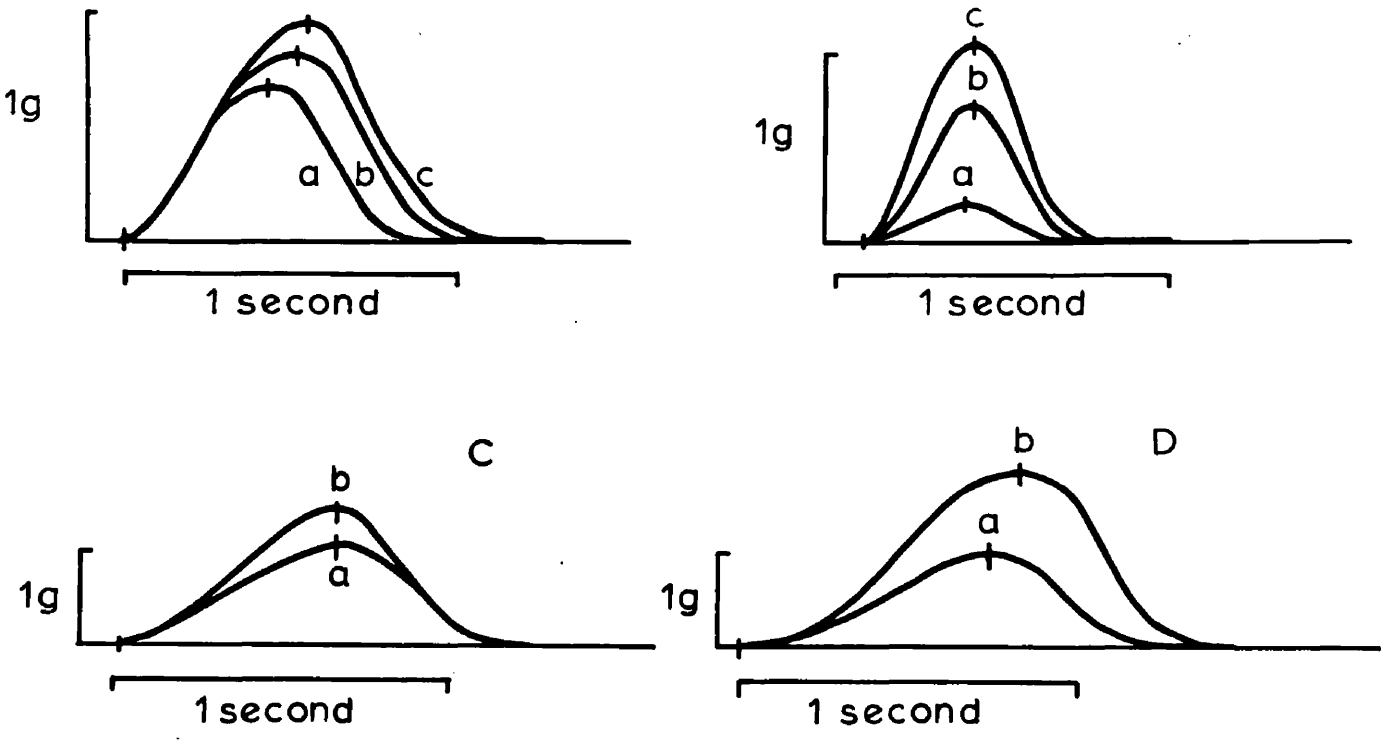

Fig. 26

Effect of various inotropic interventions on twitch tension and time to peak twitch tension.

(A) effect of varying rate of stimulation

(a) at 60/min, (b) at 30/min and (c) at 15/min.

(B) effect of varying the external Ca^{++} concentration.

(a) 0.5 mM, (b) 1.0 mM and (c) 2.0 mM.

(C) effect of altering the initial resting tension

(a) resting tension 150 mg and (b) resting tension 300 mg.

(D) effect of anoxia.

(a) ventricle perfused with N_2 bubbled Ringer, and

(b) ventricle perfused with O_2 bubbled Ringer.

Each situation was examined in a different preparation.

Except in (B) external Ca^{++} 1.0 mM and except in (A) preparation stimulated at 15/min.

time to peak tension of the twitches. However, in some ventricles increasing the external Ca^{++} concentration tended to reduce the time to peak tension as was found by Niedergerke (1956) and Naylor (1960). Reducing the external Na^{+} concentration, so that the ratio was approximately doubled, gave a slight reduction in the time to peak, similar to that found when the external Ca^{++} concentration was doubled, as shown in Fig. 28, A.

Changing the ratio $[Ca^{++}] / [Na^{+}]^2$ was found to produce a constant effect on the action of adrenaline. Firstly, with normal external Na^{+} concentration, on reducing the external Ca^{++} concentration below 1.0 mM, adrenaline always prolonged the time to peak tension and at higher concentrations adrenaline always reduced the time to peak tension. Tension records of one experiment are shown in Fig. 27. Similarly, the effect of changing the ratio $[Ca^{++}] / [Na^{+}]^2$ by altering the external Na^{+} concentration was examined. Fig. 28 shows the effect on the twitch before and during exposure to adrenaline of (A), reducing the external sodium by

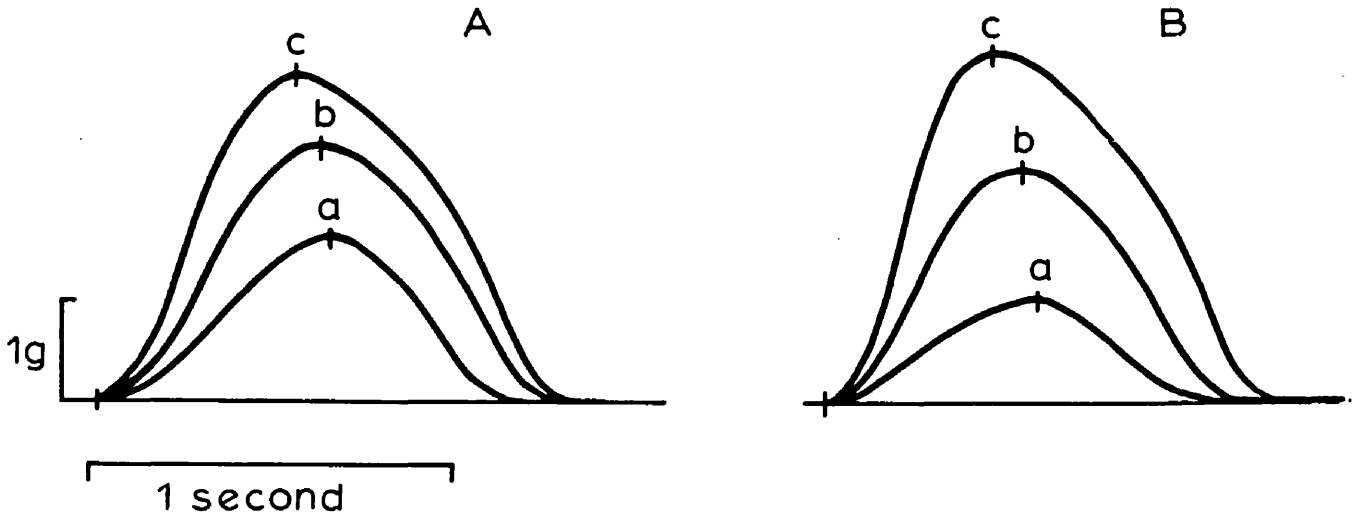

Fig. 28

Effect of altering the $[Ca^{++}]/[Na^{+}]^2$ ratio on the action of adrenaline on the time to peak twitch tension. In tracing A, the external Na^{+} concentration was reduced by one quarter, in tracing B, the external Ca^{++} concentration was doubled. Both these increase the ratio in favour of Ca^{++} by roughly similar amounts and it can be seen that the effect on the normal twitch (a) of these solutions is to increase the tension developed and to slightly reduce the time to peak tension, (b). When adrenaline is then added there is a further increase in tension and reduction in time to peak tension, (c). The effect of the two solutions, both in their own right and in combination with adrenaline, is very similar.

