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Feedback Interaction of Mechanical and Electrical Events in the Isolated Mammalian Ventricular Myocardium (Cat Papillary Muscle)* **

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Die Entlastungsexperimente ermöglichten die Abschätzung der „mechanoelektrischen Latenzzeit" des beschriebenen Rückkoppelungssystems. Diese betrug weniger als 10 msec.

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

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

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

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

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

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

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

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

Methods

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

Muscle Chamber

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

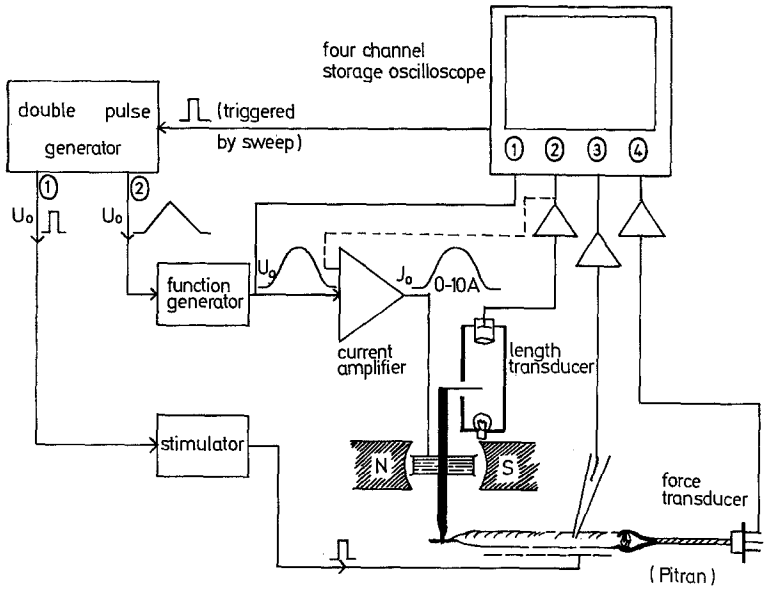

Fig. 1. See text for explanation

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

Force Transducer

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

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

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

Isotonic Lever Displacement Transducer and Load Generator

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

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

