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INFLUENCE OF PREVIOUS MECHANICAL EVENTS ON THE CONTRACTILITY OF ISOLATED CAT PAPILLARY MUSCLE

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SUMMARY

1. The influence of previous mechanical events on myocardial contractility has been investigated in the cat papillary muscle preparation.

2. When a muscle that had been producing a stable response under isometric conditions was allowed to shorten isotonically, its ability to do so increased in successive beats until it reached a steady level, which represented a potentiated state compared with that seen in the first isotonic beat and in the preceding stable isometric contractions.

3. The increase in tension development in the first isometric beat after a period of isotonic beating was used as an index of the degree of potentiation. It was found to be well correlated with the changes in other parameters that could have been used for this purpose.

4. The main determinants of the degree of potentiation produced by a period of isotonic beating were:

(*a*) the amount by which the muscle shortened. This was inversely related to the force opposing shortening (i.e. the isotonic load);

(*b*) the number of isotonic beats. There was some potentiation (about 10 %) after a single isotonic beat, but the number of beats required for maximal potentiation (up to 25 %) depended on the frequency of stimulation; about 8 beats were required at 24 min^{-1}.

5. An isotonic release during the rise of tension in an isometric response was even more effective in potentiating the next isometric beat than an afterloaded contraction against the same load. Isotonic releases at later times had a diminishing influence on tension development in the next isometric beat.

6. In the absence of stimulation, the potentiated state produced by a period of isotonic beating decayed with a half-time of about 50 sec. When the muscle was stimulated it disappeared sooner, and its rate of decay

depended on the frequency of stimulation; at 24 min^{-1} about 8 beats were required to restore contractility to its previous steady level.

7. The characteristics of the decay of the potentiated state were closely similar to those of the potentiated states that can be produced by various electrical interventions, and the possibility that all of these might have the same underlying mechanism is discussed.

8. Attention is drawn to the practical implications of this phenomenon in the design of experiments in which the muscle contracts under changing mechanical conditions.

INTRODUCTION

One of the features of cardiac muscle that makes it difficult to study from the mechanical point of view is the fact that its properties are influenced by the mechanical conditions under which it contracts. Shortening of an isolated papillary muscle during a contraction leads to an abbreviation of the active state in *that* contraction (Brady, 1965; Kaufmann, Bayer & Harnasch, 1972), as it does in skeletal muscle (Jewell & Wilkie, 1960); it also produces a transient increase in the contractility of the muscle in *subsequent* beats (Parmley, Brutsaert & Sonnenblick, 1969; Kaufmann, Lab, Hennekes & Krause, 1971).

This paper is concerned with the latter phenomenon, and our aim has been to define more precisely the conditions under which this delayed effect of shortening on contractility occurs, (*a*) as a preliminary to further studies of the relation between this phenomenon and apparently similar transient alterations in contractility that can be produced in other ways, e.g. by the passage of a weak depolarizing current through an isolated preparation (Wood, Heppner & Weidmann, 1969), and (*b*) in order to assess its implications for studies of the mechanical properties of cardiac muscle in which shortening is involved. A preliminary report on this work has already been given to the Physiological Society (Jewell & Rovell, 1972).

METHODS

The preparation. Cats weighing not more than 2 kg were anaesthetized with chloroform or sodium pentobarbitone (35 mg/kg, by I.P. injection). Their hearts were quickly removed and washed by passing them through a series of beakers containing oxygenated bathing solution (m-equiv/l.: Na 135; K 5; Ca 4·5; Mg 2; and Cl 98·5; HCO_3 24; HPO_4 2; SO_4 2; acetate 20. Glucose 1·81 g/l.; insulin 5 u./l. Solution bubbled with 5 % CO_2 in O_2; pH 7·5). The right ventricle was opened and a papillary muscle chosen to meet the following criteria: (i) maximum diameter not exceeding 1 mm; (ii) minimum unstretched length 4 mm. The muscle was removed by cutting its chorda tendinea and by undercutting at its insertion into the ventricular wall. The tuft at the ventricular end was held in a Perspex clamp (Blinks, 1965), which crushed the papillary muscle at its insertion into the tuft. The tendinous free end

was tied on to a stainless-steel or glass connector by means of the shortest possible length of thin plaited silk. The bathing solution was contained in a muscle chamber that had an internal circulation of bathing solution (Blinks, 1965), and this was immersed in a constant temperature water bath at 30° C (± 0·1° C).

Stabilization procedure. Stimuli were applied via a punctate cathode that made contact with the muscle at its base (see Blinks, 1965), and electrodes on either side of the muscle provided the return path for the current. A stimulus strength just slightly in excess of the threshold strength was used throughout the experiment; typically this was provided by an applied voltage of about 0·4 V. The preparations were 'run in' under isometric conditions at a stimulus frequency of 24 min^{-1} for 3–4 hr before the experiment began. During this period the muscle was gradually stretched until it reached the length (L_{max}) at which maximum tension development occurred, and it was then maintained at that length thereafter. At the end of the

TABLE 1. Details of preparations

The vital statistics of the cat papillary muscles used in the present experiments are given in this Table. The mean values for all twenty-two preparations are given first, and then the details for the three preparations (A, B and C) that provided the experimental data shown in all the Figures in this paper except Fig. 12 (rabbit papillary muscle). The standard conditions referred to in the last column of the Table were as follows: muscle at 30° C, stabilized at L_{max} in a bathing solution containing 2·25 mM-Ca^{2+}; stimuli of just suprathreshold strength applied via a punctate electrode at a frequency of 24 min^{-1}

	Length L_{max} (mm)	Weight M (mg)	Equivalent diameter (mm)	Stable isometric tension under standard conditions (mN mm^{-2})
All 22 preparations (mean ± S.D.)	5·39 ± 1·35	2·46 ± 1·40	0·61 ± 0·23	57·0 ± 15·5
Preparation A (Figs. 6, 9, 10)	4·5	1·7	0·69	79
Preparation B (Figs. 3, 5, 8, 13)	6·1	3·0	0·79	59
Preparation C (Figs. 7, 11)	5·0	2·5	0·80	42

experiment the muscle was weighed and its equivalent diameter calculated on the assumption that the muscle was a uniform cylinder of length L_{max} (mm) and mass M (mg) with a relative density of 1·0 (equivalent diameter = $2\sqrt{(M/\pi L_{max})}$). The mean dimensions of the twenty-two muscles used in this series of experiments are given in Table 1, together with the mean tension developed under standard conditions at the end of the 'run in' period. The vital statistics are also given for three particular muscles (Preparations A, B and C) that provided the experimental data shown in all the Figures except Fig. 12 (rabbit papillary muscle). Muscles stimulated at 24 min^{-1} could be depended upon to give tensions with ± 5 % of the value reached at the end of the 'run in' period for at least 6 hr afterwards, and all the results that will be described in this paper were obtained from muscles in this steady-state condition (see caption to Fig. 8).

The apparatus. A stainless-steel or glass connector joined the muscle to the isotonic lever (Fig. 1), which was fitted with both force and length transducers. The lever

was made of magnesium and its equivalent mass at the point of attachment of the muscle (with the connexion included) was about 80 mg. Loads were applied to the lever through a compliant support, and the lever ratio for applied load: muscle load was 9·35:1. The lever was fitted with a pneumatically operated support based on the principle used by Sonnenblick (1965). Isometric conditions of contraction were obtained by applying sufficient air pressure to the pneumatic support to hold the lever against the afterload stop. The conditions could be changed from isometric to isotonic between contractions or during a contraction (i.e. isotonic release) by cutting off the air supply to the support. The force transducer consisted of a pair of semiconductor strain gauges, which were glued to the upper and lower surfaces of the lever between the afterload stop and the muscle attachment. The length transducer