Experiments carried out on same preparation. Stimulated at 15/min.

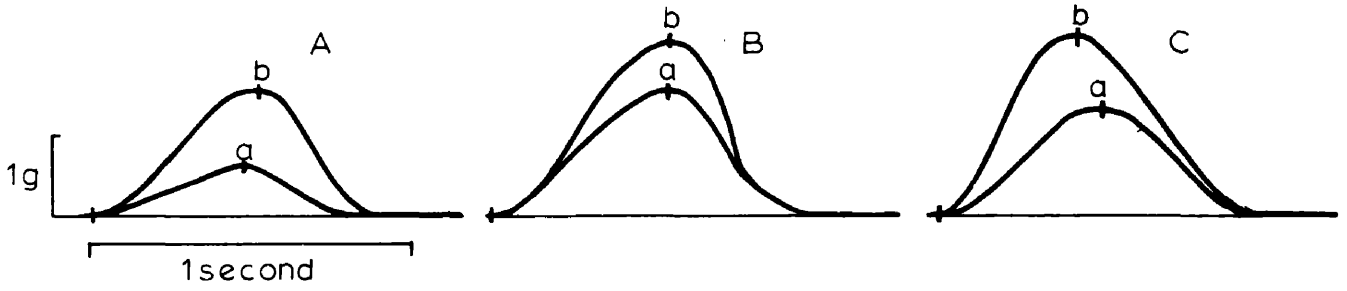

Fig. 27

The action of 1 X 10^{-6} g/ml adrenaline on the time to peak twitch tension in a superfused frog ventricular preparation at different external Ca^{++} concentrations.

(A) 0.5 mM Ca^{++} Ringer.

(B) 1.0 mM Ca^{++} Ringer.

(C) 2.0 mM Ca^{++} Ringer.

(a) normal twitch, (b) twitch after adrenaline.

This experiment was carried out on one preparation. Stimulated at 15/min.

The time to peak tension is prolonged in the low Ca^{++} Ringer, relatively unchanged in the normal Ringer and reduced in 2.0 mM Ca^{++} Ringer.

one quarter and (B), doubling the external calcium. Both increase the $[Ca^{++}]/[Na^{+}]^2$ ratio by roughly similar amounts and the figure shows the similarity of the effect on the twitch.

The results of all the experiments done where the ratio was varied by changing the external calcium are summarised in Fig. 29, where the change in time to peak tension in msec after 1 X 10^{-6} g/ml adrenaline is expressed as a function of the external Ca^{++} concentration. The variable response found with 1.0 mM Ca^{++} Ringer can be explained by the differing sensitivity of individual hearts to calcium, this concentration being at the threshold of reversal for this particular effect. As there are only a few results on the action of adrenaline on the time to peak when the $[Ca^{++}]/[Na^{+}]^2$ ratio is altered by changing the Na^{+} concentration, they have not been plotted in Fig. 29, but they are shown in Table 10. It can be seen that when the ratio is constant, the effect of adrenaline on the time to peak is similar, no matter how the final value of the ratio is obtained.

Because it had been already shown that the

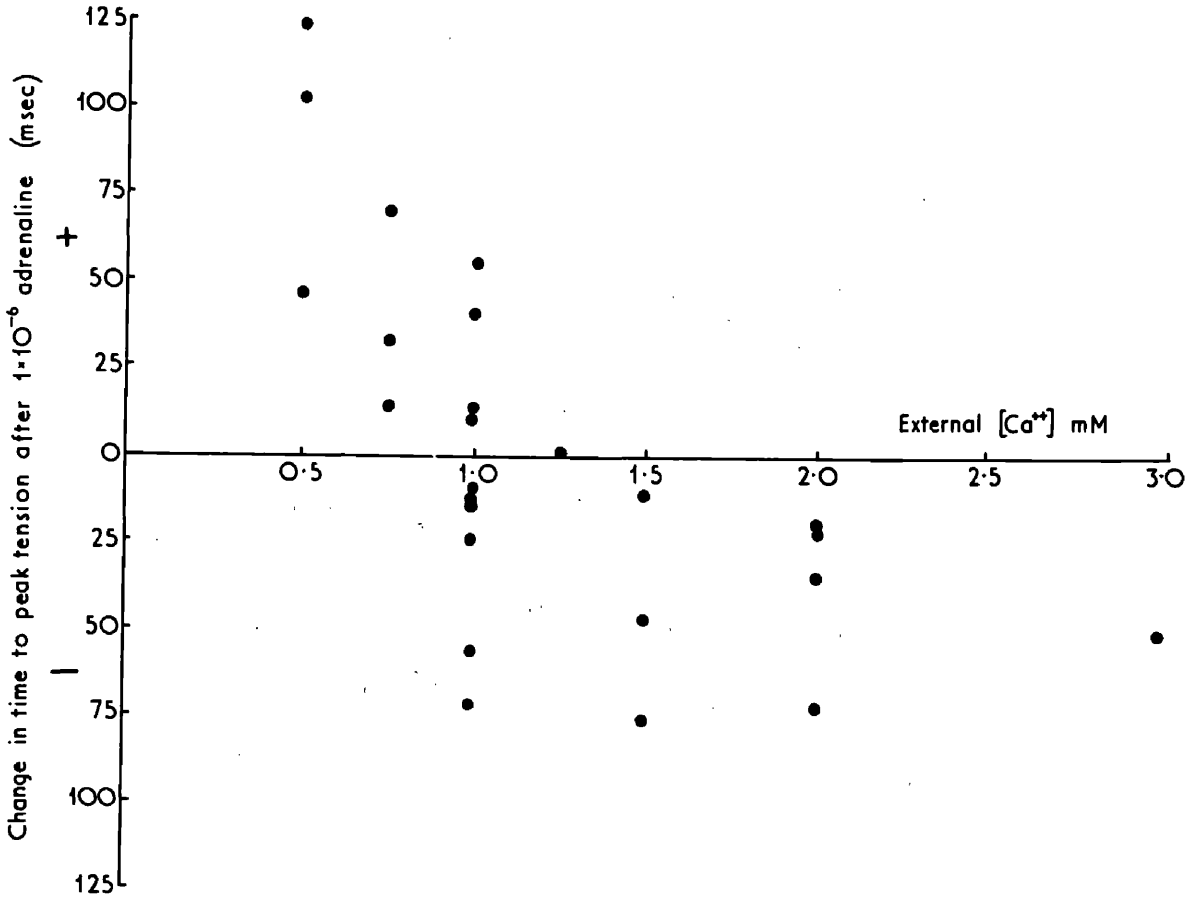

Fig. 29

Summary of the change in time to peak twitch tension brought about by 1 X 10^{-6} g/ml adrenaline in all the experiments done to test the action of varying the external Ca^{++} concentration on this phenomenon. The points above the abscissa are results where the time to peak was prolonged, and those below are results where the time to peak was reduced.

response of the action potential to adrenaline could be modified by stimulating the tissue at a low rate, the effect of adrenaline on the time to peak tension when the ventricle was stimulated at 2/min was examined. It was found that the effect of adrenaline when the $[Ca^{++}]/[Na^{+}]^2$ ratio was high could be reversed at this low rate. Whereas before it had been shown that at 'physiological' rates of stimulation adrenaline always reduced the peak tension time under these conditions, when the ventricle was stimulated at 2/min adrenaline now gave a prolongation of the time to peak tension. This is shown in Fig. 30. It was also shown that the degree of prolongation of the time to peak tension by adrenaline in low calcium Ringer could be increased by stimulating at this low rate.