Load Control Unit

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

Controlled Stretch

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

Intracellular Potential Recordings

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

Documentation and Data Evaluation

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

Material and Preparation

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

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

Solutions

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

Results

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

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

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

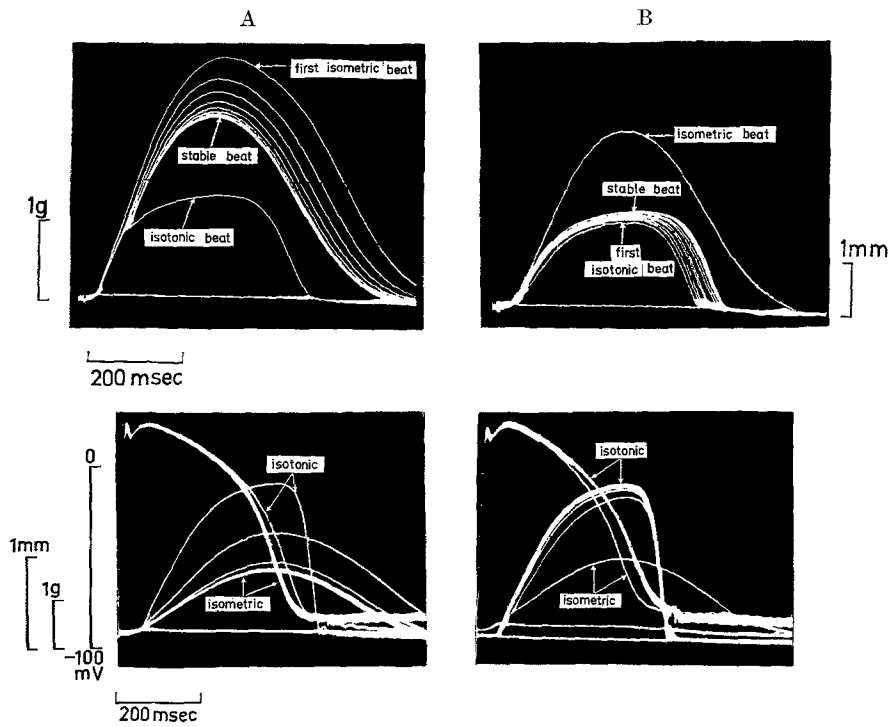

Fig. 2 A and B. Mechanical transients following a sudden change in the mode of contraction. In part I A the first beat is isotonic against a small load of 0.3 g/mm^2. The second and subsequent isometric beats show peaks of decreasing tension stabilizing at a new level after 6—8 beats. The procedure is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

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

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

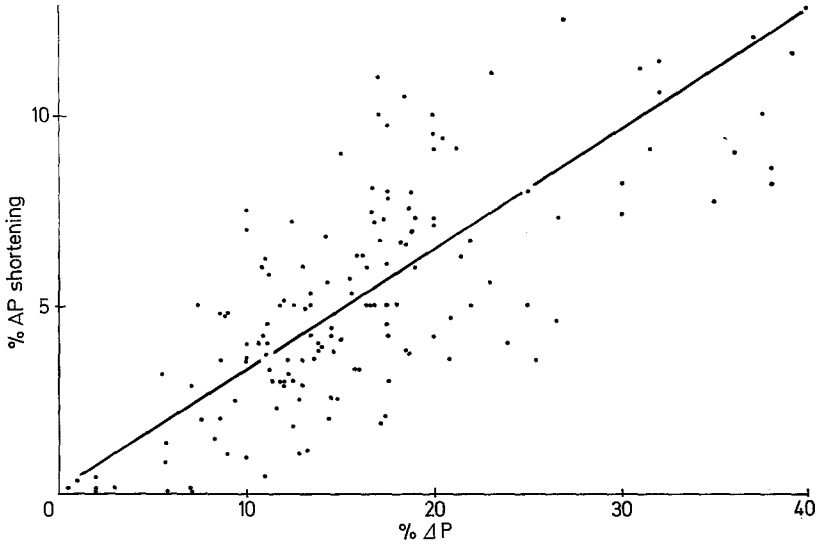

Fig. 3. The percentage change in isometric peak tension ($^0/_0$ ΔP) following a change from isotonic to isometric conditions is plotted against the $^0/_0$ abbreviation in the duration of the associated action potentials