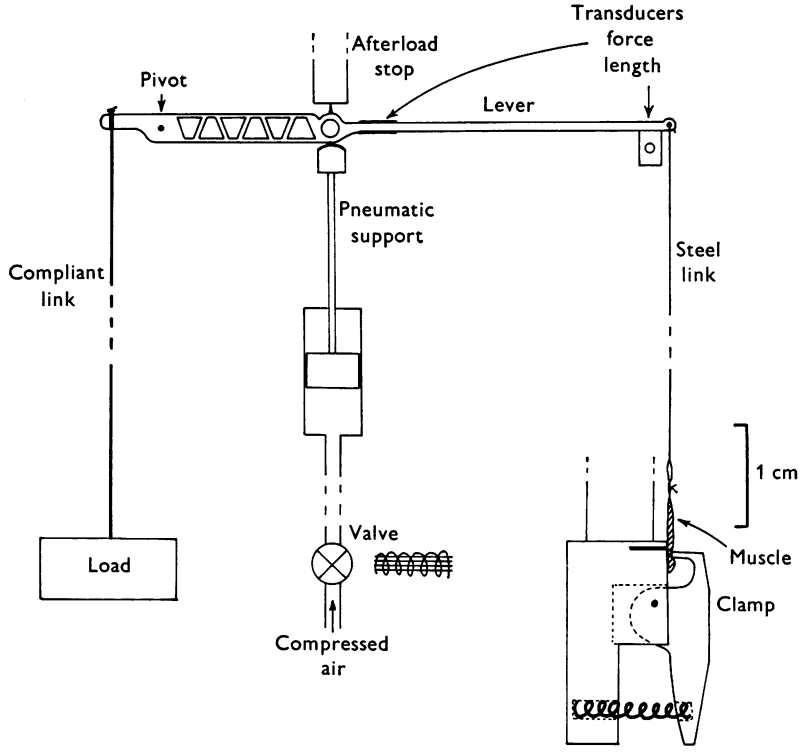

Fig. 1. Lever system for isometric and isotonic contractions. The muscle is held by a spring-loaded clamp against a Perspex block that houses a punctate electrode. The muscle is connected to the lever by means of a stainless steel or glass link, and a load is applied to the lever on the other side of the pivot through a compliant support. A pneumatic support determines whether the lever is free to move or not; the support is operated by compressed air, which is supplied via a solenoid valve. When compressed air is supplied to the support, the lever is held against the afterload stop, and the muscle will contract under isometric conditions. The support may be removed between or during contractions to allow the muscle to shorten under isotonic conditions. The lever is fitted with transducers for measuring the shortening and the tension produced by the muscle.

was a photoelectric device in which a piece of graded density film determined the amount of light falling on a photodiode. Both types of transducer have been described by Jewell, Kretschmar & Woledge (1967).

System for data acquisition and analysis. The system used (Fig. 2) was demonstrated to the Physiological Society by Jewell & Toll (1972) and has been described in detail by Toll (1973). The length and tension transducers were both operated as half-bridges in conjunction with Devices DC-6 Preamplifiers, which fed two channels of a Devices M4 recorder. The outputs of these two preamplifiers were also fed to differentiators to obtain the rates of change of tension and length, and these signals

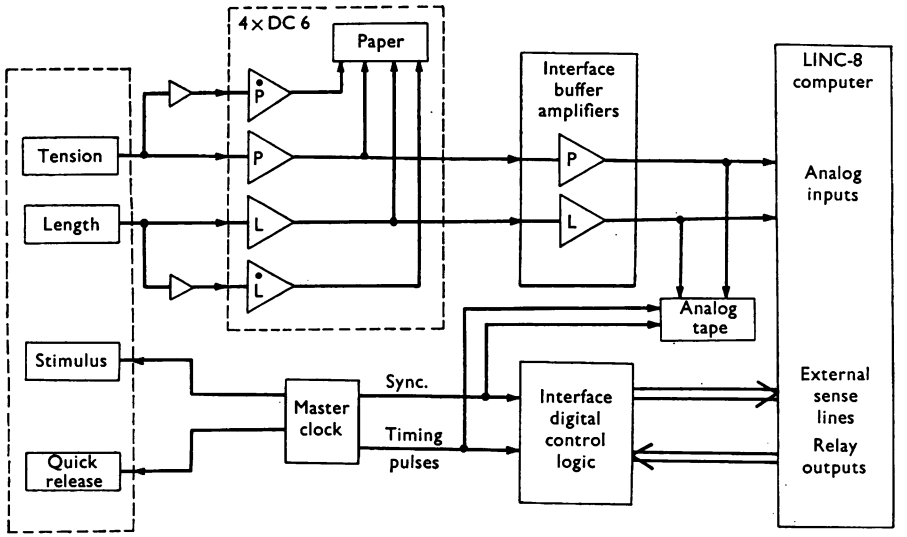

Fig. 2. Flow diagram of the recording apparatus and the interfacing with the computer (from Jewell & Toll, 1972).

were connected to the other two DC-6 preamplifiers of the M4 recorder. A display was therefore obtained on 4 channel paper of the sort shown in Fig. 3. The outputs of the four DC-6 preamplifiers were fed to a Philips ANALOG-7 tape recorder so that a complete record could be obtained for later analysis if required. However, the bulk of the signal analysis was carried out on-line by a LINC 8 computer, which was programmed as described by Toll (1973). The great advantage of the computer processing in this series of experiments was that we were often measuring small differences, which the computer was able to do with much greater objectivity than we could probably have mustered.

Analysis of results

Choice of experimental procedure. Two related approaches were possible to the phenomenon under examination: one was to consider the effect of a period of isotonic beats in an otherwise isometric sequence (Fig. 4*a*); the other was to consider the effect of a period of isometric beating in an otherwise isotonic sequence (Fig. 4*b*). The effect of the former is to increase the contractility of the preparation, and the degree of potentiation can be expressed quantitatively as the increase in the amount of shortening during the period of isotonic beating ($\Delta S/S_i$), or as the transient increase

in tension development when the conditions are restored to isometric ($\Delta P/P_s$). On the other hand if the phenomenon is examined in the way shown by Fig. 4*b*, then it can be seen that the effect of a period of isometric beating in an otherwise isotonic sequence is to reduce the contractility of the preparation, and the depression can be expressed quantitatively as the decrease in tension development during the isometric series ($-\Delta P/P_i$) or as the transient reduction in the amount of shortening when the conditions are restored to isotonic ($-\Delta S/S_s$). These two approaches are complementary, and we used the first one (Fig. 4*a*) throughout this study.

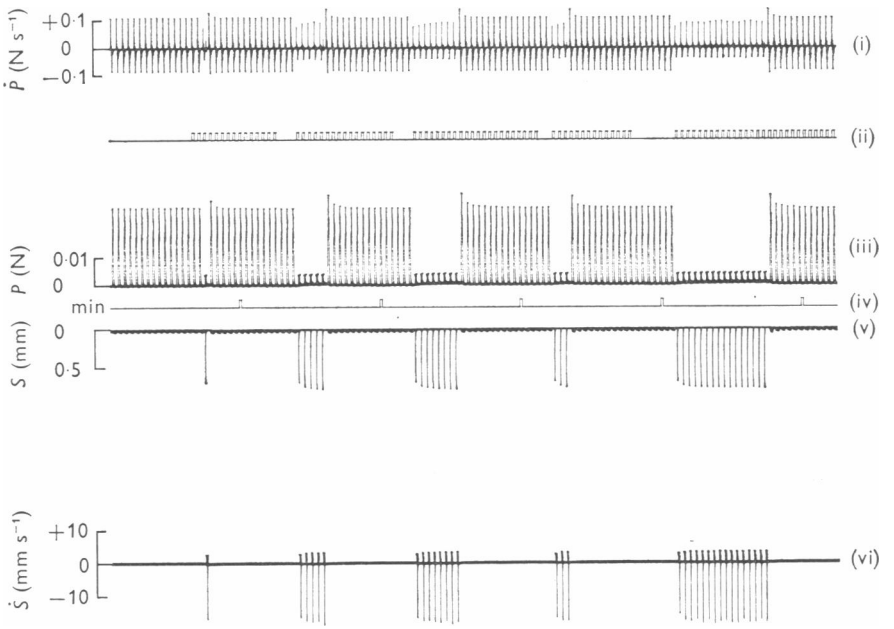

Fig. 3. Length of recording paper to show the quantities continuously monitored throughout the experiment. The six traces (from above downwards) show the following.