This dependence of the action of adrenaline on the time to peak tension on the $[Ca^{++}]/[Na^{+}]^2$ ratio could be a possible explanation for the differing results found by previous workers. A retrospective study of the papers showed that Naylor, who found a prolongation of the time to peak twitch tension after

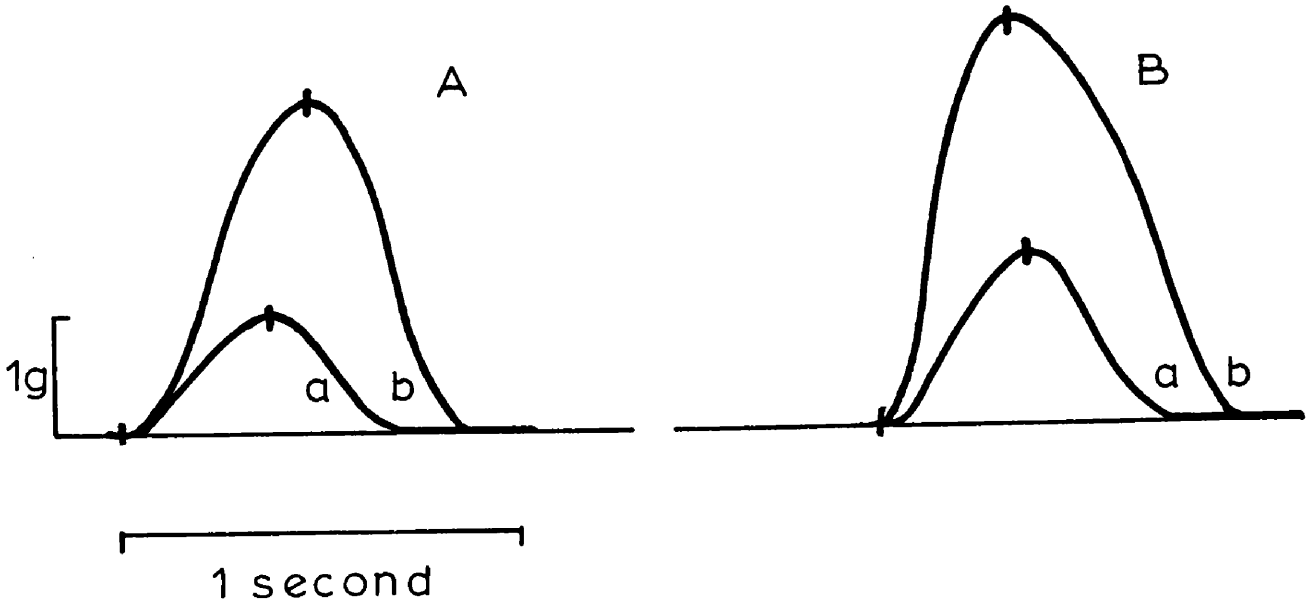

Fig. 30

The effect of two rates of stimulation on the adrenaline action on the time to peak twitch tension.

(A) Ringer 2.0 mM Ca^{++}, stimulated at 2/min.

(B) Ringer 2.0 mM Ca^{++}, stimulated at 15/min.

(a) normal twitch, (b) twitch after 1 X 10^{-6} g/ml adrenaline.

This experiment was carried out on one preparation.

As demonstrated before, the time to peak tension was reduced by adrenaline at the higher rate of stimulation, but under similar conditions except that the stimulation rate was changed to 2/min, adrenaline now caused a prolongation of the time to peak tension.

adrenaline, used Ringer containing 1.6 mM Ca^{++}, whereas both Sonneblick and Podolsky used mammalian Ringer with much higher Ca^{++} concentrations (around 5.0 mM) and they found a reduction in the time to peak.

Correlation of adrenaline effect on twitch and action potential

The effect of adrenaline on the time to peak tension, the duration of the action potential and the latent period of the tissue in Ringer containing varying Ca^{++} concentrations is summarised in Table 11. It can be seen that at all concentrations of calcium the prolongation of the action potential is considerably larger than the change in time to peak tension, which is reduced rather than prolonged in 1.0 and 2.0 mM Ca^{++} Ringers. Also the effect of adrenaline on the duration of the latent period appears to be too small to wholly explain this large discrepancy between the two factors. It seems therefore that in this case the time to peak tension is not proportional to the duration of the action potential. This will be examined more closely in the Discussion.

Ext. Ca^{++} conon. (mM)	Effect of 1 X 10^{-6} g/ml adrenaline on		
	Time to peak tension (msec)	Time to 50 % repolarisation action potential (msec)	Latent period (msec)
0.5	+ 93.0	+ 214.0	- 5.3
1.0	- 3.0	+ 131.0	- 12.2
2.0	- 38.0	+ 67.0	- 16.8

+ denotes a prolongation of the time, - denotes a reduction.

Table 11

Correlation of the action of adrenaline on the time to peak twitch tension, duration of the action potential and latent period of frog ventricular muscle. External Ca^{++} concentration varies as shown.

Stimulated at 15/min.

THE EFFECT OF ADRENALINE ON CHLORIDE FLUX

There have been several investigations carried out on the effect of adrenaline on potassium flux in different types of cardiac tissue (Hutter & Harris, in Hutter, 1957; Waddell, 1961; Stafford, 1962; McGuigan, unpublished observations). The final results vary slightly, but in all cases where some change was found, the magnitude of the change was small and unlikely to be significant. Haas & Trautwein (1963) have shown that adrenaline increased the sodium efflux from frog sinus venosus and atrium. However, there has been no published work on the effect of adrenaline on the movement of chloride across the membrane. It has been shown that chloride plays a more important part than had been hitherto thought in the control of the action potential duration (Hutter & Noble, 1961; Carmeliet, 1961). It was a possibility therefore that adrenaline might be producing its effect on electrical activity in the cell by an action on chloride movement. The experiments reported above showing that adrenaline still affected potassium contractures when the chloride of the Ringer

was replaced by sulphate gave indirect evidence against this, but several experiments were carried out to test the effect of adrenaline directly, using the radio-isotope of chloride, ^{36}Cl.

Fig. 31 shows a typical result of an experiment to measure the effect of adrenaline on chloride efflux. The activity left in the ventricle is plotted on a logarithmic scale against time and the ventricle was perfused with Ringer containing 1 X 10^{-6} g/ml adrenaline during the period shown. It can be seen that there was no effect of adrenaline on the chloride efflux. There was no difference in the efflux after adrenaline in all the experiments done, whether the ventricle was quiescent or beating.