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

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

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

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

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

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

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

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

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

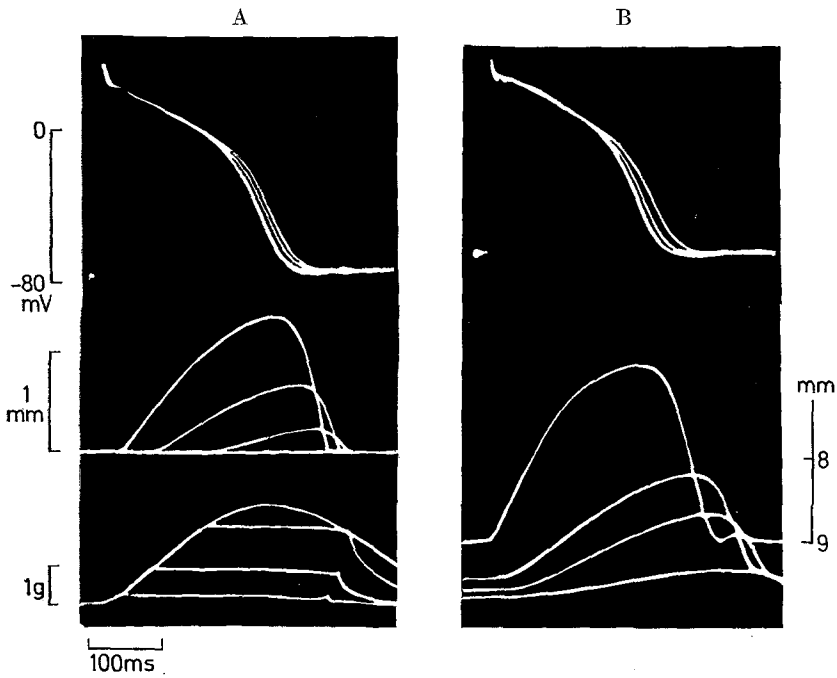

Fig. 4 A and B. Action potential duration as dependent on the shortening velocity of the muscle under different experimental conditions. In each part of the figure the upper recordings represent superimposed action potentials of 4 subsequent beats. During the experiment shown in part (A) initial muscle length was kept constant and the muscle shortened against increasing afterloads (0.3, 1.0, 2.0, 2.5 g/mm^2). The last contraction was purely isometric (isotonic displacements: middle traces, tension developed: lower traces). In part (B) increasing preloads were applied to the muscle resulting also in decreasing shortening velocity but at different initial muscle length. It can be seen that in both cases the duration of the action potential is abbreviated roughly in proportion to the decreasing shortening velocity

experiments were performed. In Fig. 6 such an experiment is shown. This figure contains the traces of four subsequent beats each starting its contractile cycle under isometric conditions (lower traces in Fig. 6). But the muscle was not allowed to complete its isometric contraction. About 150 msec after the onset of contraction the muscle was released to 0.3 g/mm^2. A particular feature of this experiment was that the speed of the release i.e. the decay of tension, was controlled and set to about -300, -150, -60, and -10 g/sec resp. in four subsequent beats. As a result the sortening velocities of the muscle during the releases show different slopes. This can be seen from the displacement recordings

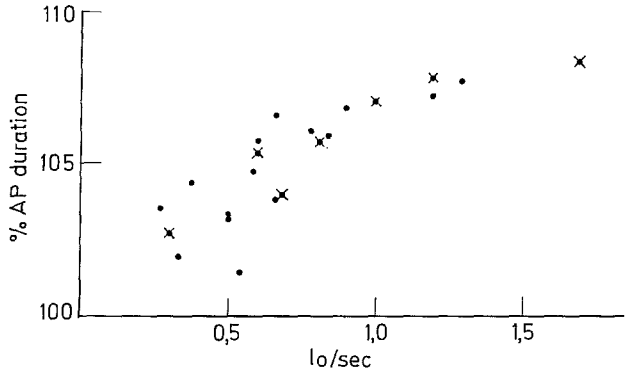

Fig. 5. Graphic evaluation of six experiments as shown in Fig. 4. The amount of changes in action potential duration is plotted against the shortening velocity of the muscle (in terms of l_o/sec). The filled circles represent experiments with increasing preloads (4 experiments performed on 4 preparations)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

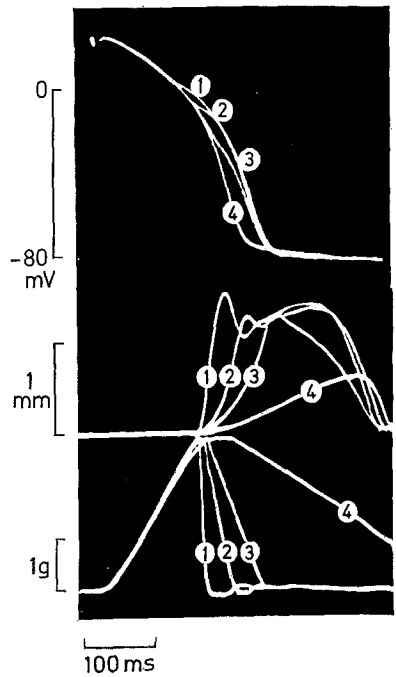

Fig. 6. Controlled release experiments. In four subsequent beats the time course of the isometric contraction (lower traces) was interrupted by a release starting about 150 msecs after the onset of the contractile cycle. The decay of tension during the release was -300 g/sec (trace *1*), -150 g/sec (trace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