(i) rate of change of tension (upward deflexion = rise of tension),

(ii) marker (computer activated) to show which contractions had their parameters stored on digital magnetic tape by the computer,

(iii) tension (upward deflexion = rise of tension),

(iv) time marker (1 min intervals),

(v) muscle length (downward deflexion = shortening),

(vi) rate of change of length (upward deflexion = shortening).

Preparation B: frequency of stimulation, 24 min^{-1}.

Definition of 'stable' beat. Pilot studies of the type illustrated by Figs. 3 and 5 showed that at a stimulus frequency of 24 min^{-1} (which we always used for routine purposes) the development of the potentiated state produced by a sudden change from isometric to isotonic conditions was essentially complete within 8 beats: similarly, after a sudden change from isotonic back to isometric conditions, the potentiated state disappeared within about 8 beats. For the purposes of computer analysis of data, the parameters measured in the eighth beat after a change from

isotonic to isometric conditions, or vice versa, were taken as representative of the 'stable' state of contractility seen in subsequent beats under either condition. This may seem a rather arbitrary choice, but it was a much simpler procedure than averaging the parameters over many beats, and we were satisfied that it introduced no greater errors than those inherent in the method of computer analysis (see Table 2).

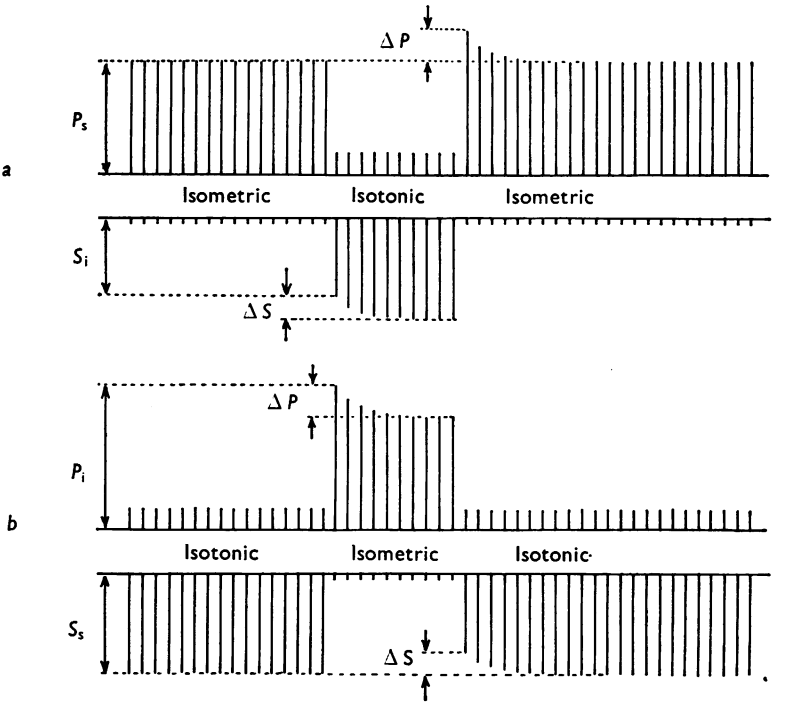

Fig. 4. Schematic diagrams to show parameters measured to characterize the change in contractility of the muscle. *a*, effect of a period of isotonic beating during an isometric series of contractions in which the stable tension developed was P_s. The shortening in the first isotonic beat is denoted by S_i and ΔS is the change in the amount of shortening during the period of isotonic beating. ΔP is the change in tension development in the first isometric beat after the isotonic series. *b*, effect of a period of isometric beating during a series of isotonic contractions in which the stable shortening was S_s. The tension developed in the first isometric beat is denoted by P_i and ΔP is the change in tension developed during the period of isometric beating. ΔS is the change in the amount of shortening in the first isotonic beat after the isometric series.

Choice of an index of contractility. In recent reviews of the meaning and measurement of myocardial contractility, Jewell & Blinks (1968) and Blinks & Jewell (1972) have argued that changes in tension development under isometric conditions provide a simple and reliable means of assessing changes in contractility produced by inotropic interventions *provided that these do not have much effect on the time to peak tension.* As we shall show later (Figs. 5*a*, 10*a*), the latter criterion was satisfied in the present study in that no consistent change was observed in the time to peak of potentiated

beats. We therefore chose the parameter $\Delta P/P_s$ (which we shall refer to as the 'tension transient') as our index of the degree of potentiation produced by a period of isotonic beating. It had the merit of being the parameter that was least subject to variation in repeat determinations made under identical conditions, and as we shall see later (Fig. 9) the other parameters that might have been used ($\Delta\dot{P}/\dot{P}_s$, $\Delta S/S_i$, and $\Delta\dot{S}/\dot{S}_i$) were all well correlated with it.

TABLE 2. Summary of parameters investigated

Parameter	Designation	Minimum detectable change: Absolute units	Minimum detectable change: As typical % of value specified
Peak tension developed:			
(*a*) in 1st isometric beat	P_i	20 μN	~0·1 % P_s
(*b*) in 8th (stable) isometric beat	P_s		
Tension transient $(P_i-P_s)\div P_s$	$\Delta P/P_s$		
Tension during isotonic shortening	P_{isot}		
Peak rate of tension development:			
(*a*) in 1st isometric beat	$\dot{P}_i$	1 mN s^{-1}	~1 % $\dot{P}_s$
(*b*) in 8th (stable) isometric beat	$\dot{P}_s$		
Tension rate transient $(\dot{P}_i-\dot{P}_s)\div\dot{P}_s$	$\Delta\dot{P}/\dot{P}_s$		
Peak shortening:			
(*a*) in 1st isotonic beat	S_i	1 μm	~0·2 % S_s
(*b*) in 8th (stable) isotonic beat	S_s		
Shortening transient $(S_s-S_i)\div S_i$	$\Delta S/S_i$		
Peak rate of shortening:			
(*a*) in 1st isotonic beat	$\dot{S}_i$	0·05 mm s^{-1}	~1·5 % $\dot{S}_s$
(*b*) in 8th (stable) isotonic beat	$\dot{S}_s$		
Shortening rate transient $(\dot{S}_s-\dot{S}_i)\div\dot{S}_i$	$\Delta\dot{S}/\dot{S}_i$		
Time parameters (tension or shortening):			
(*a*) time to peak value	T_{pk}	1 ms	~0·3 % T_{pk}
(*b*) time to 75 % relaxation	T_r	1 ms	~0·1 % T_r

Note: the additional subscripts *i* or *s* may be added to T_{pk} and T_r to denote the values of these parameters in the first and stable beats, respectively.

Parameters measured by computer. The computer was programmed to estimate the parameters listed in Table 2. The minimum detectable changes in the parameters were governed by quantification errors in the analog-to-digital converters of the computer and in the programme itself, and this Table includes values for the minimum detectable change in each parameter as a typical percentage of the parameter specified. These figures may give a spurious impression of the precision with which some parameters could be estimated, particularly the time-to-peak tension or shortening. Beat-to-beat measurements under identical conditions showed a coefficient of variation of about 1 % in time-to-peak tension (see typical statistics quoted in the caption to Fig. 10), part of which may have been genuine, but part of which was certainly due to the problem of picking a true peak value if the signal is at all noisy. However, the computer was at least as good at this as a human observer armed with a ruler and a high speed record of the mechanical response.