The effect of adrenaline on the influx of ^{36}Cl was also examined and again there was no apparent change in the influx during exposure to adrenaline.

No attempt was made to analyse these results further, as it was not considered necessary to know the actual values of the chloride flux through the membrane.

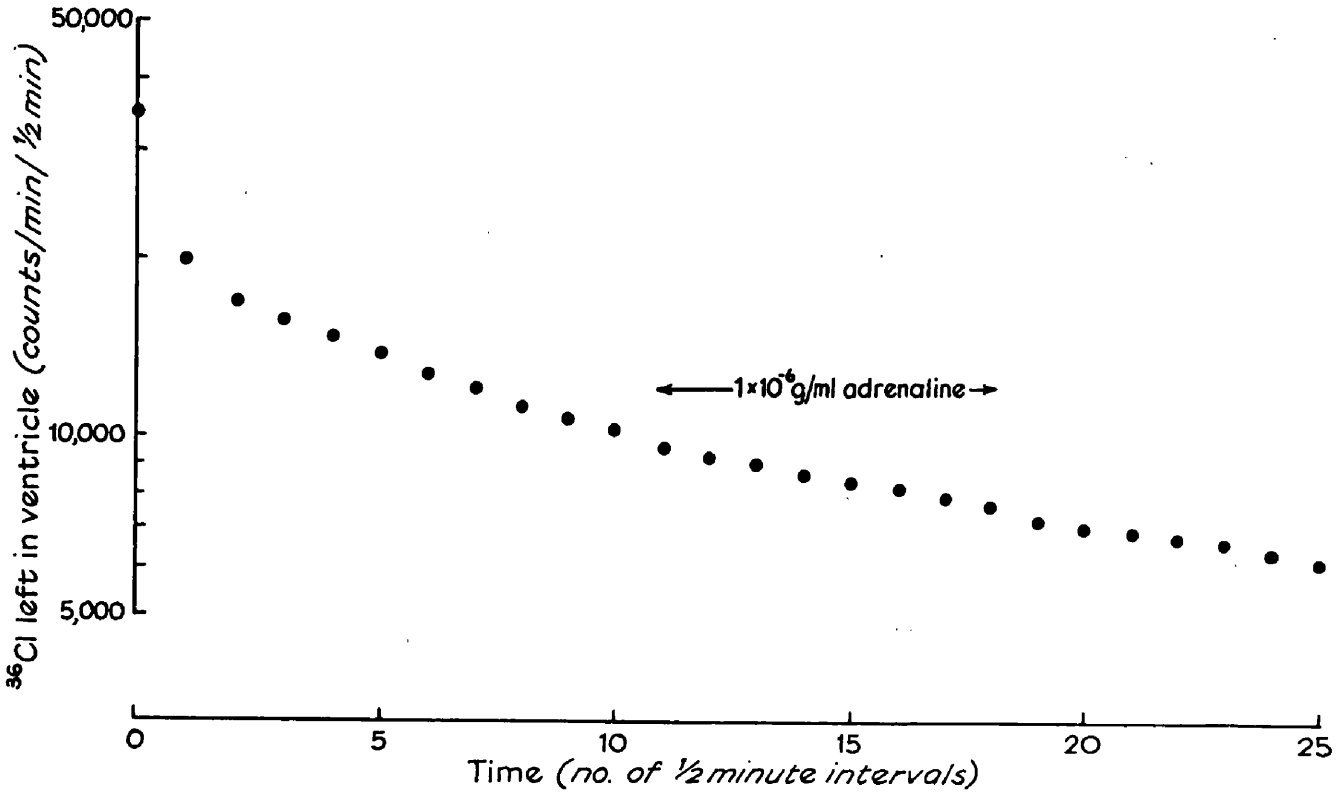

Fig. 31

Effect of 1 X 10^{-6} g/ml adrenaline on chloride efflux from a perfused frog ventricle. The ventricle was loaded with ^{36}Cl by soaking it in Ringer made up with $Na^{36}Cl$. Perfusion with Ringer was then started, at the point marked on the graph the Ringer contained adrenaline. The total activity washed out of the ventricle each half minute was counted and summation of each of these values gave an estimate of the amount of radio-activity left in the ventricle at each half minute interval. The ^{36}Cl left in the ventricle is therefore plotted against time in the graph above, and it can be seen that during the time when the ventricle was exposed to adrenaline the rate of loss of ^{36}Cl was not altered.

DISCUSSION

DISCUSSION

The purpose of this thesis was to examine the effects of adrenaline on mechanical activity and electrical events in frog ventricular muscle and also to examine the inter-relationship between these effects. In a preliminary experiment the effect of adrenaline on potassium contractures was tested, as this did not appear to have been done before. The result, that adrenaline reduced the developed tension, was unexpected and led to a more complete investigation of this phenomenon. It is proposed therefore to discuss this part of the experimental work first and to postulate a possible mechanism for the effect.

The action of adrenaline on potassium contractures

The dependence of contracture tension on membrane potential is well known, (Kuffler, 1946; Niedergerke, 1956; Hodgkin & Horowicz, 1960). The characteristic relationship between the two in a superfused frog ventricular preparation is shown in Fig. 6, (from Lamb & McGuigan, 1966). It was found that after the ventricle had been exposed to adrenaline,

the contracture tension developed with each concentration of excess KCl was markedly reduced. This meant that the relationship between tension and potassium concentration had been altered and it was now necessary to seek possible causes for this change.

Dudel & Trautwein (1957) showed that adrenaline hyperpolarised the resting membrane potential of dog auricular fibres and Otsuka (1958) and Trautwein & Schmidt (1960) found similar results in sheep and dog Purkinje fibres respectively. These results were confirmed for frog ventricle in this thesis and it was also found that adrenaline still hyperpolarised the membrane when the potential had been altered by exposing the tissue to high potassium solutions. This seemed a possible explanation for the reduction in contracture tension after adrenaline, the increase in membrane potential caused by adrenaline only allowing development of a smaller amount of tension. When the relation between tension and potential during exposure of the ventricle to adrenaline was plotted it was found that the points,

for each concentration of potassium tested, fell along the curve connecting the pre-adrenaline plots (Fig. 15). This is consistent with the hypothesis that the action of adrenaline on the contractures is mediated through the action on the membrane potential and that under these conditions adrenaline is not altering the relation between tension and potential. The full significance of this point will be discussed later, in connection with the action of adrenaline on the twitch.

A possible explanation for this hyperpolarisation of the membrane by adrenaline will now be discussed. The resting membrane potential can be described by the Goldman Constant Field equation,

$$V = \frac{RT}{F} \log_e \frac{[K_o] + \alpha [Na_o]}{[K_i] + \alpha [Na_i]}$$

where α is the ratio of the sodium and potassium permeabilities and the other symbols have their usual significance, (see Adrian, 1960). Chloride, although probably not passively distributed in heart muscle (Lamb, 1961), is unlikely to much affect the membrane potential as its permeability is low compared to that of potassium (Hutter & Noble,

1961; Carmeliet, 1961). There is also good evidence against the action of adrenaline on the membrane potential being due to an effect on chloride movement. Trautwein (1963) has shown that adrenaline still increases the slope of the pre-potential of frog sinus venosus cells when the extracellular chloride is replaced by a large anion to which the cell is impermeable. It has been shown in this thesis that the action of adrenaline on potassium contractures in frog ventricle is unaltered by replacing the chloride of the Ringer with sulphate and also experiments done with the radio-isotope of chloride, ^{36}Cl, have failed to show any alteration in chloride flux across the membrane under the influence of adrenaline.