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

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

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

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

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

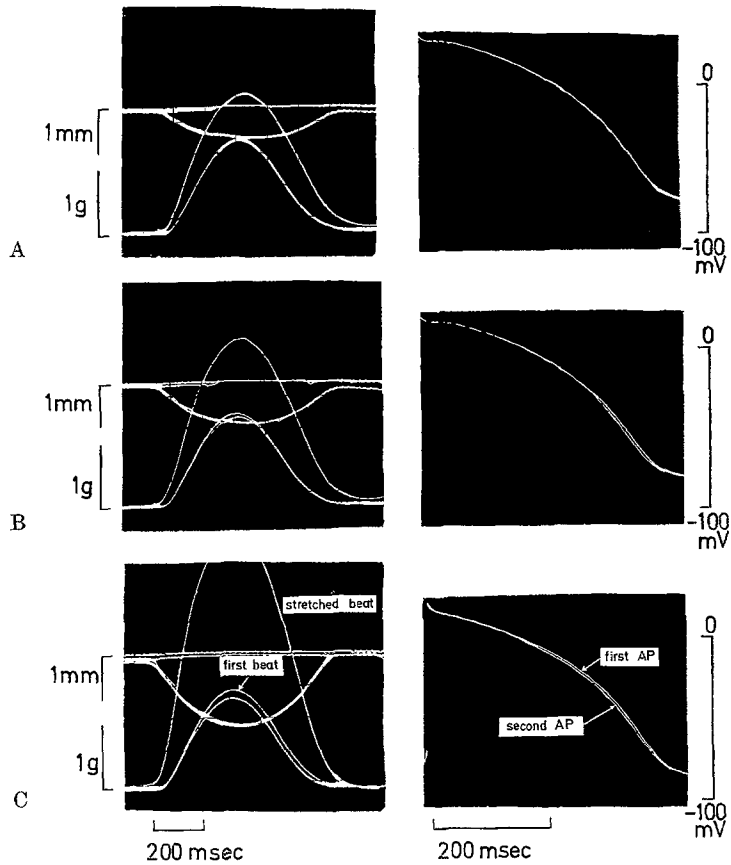

Fig. 7 A—C. Controlled stretch experiments performed on a papillary muscle of 7 mm in length and 0.9 mm in diameter. Each recording in the left column contains three superimposed traces of mechanical activity. The first beat is isometric, the second is performed under a controlled stretch (increasing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