RESULTS

General features of the change in contractility

The record shown in Fig. 3 illustrates many features of the phenomenon under examination, but changes in the time course of the contraction are not apparent on such a slow speed record. A more complete picture is presented in Fig. 5, which shows the results of computer analysis of data similar to the last isotonic series on Fig. 3. Fig. 5*a* shows how the various measured parameters changed during the period of isotonic beating, and

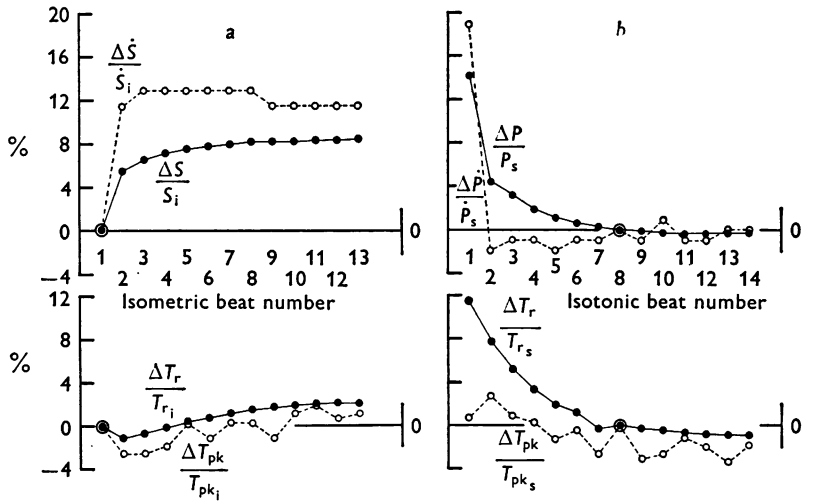

Fig. 5. Graphs plotted from the computer print-out to show how the various measured parameters defined in Table 2 changed on a beat-to-beat basis (*a*) when the conditions were changed from isometric to isotonic, and (*b*) when the conditions were changed back from isotonic to isometric. In each set of graphs the circled points indicate the 'reference' beat; all the differences shown by the other plotted points are the percentage deviations from the values of the various parameters in the reference beats. *Preparation B*: frequency of stimulation 24 min^{-1}.

Fig. 5*b* shows the corresponding changes that followed the restoration of isometric conditions. During the period of isotonic beating, the amount of shortening increased progressively to reach a steady level (8 % greater than in the first isotonic beat) after about 8 beats, whereas the increase in peak velocity of shortening (12 %) was complete after 2 or 3 beats. The time to 75 % relaxation increased slightly with the number of isotonic beats, but time-to-peak shortening showed no definite trend in view of the amount of beat-to-beat variation in the estimate of this parameter. When the conditions were restored to isometric (Fig. 5*b*), the tension developed

showed an initial 14 % increase over the steady level, but this disappeared over the following eight beats. The peak rate of rise of tension in the first isometric beat was also markedly (19 %) raised above the steady level, but the next and later isometric beats were not potentiated in that respect. The time to 75 % relaxation was initially prolonged, but it returned to its steady level over the next eight or so beats; the estimate of the time-to-peak tension was too noisy to reveal any significant trend.

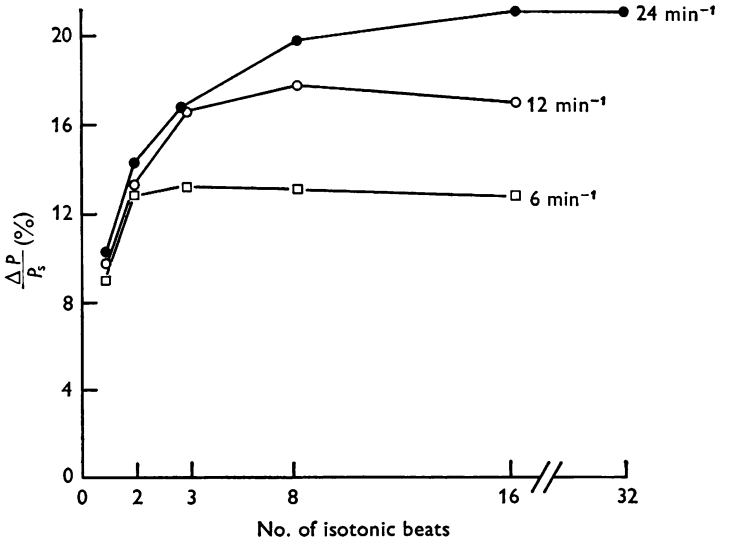

Fig. 6. Effect of number of isotonic beats on the tension transient ($\Delta P/P_s$ as %). The tension transient was measured after various numbers of isotonic beats against a fractional load, $P_{isot}/P_s \approx 0{\cdot}1$ (0·07 at 12 min^{-1} and 24 min^{-1}, and 0·06 at 6 min^{-1}). Each point is the mean of two observations made in a random sequence. *Preparation A.*

Number of isotonic beats

Fig. 5*a* shows that the increase in contractility develops over several isotonic beats, as judged by the ability of the muscle to shorten. When the tension transient was determined after different numbers of isotonic beats (Fig. 6), a substantial effect on contractility was always seen after a *single* isotonic beat, but several beats were required to obtain a maximal effect. From Fig. 6 it appears that the *time* required for the full development of the potentiated state was about 30 seconds, whatever the frequency of stimulation, but this was not so for all preparations. Many attempts were made to clarify the influence of frequency of stimulation on the development of the potentiated state, but no consistent picture emerged from these studies.

In the experiment illustrated by Fig. 6, the maximum degree of potentia-

tion increased with the frequency of stimulation, but this was not always the case over the range 12–24 min^{-1}. When the maximum degree of potentiation at different frequencies of stimulation was estimated from data of the type shown in Fig. 8 by extrapolating to zero fractional load, the values obtained were as follows (mean $\pm$ S.E. for n preparations): $16{\cdot}6\,\% \pm 0{\cdot}93\,\%$ ($n = 7$) at 24 min^{-1}; $15{\cdot}8\,\% \pm 1{\cdot}6\,\%$ ($n = 6$) at 12 min^{-1}; and $12{\cdot}8\,\% \pm 0{\cdot}92\,\%$ ($n = 3$) at 6 min^{-1}. There was no significant difference between the mean values for 24 and 12 min^{-1}, but the value at 6 min^{-1} was significantly different ($P < 0{\cdot}05$) from both.

Time course of decay of potentiated state

Fig. 5*b* shows the time course with which the potentiated state disappeared at a particular frequency of stimulation (24 min^{-1}). Two obvious questions that arise are (i) what happens to the potentiated state if the muscle is not stimulated, and (ii) how is the decay affected by altering the frequency of stimulation. These questions were answered by means of similar experiments which are illustrated by inset diagrams in Fig. 7. The problem to be overcome in both experiments is the fact that the contractility of the preparation is disturbed by *any* alteration in the pattern of stimulation. It is therefore necessary to set up suitable control sequences with which the test sequences can be compared. The procedure for studying the decay of the potentiated state in the resting muscle was as follows (see inset to Fig. 7*a*): a control sequence was obtained by interrupting an isometric series of beats for a given period of time and then recording the beats when stimulation was resumed (numbered 1, 2, 3, 4, etc.). A test sequence was then produced by interrupting the stimuli for the same period of time immediately after a conditioning series of isotonic beats. The test and control sequences were compared, and the differences between them ($\Delta P'$) calculated on a beat-by-beat basis. The difference between the *first* beat of the test and control sequences (expressed as $\Delta P'/P_s$) is plotted in Fig. 7*a* against the time for which stimulation was discontinued. The rather 'noisy' quality of the data was an inevitable consequence of taking small differences, but the general form of the decay of the potentiated state emerged quite clearly. The half-time of the decay was about 50 seconds.