With normal external potassium concentrations the membrane potential might be increased by increasing the ratio P_K/P_{Na} thus bringing V nearer to the equilibrium potential for potassium. This would require an increase in P_K or a decrease in P_{Na} or both. There have been several studies of the effect of adrenaline on potassium flux in various

types of cardiac muscle. The results have been variable. Hutter & Harris (quoted in Hutter, 1957) and McGuigan (unpublished observations) found no change in flux after adrenaline, whereas Waddell (1961) and Stafford (1962) found some increase under certain conditions. This increase, however, was quantitatively too small (of the order of 10%) to make much difference to the membrane potential. It has been argued that the pacemaker effects of adrenaline would require an increase in P_{Na} rather than a decrease (Hutter & Trautwein, 1956) although this has not been established. However, when the membrane is depolarised with excess potassium, the equilibrium potential for potassium and the membrane potential approximate to each other (Adrian, 1960; Anderson, 1966) and so under these conditions changes in P_K or P_{Na} could not increase the membrane potential. Therefore it seems unlikely that permeability changes could account for the effect of adrenaline in hyperpolarising the membrane.

Recently evidence has been accumulating in the literature that the sodium pump may make a direct

contribution to the membrane potential. This is the 'electrogenic' action of the pump, meaning that the pump is not electrically neutral and therefore that its action is directly responsible for the generation of a potential across the membrane. Kernan (1962) and Keynes & Rybová (1963) loaded frog sartorius muscles with sodium and then allowed them to recover in normal solutions. The measured membrane potential was significantly larger than the calculated equilibrium potential for potassium. Once the excess sodium had left the cell the membrane potential returned to the normal value. Keynes & Rybová were able to block this hyperpolarisation of the membrane potential by exposing the muscle to ouabain. Mullins & Awad (1965) carried out similar experiments except that they replaced the extracellular chloride with sulphate in order to exclude any change in chloride flux which might cause this effect. Adrenaline may therefore be able to bring about the observed change in the membrane potential by increasing the contribution to the membrane potential from this

'electrogenic' sodium pump and there is evidence that adrenaline may be producing its effect by increasing the active extrusion of sodium across the membrane. Trautwein & Schmidt (1960) found that the hyperpolarisation of the membrane of Purkinje fibres by adrenaline could be blocked by exposing the tissue to DNP or iodoacetate and also by replacing the intracellular sodium with lithium, which is not actively extruded from the cell by the sodium pump mechanism. Then Hass & Trautwein (1963) showed that adrenaline increased the sodium efflux from frog sinus or atrium. This could also be blocked by exposure of the preparation to DNP or iodoacetate. Experimental work for this thesis has shown that the hyperpolarisation of the membrane by adrenaline could be increased by replacing the extra-cellular sodium with sucrose, thus probably increasing the ease with which sodium could leave the cell. This observation requires further discussion. Both Adrian (1956) and Niedergerke (1966) have drawn attention to the increased frequency of the development of tip

potentials in microelectrodes during recording in solutions of low ionic strengths. No measurements of tip potentials were made during this work. However, the microelectrode was checked regularly and potential readings discarded if the resistance had increased, as it was known that this increased the likelihood of tip potential interference. Therefore, although unlikely, the initial hyperpolarisation of the membrane observed when the normal Ringer bathing the tissue was replaced with sodium-free Ringer could have been due to development of a tip potential. On the other hand the recordings of the membrane potential before and during exposure of the ventricle to adrenaline were made in the same solution and with the same microelectrode, so there was no change in the ionic strength of the bathing fluid and the increased potential recorded is not likely to be due to tip potential interference. It was separately tested that addition of adrenaline to a Ringer solution does not alter the characteristics of the microelectrode.

The last piece of evidence in favour of an

increased active extrusion of sodium under the influence of adrenaline is that the hyperpolarisation of the membrane can be blocked by prior exposure of the ventricle to ouabain. The work of Trautwein and his associates using DNP and iodoacetate shows only that metabolic energy is required for this process, but it is known that the action of ouabain is mediated specifically through its effect on the sodium pump. All this therefore supports the assertion that the hyperpolarisation of the membrane produced by adrenaline is associated with an increase in activity of the 'electrogenic' sodium pump, and therefore that the hyperpolarisation may be due to an increase in the potential resulting from the activity of the pump.

An important point about the interaction between adrenaline and ouabain which should be noted here is that after exposure of the ventricle to ouabain, the addition of adrenaline to the bathing fluid caused a depolarisation of the membrane rather than the normal hyperpolarisation. Adrenaline would therefore appear to be also producing another effect on the

cell which tends to depolarise the membrane in the absence of the over-riding hyperpolarisation due to increased sodium pump activity. This is unlikely to be an effect on either potassium or chloride movement across the membrane in view of the findings given above. One very possible explanation would be an increase in the membrane permeability to sodium; an effect which would depolarise the cell. The slight depolarisation of the membrane and increased slope of the prepotential in pacemaker tissues (del Castillo & Katz, 1955; Hutter & Trautwein, 1956) could be most readily explained by a similar increase in the permeability to sodium. In ventricular cells the resting permeability to sodium is probably low and therefore any increase in permeability to sodium will tend to be relatively small and is quite likely to be masked by increased sodium pump activity. On the other hand Trautwein & Kassebaum (1961) have shown that the resting conductance of Purkinje fibres is appreciably larger, and in them and other types of pacemaker tissue, it might be expected that an

increase in permeability would predominate over increased active extrusion of sodium and that the end result would be a reduced membrane potential. This point will be dealt with further when the effects of adrenaline on the action potential are discussed.

The mechanism of adrenaline action on twitch tension

Despite much research into the problem of the action of adrenaline in increasing the twitch tension, there has still been no completely acceptable theory formulated to explain the action at the cellular level. Workers studying the dynamics of cardiac contraction and the effect of adrenaline on the contraction (Podolsky, 1962; Sonnenblick, 1962) state that adrenaline increases the velocity of onset of the active state in the contractile element (having excluded changes in the series elastic compliance) and possibly also increases the peak tension of the contractile element, without giving any evidence or theories as to the underlying mechanism of this effect. The commonly held view that adrenaline made available more energy, in the

form of high energy phosphate compounds, for the contractile mechanism by its action on the adenyl cyclase system and the formation of 3'5' AMP (see Haugaard & Hess, 1965) seems to have been effectively disproved by the work of Øye (1965) and Williamson (1966). They have shown that the positive inotropic effect of adrenaline on the cell begins to develop an appreciable time before there is any increase in phosphorylase activity in the cell.