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

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

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

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

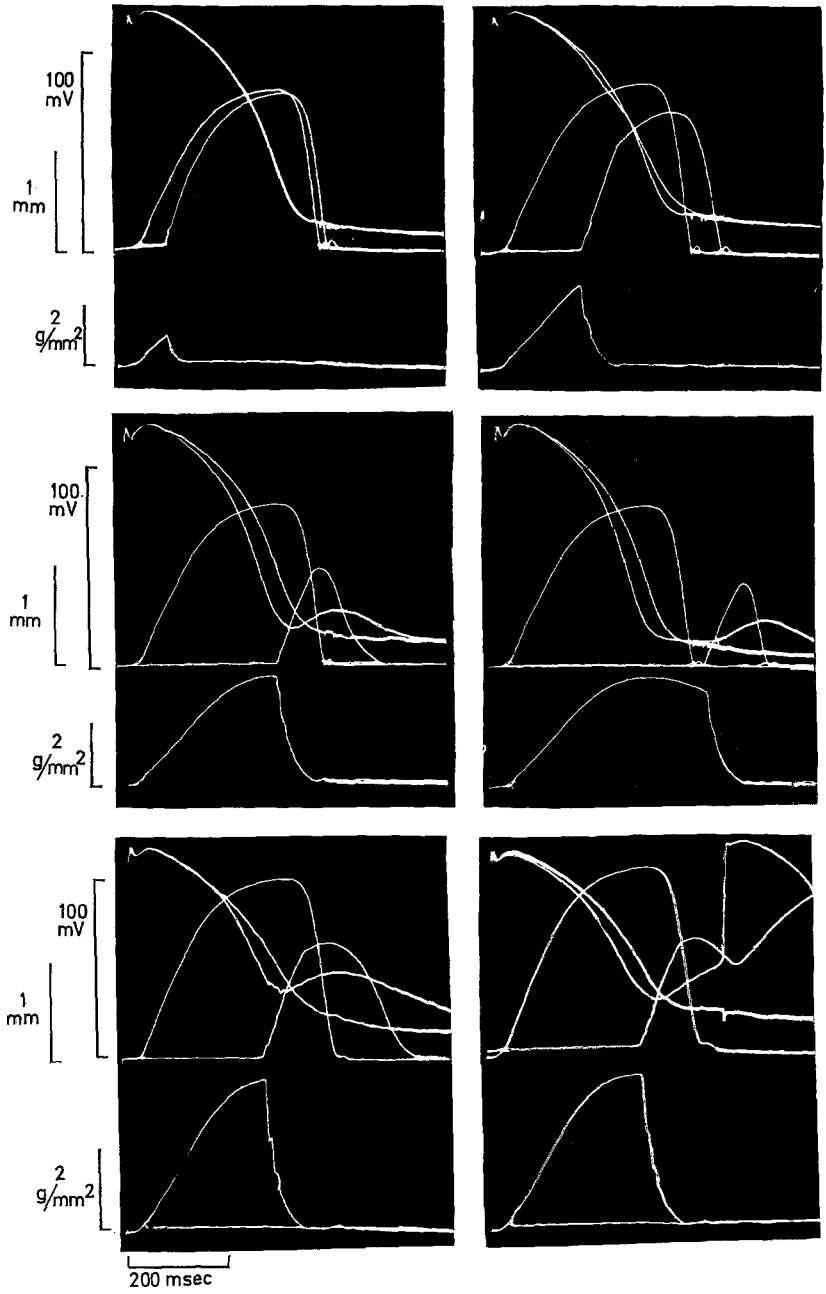

Fig. 8

the appropriate time constant of tension decay. In the experiments shown in Fig. 8 the time constant governing the exponential decay of tension was set to 20 msec resulting in a shortening velocity, which was much higher than that during the preceding isotonic beat at 0.3 g/mm^2. For instance during the experiment demonstrated in the upper right part of Fig. 8 the shortening velocity during the release was 1.9 l_i/sec as compared with a value of 1.1 l_i/sec developed during the preceding isotonic beat at 0.3 g/mm^2. Each part of Fig. 8 also contains two superimposed action potentials belonging to the isotonic and to the subsequent released isometric beat. Comparing the time course of each pair of action potentials one can differentiate two effects: the first is an abbreviation of the action potential associated with switching from isotonic to isometric conditions [already described in chapter (A)]. However in these experiments the muscle is not allowed to complete its isometric contraction but is released in such a way that a fast "isovelocic" displacement takes place starting at different points during the isometric cycle. As a consequence of this release the action potential is prolonged as expected from previous results but is now longer than that of the normal isotonic beat. This is seen in the upper recording of Fig. 8 where the release was made 100 and 170 msec after the onset of the isometric contraction. The action potential associated with these releases crossed over the "isotonic" one about half way down the repolarization phase. This shows that by shifting the shortening velocity of the muscle towards V_{max} the duration of the action potential can in fact be further increased.

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

Fig. 8. Quick release experiments performed on two different preparations. In each recording the first beat is purely isotonic against a 0.3 g/mm^2 load. The second beat begins isometrically and is released at different times after its onset to 0.3 g/mm^2 load, so that the contraction is completed isotonically. The decay of tension in these release experiments is exponential in order to obtain a nearly uniform shortening velocity during the release (for comparison see also Fig. 6). The corresponding action potential recordings exhibit two different effects. During the isometric period the action potential is again shortened (as previously described). But a few msecs after the start of the release a depolarizing current interrupts this trend producing a further prolongation (upper right part), or a new wave of depolarization (middle, left, and right part). In the lower parts an experiment is shown where this depolarization reached threshold and initiated a new propagated action potential