The effect of changing the frequency of stimulation was investigated by means of similar experiment, which is illustrated in the inset to Fig. 7*b*. The frequency was changed from a standard value (24 min^{-1}) to a new value in the middle of a series of isometric beats (control sequence) or immediately after 12 isotonic contractions plus 1 isometric beat (test sequence). The two sets of data were compared on a beat-to-beat basis: beat 0 was the last one at 24 min^{-1} in each sequence (an isometric beat in both cases), and the difference between them showed the increase in con-

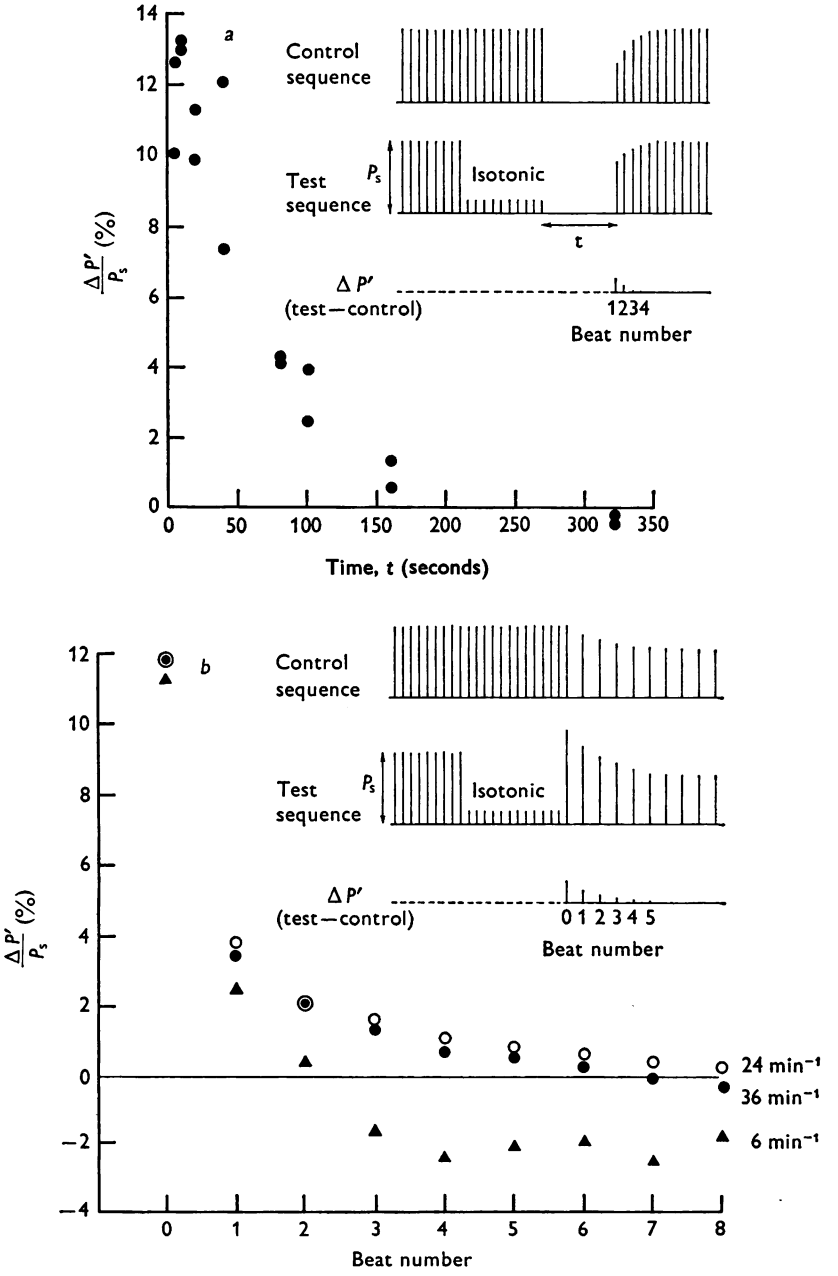

Fig. 7. For legend see opposite page.

tractility due to the period of isotonic beating; the differences were calculated for corresponding pairs of isometric beats (number 1 to 5) at the new frequency of stimulation; and these have been plotted in Fig. 7*b* for changes from 24 min^{-1} to 36 min^{-1} and 6 min^{-1} (12 min^{-1} and 3 min^{-1} were also studied, but these results have been omitted from Fig. 7*b* in the interests of clarity). The decay of the potentiated state required about the same number of beats at 36, 24 and 12 min^{-1}, but fewer beats were needed at 6 and 3 min^{-1}. Unfortunately, the interpretation of these results is complicated by the fact that some depression of contractility occurred when the frequency of stimulation was reduced to 6 or 3 min^{-1} after the period of isotonic beating. (This was also a common problem when studies of the sort illustrated in Fig. 4*a* were made at low frequencies of stimulation throughout the isometric-isotonic-isometric sequence of beats.) Nevertheless, it is quite clear from the results in Fig. 7 that the potentiated state disappeared much more rapidly when the muscle was stimulated than it did when the muscle was allowed to rest after a period of isotonic beating.

Effects of varying the isotonic load

In all the experiments described so far, the force opposing shortening during the period of isotonic beating was adjusted to give a near maximal improvement in contractility. Fig. 8 shows the results of an experiment in which the magnitude of the tension transient was determined after a fixed number of isotonic beats against a variety of loads taken in random order. The tension transient is plotted as a function of the fractional isotonic (lower abscissal scale) load, and as a function of the amount of shortening in the corresponding isotonic beats (upper abscissal scale). The two relations are very similar because there is an almost linear inverse

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Legend to Fig. 7

Fig. 7. Decay of the potentiated state (*a*) in the absence of stimulation, and (*b*) at different frequencies of stimulation. Inset diagrams illustrate the experimental procedure used to obtain the data shown in the graphs. In *a* the control sequence shows the effect of stopping stimulation for a period, *t*. The test sequence shows what happens when the cessation of stimulation is preceded by a period of isotonic beating. The differences between the tensions developed in test and control beats when stimulation is resumed is shown at the bottom of the inset diagram. The graph shows how $\Delta P'$ for the first beat (as a % of P_s) varies with the time, *t*, for which stimulation was stopped. *Preparation C*: frequency of stimulation 24 min^{-1}; P_{isot}/P_s = 0·19. In *b* similar control and test sequences are shown for a change in the frequency of stimulation. The graph shows how the difference between test and control beats ($\Delta P'$ as a % of P_s) changes on a beat-to-beat basis at different frequencies of stimulation. *Preparation C*: frequency of stimulation initially 24 min^{-1}; P_{isot}/P_s = 0·19.

relation between the amount of shortening in isotonic beats and the fractional load. As a reasonable approximation, it can be said that the tension transient is directly proportional to the shortening of the muscle and inversely proportional to the tension developed during the period of isotonic beating.

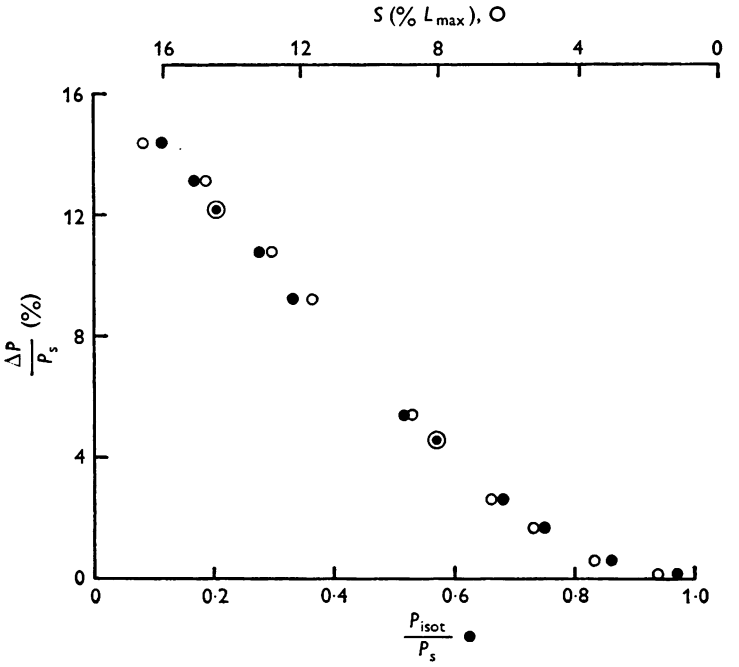

Fig. 8. Effect of isotonic conditions on the tension transient, which was measured after 12 isotonic beats against various loads taken in random order. The solid circles show how the tension transient ($\Delta P/P_s$ as a %) varied with the fractional load (P_{isot}/P_s, lower abscissal scale), and the open circles show how it varied with the amount of shortening of the muscle (S as a % L_{max}, upper abscissal scale). Each point is the mean of four observations, apart from two points which are the means of two. The decline in P_s during the 2080 responses required to complete this study was less than 1 %. *Preparation B*: frequency of stimulation 24 min^{-1}.

In experiments where the load opposing shortening was not varied (i.e in all the Figures except Figs. 8 and 9), it was adjusted as closely as possible to make $P_{isot}/P_s \approx 0{\cdot}1$ as a standard condition. This gave a near-maximal increase in contractility and it was a good deal easier to attain as an experimental condition than an isotonic load of zero.