Fleckenstein (1963) ascribes the action of adrenaline to (a) a prolongation of the action potential, which effectively increases the period of time for entry of calcium into the cell and (b) 'direct inotropic actions synergistic to calcium that cannot be explained solely by an influence on excitation.' This latter statement was based on the observations of Antoni & Engstfeld (1961) who showed that adrenaline only partially restored to normal the action potential of cat papillary muscle fibres which had been exposed to Ringer containing excess KCl, whereas the twitch tension was restored

to supernormal values. These statements, though vague, constitute a definite attempt to equate the action of adrenaline to the known mechanisms of excitation-contraction coupling, Fleckenstein postulating that adrenaline produces an intracellular increase in the amount of the 'link' substance calcium between the excitation process and the contractile mechanism, due firstly to the effect on the action potential duration and secondly to some other unspecified action apparently of a similar nature to the inotropic action of increasing the external calcium concentration.

Further evidence is presented in this thesis for an interaction between calcium and adrenaline on twitch tension. Thus, Figs. 24 and 25 show that the action of adrenaline on twitch tension is greater but takes longer to develop at low external Ca^{++} concentrations. Once the concentration is increased to approximately 1.0 - 2.0 mM the percentage increase in tension and time for full development of tension remain relatively constant. It has also been shown that altering the ratio $[Ca^{++}]/[Na^{+}]^{2}$ by

changing the external Na^+ concentration affects the tension development and the time for development of the peak inotropic effect in a similar fashion. Lüttgau & Niedergerke (1958) postulated that the ratio $[Ca^{++}] / [Na^+]^2$ at a superficial site within the cell controls the mechanical tension developed by the contractile element. The greater effect of adrenaline on the twitch tension when the ratio is low suggests that a possible mode of action of adrenaline could be to produce an increase in the ratio at the 'active' site in the cell.

The work done on the effect of adrenaline on the action potential characteristics, the time to peak tension of the twitch and on the correlation between the two, shows that here again the ratio $[Ca^{++}] / [Na^+]^2$ of the bathing solution controls the response to adrenaline to a marked degree.

The effects of adrenaline on the cell membrane The experimental results of the effect of adrenaline on the action potential of frog ventricular muscle fibres can be easily summarised. Adrenaline gives

a dose-dependent increase in the magnitude of the overshoot and also increases the duration of the repolarisation phase of the action potential. This effect is modified by the external Ca^{++} concentration, the smallest overshoot and the smallest increase in duration of the action potential occurring at the highest calcium level. When the ventricle is stimulated at a low rate (2/min) instead of the usual 15 - 30/min the effect of adrenaline on both parameters of the action potential is increased at all concentrations of calcium, but most markedly at the lower values. The effect of adrenaline on the action potential when the ratio $[Ca^{++}]/[Na^{+}]^2$ is altered by changing the external Na^{+} concentration was not examined, as time did not permit this. Niedergerke & Orkand (1966) have examined the effect on the action potential of frog ventricular fibres of changing the ratio by altering the Na^{+} concentration and have found that the results are more complex than those found when the external Ca^{++} concentration is altered, as the action potential not only depends on the ratio $[Ca^{++}]/[Na^{+}]^2$, but also

on the absolute concentration of sodium ions present in the bathing Ringer. It would be expected therefore that the effect of adrenaline on the action potential under these conditions would be similarly complicated.

Because of this dependence of the adrenaline effect on the external Ca^{++} concentration, it is necessary first to discuss the effect of varying the external calcium on the action potential in the absence of adrenaline. Fig. 20 shows that as the external Ca^{++} concentration is increased so the overshoot magnitude falls and the duration of the action potential is reduced. Niedergerke & Orkand (1966) found similar results using similar stimulation rates (circa 15/min). However, when the ventricle was stimulated at very low rates (less than 1/min) they found that increasing the external calcium gave an increase in the overshoot amplitude and a complicated pattern of alteration of the action potential duration. They examined this in more detail and found that this effect of calcium was basically due to an increase in the

ratio $[Ca^{++}]/[Na^{+}]^2$ of the external fluid, in turn controlling the ratio at an 'active' superficially located site in the cell. They postulate that the ratio $[Ca^{++}]/[Na^{+}]^2$ at the membrane controls the permeability ratio P_{Na}/P_K so that an increase in the first ratio brings about an increase in the second. This increase in the permeability ratio would increase the sodium current during the action potential and bring about an increase in the overshoot magnitude and the action potential duration as was found. Niedergerke & Orkand further postulate that the depressant effect of increased external calcium at higher rates of stimulation (as was found in the work of this thesis) is due to an accumulation of calcium inside the cell rather than at the superficial site and this accumulated calcium then produced a reduction in the permeability ratio P_{Na}/P_K.

With these statements in mind it is possible to make two points about the action of adrenaline on the action potential, (a) as the effect of adrenaline is greater at low external Ca^{++}

concentrations and also at low rates of stimulation, it appears likely that adrenaline is increasing the inward movement of calcium into the cell to such an extent that the depressant action of calcium on the action potential can be demonstrated and (b) that as adrenaline does produce an increase in the overshoot magnitude and the duration of the action potential even in high calcium Ringer and at the faster rates of stimulation, this effect of adrenaline cannot be mediated purely by increasing the ratio $\left[Ca^{++}\right] / \left[Na^{+}\right]^2$ at the 'active' site of the cell, as under these conditions Niedergerke & Orkand have shown that this would lead to a rapid accumulation of calcium inside the cell with consequent depression of the action potential. There is some depression of the action potential due to this phenomenon but there must be another change occurring which can over-ride this and give the effects on the overshoot and duration of the action potential quoted above.

Therefore it appears necessary to postulate that adrenaline is increasing the inward sodium

current during the action potential by an action not purely dependent on a change in the $[Ca^{++}]/[Na^{+}]^2$ ratio at the membrane. As mentioned previously there is some evidence that adrenaline increases sodium influx from the experiments done to test the action of adrenaline on the cell in the presence of ouabain. It was shown that under these conditions adrenaline gave a depolarisation of the membrane instead of the usual hyperpolarisation. This is most probably due to an increase in the membrane permeability to sodium, as there is good evidence that the movements of potassium and chloride are not appreciably altered. The interaction between adrenaline and ouabain on the action potential has not yet been tested.

The present evidence therefore suggests that adrenaline is increasing the membrane permeability to both sodium and calcium ions. This could be a simpler process than it appears at first sight, in view of the work of Lüttgau & Niedergerke (1958). They showed that the force of contraction of cardiac muscle was dependent on the ratio $[Ca^{++}]/[Na^{+}]^2$

of the external fluid. They explained this by postulating that both calcium and sodium combine with the same receptor site at the membrane and that they compete with each other at these sites. When the membrane is depolarised, as by an action potential, the calcium-receptor complex passes into the cell and activates the contractile process. Niedergerke (1963) has shown that increasing the ratio by increasing the external Ca^{++} or by reducing the external Na^{+} concentrations had the effect of increasing the calcium influx into the cell.

As sodium and calcium enter the cell at these same sites, adrenaline would only have to increase the efficiency or availability of these carrier sites in some way to produce an increase in both the sodium and the calcium influx. This would give an increased sodium current which would explain the action potential changes and an increase in the amount of calcium available for activation of the contractile elements which would explain the increase in twitch tension. There would also be a very quick accumulation of calcium inside the cell

giving some depression of the action potential, as shown by Niedergerke & Orkand (1966a) and as found in this thesis.

The effect of adrenaline on the time to peak twitch tension There is considerable evidence accumulating in the literature that the action potential controls the development of tension in the contractile element of the cell, acting as a switch to turn on and off the contractile element tension. Therefore it was thought that there should be a relation between the time to peak tension of the twitch, taken to represent the time of activity of the contractile element - the active state, and the duration of the action potential. In fact there has been found to be a relationship between the two in many cases (see Results section for references), correlation during an inotropic intervention being striking in some examples.