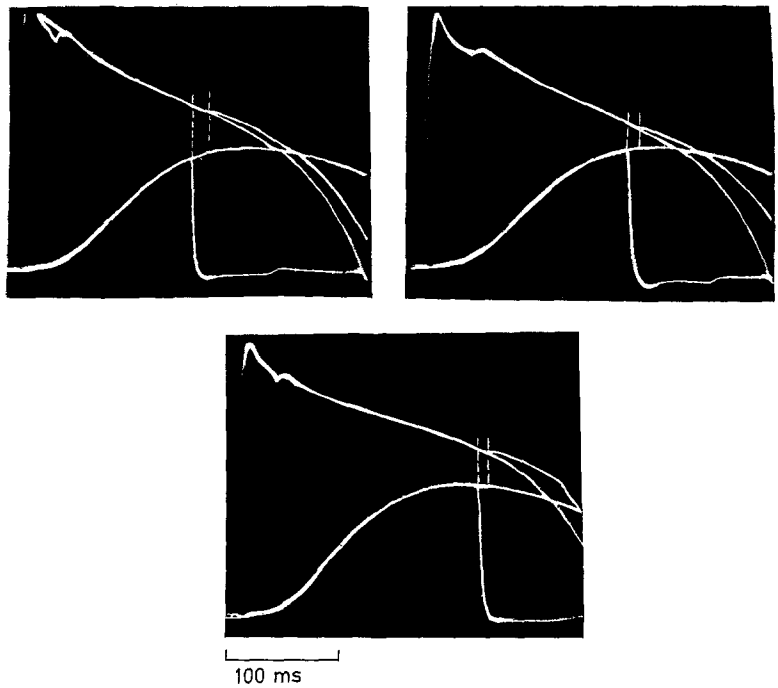

Fig. 9. Quick release experiments. The period of the "mechano-electrical delay" could be estimated by measuring the interval between the onset of the release and the first detectable deviation of the action potential at a high sweep speed. In 14 such experiments this interval averaged 8.9 $\pm$ 1.9 msec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

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

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

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

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

Discussion

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

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

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

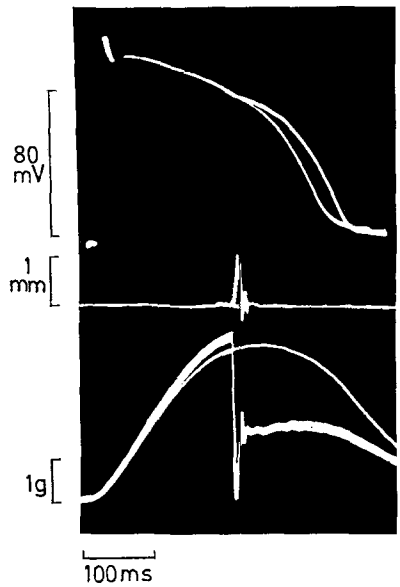

Fig. 10. Tension and action potential recordings of a papillary muscle. The first tension curve is a completed isometric contraction, whereas the second is interrupted (190 msec after its onset) by a 5 msec release period. Isometric contraction at the same muscle length is thus resumed. An "uncoupling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

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

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

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

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

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

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

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

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

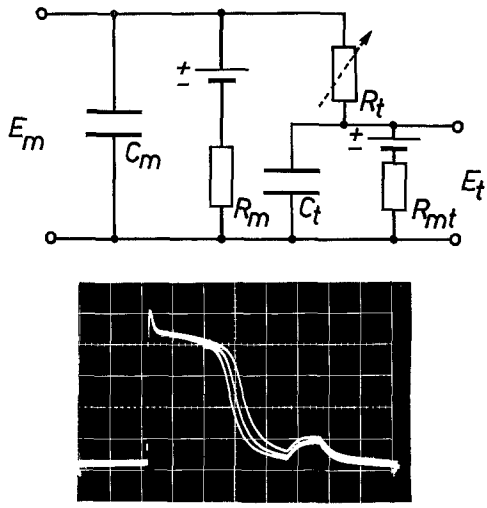

Fig. 11. The upper part shows a simplified equivalent circuit of the ventricular cardiac fibre. C_m and R_m represent the capacitance and the resistance of the plasma membrane. R_t and R_{mt} the capacitance and the resistance of the transverse tubular membranes. The series resistor R_t is thought to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

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

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

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

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

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