Inter-relation of parameters

As altering the isotonic load provided the best way of regulating the increase in contractility during the period of isotonic beating, it was used as a basis for examining the inter-relations among the various measured parameters. Reasons have already been given for the choice of the tension transient, $\Delta P/P_s$, as the standard parameter for assessing the change in contractility. In Fig. 9*a*, *b*, *c*, graphs have been plotted to show how the other measured parameters were related to the tension transient when the

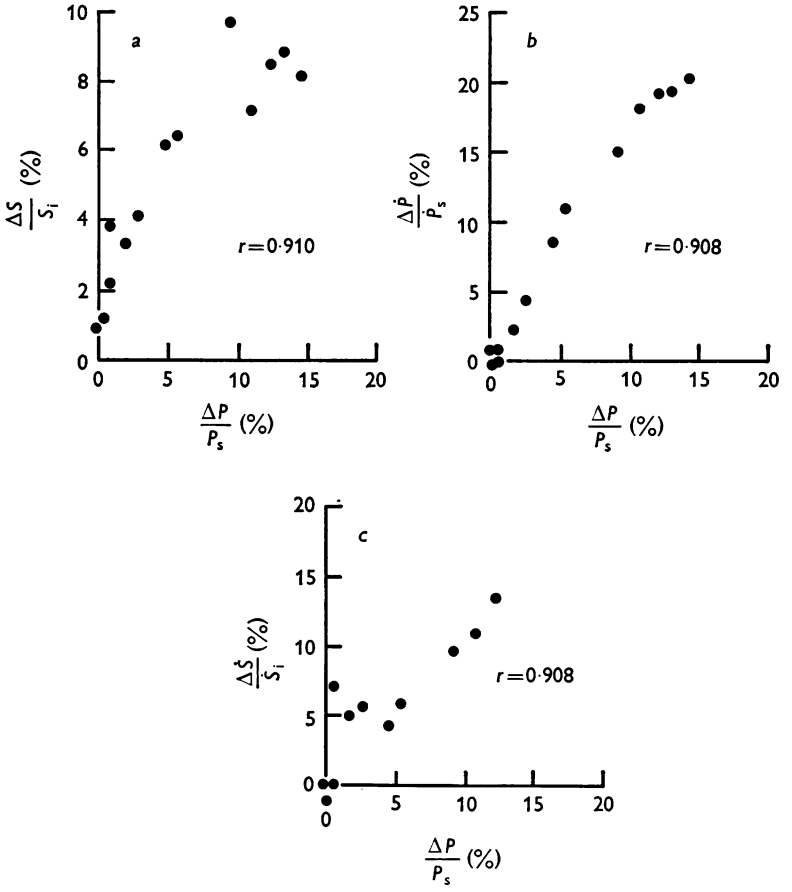

Fig. 9. Inter-relations among other parameters that were measured during the experiment illustrated by Fig. 8. The various parameters that could be used to characterize the change in contractility (see Table 2) are each plotted against the tension transient. (*a*) $\Delta S/S_i$ *vs.* $\Delta P/P_s$, (*b*) $\Delta \dot{P}/\dot{P}_s$ *vs.* $\Delta P/P_s$, (*c*) $\Delta \dot{S}/\dot{S}_i$ *vs.* P/P_s. Each point is the mean of two observations. *Preparation A*: frequency of stimulation 24 min^{-1}.

load opposing shortening of the muscle during the isotonic period was varied. It can be seen that they were all quite well correlated with the tension transient, and therefore with each other. Fig. 10 shows how the time parameters measured on the first isometric beat after a period of isotonic beating changed with the tension transient when the isotonic load was varied. There was a good correlation between the duration of the contraction and the tension transient (Fig. 10*b*), but no correlation between

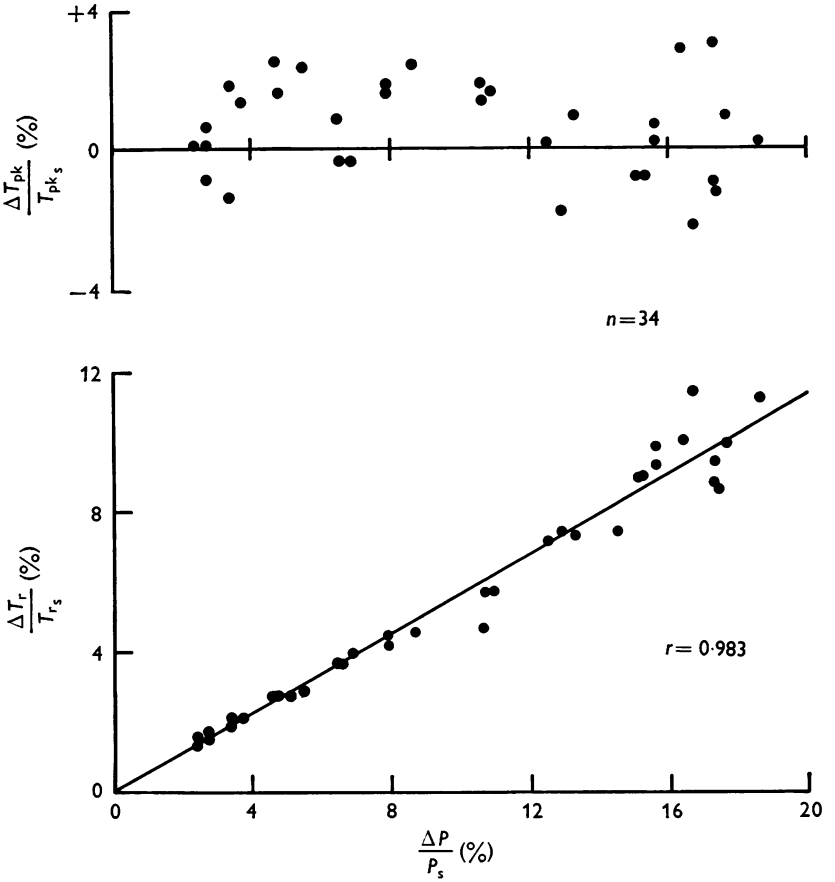

Fig. 10. Relations between time parameters and the tension transient. These data were obtained from an experiment like the one illustrated by Figs. 8 and 9. The change in time-to-peak tension, $\Delta T_{pk}/T_{pk_s}$, and in the time to 75 % relaxation, $\Delta T_r/T_{r_s}$, in the first isometric beat after an isotonic series against various fractional loads have been plotted against the corresponding tension transients ($\Delta P/P_s$ as a %). The values for T_{pk_s} and T_{r_s} in this experiment (mean ± S.D.) were 409·6 ± 3·8 ms and 779·1 ± 5·55 ms, respectively. *Preparation A*: frequency of stimulation 24 min^{-1}.

the time-to-peak tension and the tension transient under these conditions (Fig. 10*a*).

Isotonic release studies

One of the points of interest that emerged from Fig. 6 was that a single isotonic beat has an appreciable effect on the contractility of the muscle. This raises the question of at what time during the mechanical response shortening needs to occur in order to influence the contractility of the muscle in subsequent beats. We studied this by making isotonic releases against a fixed load at different times during isometric contractions. Fig. 11*a* shows how the potentiation of the next isometric beat varied with the time of release, and Fig. 11*b* shows how the shortening of the muscle in the releases varied with the time of the release. In Fig. 11*c* the tension transients from Fig. 11*a* have been plotted against the corresponding shortenings from Fig. 11*b*. The dashed lines show the tension transients that might have been expected from relation between tension transient and shortening shown in Fig. 8, in which the isotonic load was the factor that determined the amount of shortening. When releases were made more than 340 ms after stimulation (cf. isometric T_{pk} = 400 ms), the magnitude of the tension transient was about what would be predicted from Fig. 8. However, when releases were made earlier than this, the magnitude of the tension transient was greater than expected from Fig. 8, and in some cases greater than that produced by an afterloaded isotonic contraction against the same isotonic load.