However, the results of experiments carried out for this thesis on the effect of adrenaline on the time to peak twitch tension cast some doubt on this relationship. It has been shown that adrenaline

consistently prolongs the action potential under all the experimental conditions tried. On the other hand it gives a prolongation of the peak tension time only under certain conditions, while in others the time to peak is reduced. This variation in the effect of adrenaline has been shown to be a function of the ratio $[Ca^{++}] / [Na^{+}]^2$ in the extracellular fluid, increasing the ratio causes adrenaline to consistently reduce the time to peak twitch tension and reducing the ratio has the opposite effect.

Assuming that the duration of the action potential does control the duration of the active state in the contractile element, the active state after exposure of the tissue to adrenaline must be prolonged. Therefore there must be other factors affecting the tissue as a whole which tend to reduce the time to peak tension after exposure to adrenaline and which are most marked at high $[Ca^{++}] / [Na^{+}]^2$ ratios. The following factors are likely to be important:-

(a) it has been shown that the prolongation of the

action potential duration by adrenaline is least marked in high Ca^{++} Ringer. This means that the duration of the active state is prolonged less and therefore that any factor reducing the time to peak tension after adrenaline will have a relatively greater effect.

(b) the velocity of onset of tension development is increased by adrenaline. This is also seen when the external Ca^{++} concentration is increased, getting faster as the concentration rises. There has been some evidence (given above) that the action of adrenaline is produced by increasing the effective amount of calcium within the cell. As would be expected, the velocity of shortening after adrenaline is faster at high Ca^{++} concentrations than at low. It is impossible to tell from these experiments whether the increase in shortening velocity is due to an intrinsic increase in the speed of shortening of the contractile element itself, or whether it is due to a change in the compliance of the series elastic component of the cell. Sonnenblick (1962) who

examined isotonic twitches in cat papillary muscle preparations under various afterloads, found no change in the series elastic compliance during exposure of the tissue to noradrenaline and therefore subscribes to the former view. This increase in shortening velocity will tend to reduce the time to peak twitch tension even though the duration of the active state is prolonged. This effect will be most marked at high external Ca^{++} concentrations, where also the increase in the duration of the action potential - and hence the active state - is least, and is therefore another possible explanation for the reduction in time to peak tension after adrenaline in high calcium Ringer despite the prolongation of the action potential.

(c) finally, the work of this thesis confirms for frog ventricle the results given in the literature (see Results section for references) that conduction in excitable tissue is slowed by increasing the external calcium and also that adrenaline increases the conduction velocity. This change in the

conduction velocity with increasing Ca^{++} concentrations is a possible explanation for the observation that there is little change in the time to peak twitch tension over the small range of calcium concentrations tested in this thesis (Fig. 26, B), whereas there is a marked change in the duration of the action potential in the same solutions, and by inference, in the duration of the active state. Thus in high calcium solutions the action potential duration is reduced, but the time to peak twitch tension of the whole tissue is little altered because the spread of excitation throughout the tissue is prolonged. It was also found that the percentage reduction in conduction velocity by adrenaline is greater at high Ca^{++} concentrations and will therefore play a more important part in altering the time to peak twitch tension (see Table 9). The marked increase in conduction velocity after adrenaline will speed up the excitatory process throughout the ventricle and with better synchronisation of the contraction of individual cells, the time to peak twitch tension

of the whole tissue will be reduced.

It is important to note here that the reduction in time to peak tension after adrenaline in high calcium solutions could be converted into a prolongation by stimulating the heart at a very low rate (2/min). Niedergerke (1963) has shown that at low rates of stimulation the entry of calcium into the cell is reduced. This has a twofold effect in this case (a) the action potential after adrenaline is increased by a greater amount as the depressant action of calcium on the action potential is reduced, and (b) the velocity of onset of tension is slowed (Fig. 30). Both these factors could cause this reversal of the change in time to peak tension.

The results of these experiments show that there are definite limitations to the view that the time to peak twitch tension can be taken as a measure of the action potential duration and hence of the active state duration. Although in many cases a correlation between the two has been found, this may be partly fortuitous. In the first place

the time to peak twitch tension is dependent on the velocity of shortening of the tissue, which although it may be controlled by alterations in the speed of onset of contraction in the contractile element, also depends on the series elastic element of the cell. In an isometric twitch it is impossible to separate these and a change in the time to peak tension could be due to an alteration of one or other or both of these factors. Also it is possible for the time to peak twitch tension to be complicated by other factors, for instance the change in conduction velocity quoted above. Due to this therefore it can be seen that the time to peak twitch tension under certain conditions can be altered by an inotropic intervention in the opposite direction to the change produced in the duration of the action potential.

Conclusions

Finally, therefore, it is postulated that the mechanism of action of adrenaline in increasing the twitch tension of cardiac muscle may be

something like the scheme shown in Fig. 32. Here, probably as the very first step, adrenaline through the medium of the adenyl cyclase system increases the amount of 3'5' AMP inside the cell. The permeability of the membrane to sodium and calcium is increased, perhaps due to this augmentation of 3'5' AMP activity - it may be that 3'5' AMP in some way increases the efficiency of the postulated sodium-calcium carrier at the membrane. At the same time the activity of the sodium pump mechanism is increased, again probably due to the increase in 3'5' AMP activity, either as a direct effect on the mechanism or by its action on glycolysis to make available more high energy phosphate substances for metabolic processes within the cell. The effect of the increased sodium pump is to bring about an increase in the resting membrane potential of the cell by an 'electrogenic' movement of sodium out of the cell. The increased calcium at this site leads to increased speed of activation of the myofibrils, which increases the velocity of shortening of the