DISCUSSION

The work reported in this paper has confirmed the finding of Parmley *et al.* (1969) that the contractility of an isolated papillary muscle from cat heart depends on the mechanical conditions under which it contracts. Thus, when a muscle that had been producing a stable response under isometric conditions was allowed to shorten against a small load (Figs. 3–5), its ability to do so increased in successive beats until it reached a steady level, which represented an enhanced state of contractility compared with that seen in the first isotonic beat and in the preceding stable isometric contractions.

Duration of potentiated beats

The lack of prolongation in the time-to-peak tension (Fig. 10*a*) is one of the few respects in which our results differ from those of Parmley *et al.* (1969) in the area of overlap between our experiments. They observed a mean prolongation of 6·5% in time-to-peak tension. Our computer programme would certainly have detected a change of this magnitude (see Table 2); but none was observed and we can find no obvious explanation

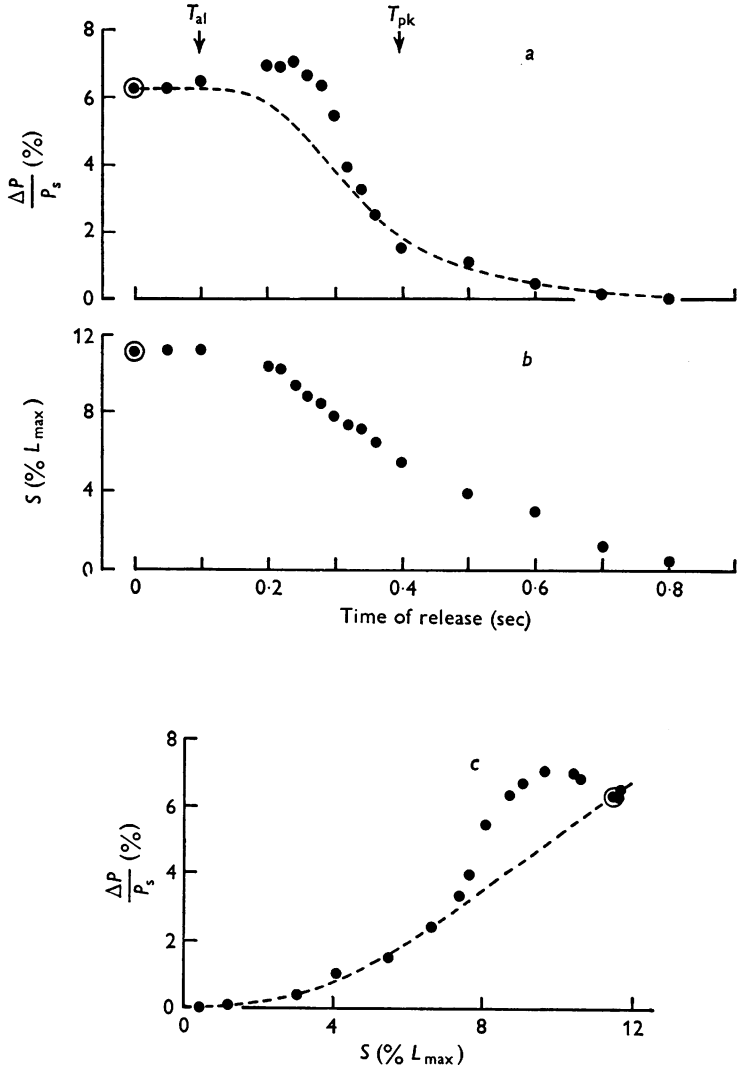

Fig. 11. Production of tension transients by single isotonic releases against a fractional isotonic load $P_{isot}/P_s = 0{\cdot}19$ at various times after stimulation. *a*, graph of the tension transient ($\Delta P/P_s$ as a %) against the time of release. The arrows, T_{al}, and T_{pk}, show the time at which the load was lifted in an afterloaded isotonic contraction and the time at which peak tension occurred in a stable isometric contraction, respectively. *b*, graph of muscle shortening (S as % L_{max}) against the time of release. *c*, graph of tension transient against muscle shortening compiled from *a* and *b*. Each point is the mean of two or three observations. In all the panels, the circled point shows the situation in an afterloaded isotonic contraction. The dashed lines in *a* and *c* show the tension transients that might have been expected on the basis of the amount of muscle shortening shown in *b* if the relation shown in Fig. 8 is obeyed under these conditions. *Preparation C*: frequency of stimulation 24 min^{-1}.

for this minor discrepancy between the two sets of experimental results. We found, as they did, that the duration of the potentiated beats was increased, and from Fig. 10*b* it can be seen that the prolongation was directly proportional to the degree of potentiation produced by the period of isotonic beating.

Inotropic interventions vary in their effect on time-to-peak tension (see Blinks, Olson, Jewell & Braveny, 1972). Most of them (e.g. sympathomimetic amines, increase in frequency of stimulation) decrease the time-to-peak tension, but an increase in the concentration of ionized calcium in the bathing solution leaves it unchanged, and high concentrations of methyl xanthines (e.g. caffeine) increase the time-to-peak tension of potentiated beats. In this respect, a period of isotonic shortening as an inotropic intervention has the same characteristics as increasing the extracellular calcium concentration.

Decay of potentiated state

The potentiated state produced by a period of isotonic beating disappeared with a half time of about 50 sec under resting conditions (Fig. 7*a*), but much more rapidly if the muscle was stimulated (Fig. 3, 5*b*). In fact most of the potentiation was 'dissipated' by the first isometric beat. When different frequencies of stimulation were examined, the disappearance of the potentiated state was found to be essentially beat dependent (Fig. 7*b*), except perhaps at the lowest frequency of stimulation (6 min^{-1}) where the interval between stimuli (10 sec) was sufficient to allow the effect of the resting decay to become significant.

The characteristics of the decay of the potentiated state produced by a period of isotonic beating are strikingly similar to those reported by Wood *et al.* (1969) for the decay of the potentiated states produced by passing a weak depolarizing current through ventricular trabeculae of calf and sheep hearts, by a burst of high frequency stimulation, and by paired pulse stimulation (see their Fig. 12). This suggests that all these means of increasing the contractility of cardiac muscle preparations may act through the same mechanism. It should be noted, however, that the potentiations which could be produced by these electrical interventions were at least an order of magnitude greater than that produced by a period of isotonic beating.

Determinants of the degree of potentiation

We have found that the principal determinants of the degree of potentiation produced by a period of isotonic beating are the length and tension changes that occur in the muscle in the isotonic beats (Fig. 8), the frequency of stimulation (Fig. 6), and the number of isotonic beats (Figs. 3, 6).

Fig. 8 shows that the tension transient was directly related to the amount

of shortening of the muscle under isotonic conditions and inversely related to load opposing shortening. Our estimates of the tension transient that might be expected as a result of a period of isotonic beating against zero load were all rather lower than those obtained experimentally by Parmley *et al.* (1969), but our results agree with theirs in showing significantly less potentiation at a frequency of stimulation of 6 min^{-1} than at 12 min^{-1} or 24 min^{-1}. In fact at low frequencies of stimulation we sometimes found that the initial potentiation after a period of isotonic beating was followed by a longer lasting period of slight depression. Biphasic responses of this type were not studied in any detail.

As one might expect from the way in which the potentiated state developed during the period of isotonic beating (Fig. 5*a*), the tension transient depended on the number of isotonic beats. What is remarkable is that there was a transient disturbance of contractility after a single isotonic beat (Figs. 3, 6), even when the shortening occurred in a quick release late on in the mechanical response (Fig. 11). The number of isotonic beats required to produce the maximum possible potentiation at a given frequency depended on the frequency of stimulation (Fig. 6), and we had hoped to obtain some useful inferences about the mechanism of the potentiation from such experiments. Unfortunately, as reported in the Results section, no consistent pattern emerged from studies of different preparations.