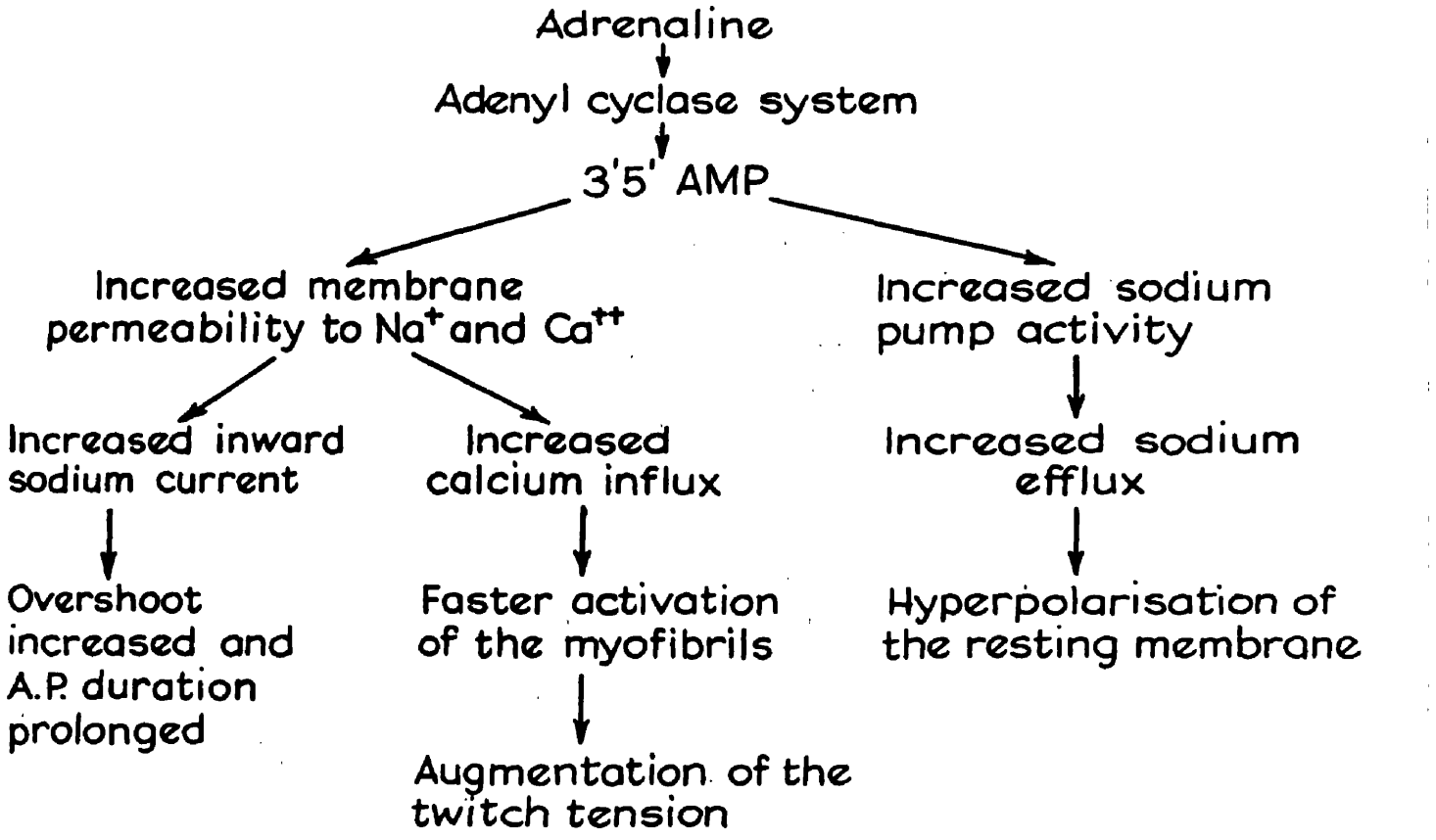

Fig. 32

Proposed hypothesis of the mode of action of adrenaline on a cardiac muscle cell.

fibre (and perhaps also the rate of shortening of the series elastic element) and in this way increases the twitch tension. At normal Ca^{++} concentrations it is thought that adrenaline does not increase the peak tension developed in the contractile process, as it has been shown that adrenaline does not alter the contracture tension/membrane potential relationship under these conditions. Unfortunately experiments designed to examine the tension/potential relationship under conditions of high and low Ca^{++} concentrations were inconclusive. However, as the percentage increase in twitch tension after exposure to adrenaline is greater when the external calcium is reduced below 1.0 - 2.0 mM, there may be some increase in contractile element tension found in this case. In other words, there is not, before adrenaline, enough calcium present inside the cell to fully activate the myofibrils. At normal and supernormal calcium levels there is enough calcium present normally, and the only way adrenaline can produce its inotropic effect is to increase the speed of

activation of the myofibrils, or in some other way to increase the velocity of shortening of the mechanical part of the cell and make more of the active state tension available as external work.

SUMMARY

SUMMARY

1. Experiments were carried out to examine the effect of adrenaline on mechanical activity and electrical events in frog ventricular muscle.

2. Mechanical activity was observed in a superfused preparation, which has the advantage over other preparations that the ionic environment of all the cells can be quickly altered.

3. Resting and action potentials of individual cells under various conditions were measured using intracellular microelectrodes.

4. The effect of adrenaline on chloride flux across the cell membrane was examined using the radio-isotope of chloride, ^{36}Cl.

5. When the tissue is exposed to Ringer containing excess KCl, there is a rapid depolarisation of the cells leading to development of a contracture. There is a characteristic relationship between developed tension and the logarithm of the external potassium concentration or the membrane potential.

6. Adrenaline reduced the contracture tension developed for each concentration of potassium tested,

the average reduction being 37.5%. This reduction was not due to the spike of tension which preceded development of the contracture tension after adrenaline.

7. There was no change of the adrenaline effect on contracture tension when the chloride of the Ringer was replaced with sulphate.

8. Adrenaline also reduced the contracture tension developed when the tissue was exposed to sodium-free Ringer.

9. Adrenaline hyperpolarised the membrane of the ventricular cells; this was found in normal Ringer, Ringer containing excess KCl and sodium-free Ringer.

10. The effect of adrenaline is to change the relationship between contracture tension and membrane potential for each concentration of excess potassium along the slope of the curve joining the pre-adrenaline plots. This suggests that the effect of adrenaline on contracture tension may be mediated through the effect on the membrane potential, such that the new increased potential after adrenaline only allows development of the smaller amount of tension which would be expected

according to the tension/potential relationship.

11. Ouabain in toxic doses blocks the hyperpolarising action of adrenaline, in the presence of ouabain the membrane is depolarised by exposure to adrenaline.

12. The beta-blocking agent pronethalol could block the effect of adrenaline on twitch, contracture and membrane potential.

13. It is postulated that the hyperpolarisation of the cell membrane by adrenaline may be due to an increased activity of the 'electrogenic' sodium pump.

14. The effect of adrenaline on the action potential of frog ventricular cells was to give an increase in the magnitude of the overshoot and to increase the duration of the action potential. This effect was dose-dependent.

15. It was found that this effect on the action potential was dependent on the $[Ca^{++}]/[Na^{+}]^{2}$ ratio of the external fluid, being greater at low values of the ratio. The effect was also greater at each value of the ratio when the ventricle was stimulated

at a low rate (2/min) instead of the usual 15/min.

16. Adrenaline increased the conduction velocity of the tissue, the effect being more marked when the external Ca^{++} concentration was high.

17. It was noted that during the course of an experiment the twitch in normal Ringer decreased quickly as hypodynamia developed; however the twitch in the presence of adrenaline remained constant throughout.

18. This effect of adrenaline on the twitch tension was also found to be affected by the ratio $[Ca^{++}]/[Na^{+}]^2$ of the perfusing Ringer. The percentage increase in twitch tension after adrenaline was greater at low values of the ratio and the time taken to produce the maximum effect was prolonged under these conditions.

19. In normal Ringer adrenaline produced a variable change in the time to peak twitch tension. However, at low $[Ca^{++}]/[Na^{+}]^2$ ratios, adrenaline gave a constant prolongation of the time to peak tension, and <u>vice versa</u>. When the ventricle was stimulated at 2/min adrenaline gave a prolongation of the time to peak

tension even when the ratio $\left[Ca^{++}\right] / \left[Na^{+}\right]^2$ was twice normal.

20. The results presented above are thought to cast some doubt on the view commonly held that the time to peak twitch tension can be taken as a measure of the duration of the action potential.

21. Experiments with ^{36}Cl have failed to show any change in chloride flux across the membrane during exposure of the tissue to adrenaline.

22. In the light of the results given above an attempt is made to explain the action of adrenaline on twitch tension and action potential on an ionic basis, and a postulated scheme of the action is given in the Discussion.

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