The mechanism of the potentiation

The fact to be explained is that the contractility of the preparation in a given beat is influenced by mechanical events in preceding beats. Parmley *et al.* (1969) examined and dismissed the possibility that this behaviour might be the result of changes in the length of the contractile component of the muscle due to the presence of a series viscous element. The other possibility they considered was that mechanical events might influence the 'availability of intracellular calcium'. One of the arguments they advanced against an explanation along these lines was that an increase in contractility induced by raising the concentration in the bathing medium is always accompanied by a decrease in the time-to-peak tension, whereas the potentiation they observed after a period of isotonic beating was always accompanied by a slight prolongation in the time-to-peak tension. We would not accept this as a serious argument because there is evidence that calcium-induced increases in contractility are not accompanied by any change in the time-to-peak tension (Blinks *et al.* 1972), and in our experiments changes in contractility produced by shortening of the muscle were not accompanied by a change in the time-to-peak tension (Fig. 10*a*).

Kaufmann *et al.* (1971) found that the action potentials that accompany isotonic beats of a cat papillary muscle preparation are slightly longer in

duration than those associated with isometric beats. If the effect of this is to allow a greater entry of calcium into the muscle fibres during isotonic beats, then the enhancement of contractility that results from shortening is readily explained by an extension of the detailed hypothesis advanced by Wood *et al.* (1969) to explain the potentiation produced by the various electrical interventions mentioned previously. What these interventions have in common (including shortening if it prolongs the action potential) is that they cause the cell membranes to be depolarized for a higher proportion of the time, and might therefore allow the releasable calcium within the cells to increase due to greater entry of calcium from the extracellular space. In fact there is very little direct evidence in favour of this hypothesis, but it is consistent with all the known facts about excitation-contraction coupling in mammalian heart muscle (see Nayler & Merrillees, 1971, for review). It is of interest to note that changes in the calcium concentration in the bathing solution appear to have no important effects on the potentiating influence of preceding mechanical events (Parmley *et al.* 1969).

It is not at all clear yet how mechanical events influence the action potential. Kaufmann *et al.* (1971) arrived at the conclusion that 'the instantaneous force–velocity relation of the contractile element contains the controlling parameter', but we can see no reason for attributing the phenomenon to the behaviour of a hypothetical element in an analogue model of muscle. All our results (and, we think, all their's) are consistent with the much simpler idea that either shortening of the muscle or lack of tension development, or both, are the controlling factors (see Fig. 8). In a structure of the geometrical complexity of cardiac muscle, it is quite likely that force transmission occurs through the sarcolemma, as well as from the filaments of one cell to those of the next via the desmosomes of the intercalated disk. As the sarcolemma also includes the cell membrane in which the electrical events take place, it is not unreasonable to suppose that variations in membrane distortion due to changes in force transmission might (through permeability changes) allow mechanical events to influence electrical events. Kaufmann *et al.* (1971) report controls that they consider exclude such a simple explanation, but none of the controls is entirely appropriate to the situation under consideration.

Our quick release studies (Fig. 11) are of interest in this respect. What they show is that releases made between 200 and 300 ms after stimulation resulted in larger tension transients in the isometric beat than afterloaded isotonic contractions against the same load. Although the amount of shortening was less in the releases than in the afterloaded contraction, there was always a very rapid recoil of the muscle – and an equally rapid fall of tension. If mechanical distortion of the cell membrane is

the crucial factor in the potentiation mechanism, then perhaps one would expect that isotonic releases occurring at a time when the tension in the muscle is high might be more effective than afterloaded isotonic contractions in producing potentiation of the next beat. Kaufmann *et al.* (1971) certainly observed dramatic effects of late releases on the membrane potential (their Fig. 8) but no information is given about the potentiating effect of these on subsequent beats.

One important way in which lightly-loaded isotonic contractions of cardiac muscle preparations differ from isometric contractions is that they involve a much smaller expenditure of energy (McDonald, 1966; Gibbs, Mommaerts & Ricchiuti, 1967; Coleman, 1968), and we wondered if this might provide the basis of a possible mechanism through which mechanical events might modulate the contractility of the muscle. In the course of private discussion, Dr J. B. Chapman made an interesting suggestion to us along these lines. The state of reduction of pyridine nucleotides in cardiac muscle preparations is influenced by mechanical events (Chapman, 1972), and the calcium uptake of mitochondria is linked to the state of reduction of mitochondrial pyridine nucleotides (Chance, 1965; Lehninger, Carafoli & Rossi, 1967).

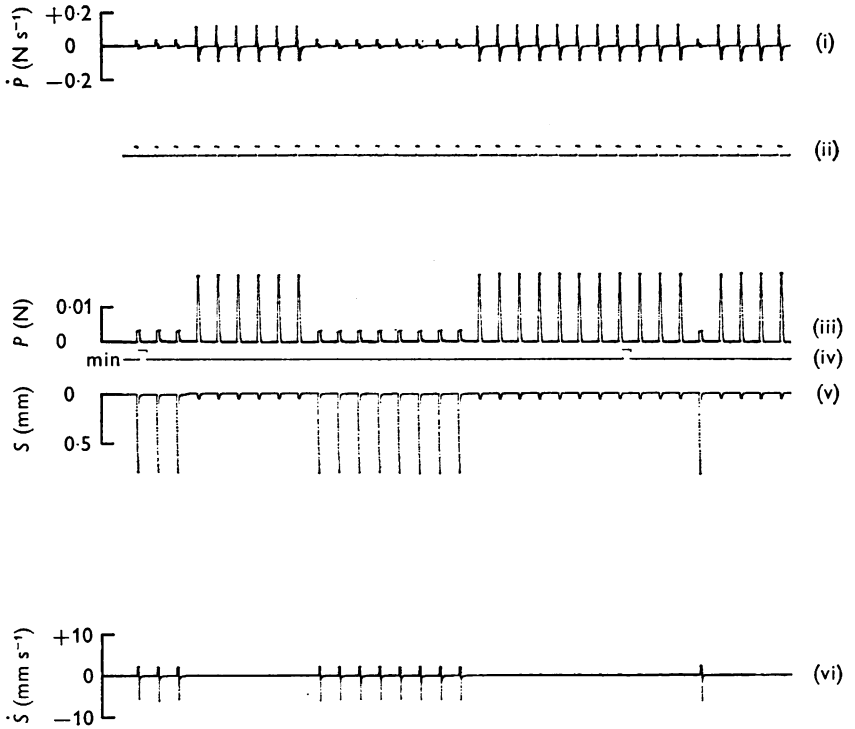

Fig. 12. Effect of a period of isotonic beating on tension production by a *rabbit* papillary muscle (L_{max} = 4·0 mm, M = 2·5 mg; frequency of stimulation, 24 min^{-1}). The quantities monitored are the same as in Fig. 3, with which traces (i) to (vi) should be compared. In this preparation there was no potentiation of tension production after a period of isotonic beating.

These two effects together provide a way in which mechanical events could regulate the distribution of calcium between different intracellular compartments, and perhaps thereby influence contractility. Preliminary experiments by Dr Chapman to test this hypothesis were encouraging: he found that changes of mechanical conditions from isotonic to isometric, and vice versa, were accompanied by changes in the state of reduction of pyridine nucleotides, and these changes occurred with a similar time course to those shown in Fig. 5. However, his studies were made on *rabbit* papillary muscles, and when we made mechanical studies to check that the characteristics of the potentiated state in rabbit muscle were the same as those in cat muscle we were disappointed to find that the contractility of this preparation is *not* affected by mechanical events (compare Fig. 12 with Fig. 4). Nor, apparently, is it possible to potentiate the mechanical response of this preparation by electrical interventions of the sort discussed previously (J. W. Bassingthwaite & G. W. Beeler, private communication). It should be noted that a similar lack of reactivity was reported for rat papillary muscle by Parmley *et al.* (1969). These non-reactive preparations are useful tools for analysing potentiating mechanisms, and they have yet to be fully exploited: for example, it would be very interesting to know whether the action potentials of these preparations are influenced by mechanical events.

In spite of all these uncertainties, it seems very likely that the dependence of the state of contractility of cat papillary muscle on preceding mechanical events is due to variations in the amount of releasable calcium. Until more direct experimental evidence is available in this area, further speculation about the exact mechanism seems unwarranted.

Practical implications of the phenomenon

If the contractility of certain preparations of cardiac muscle depends on previous mechanical events, then any procedures used to obtain mechanical data (e.g. a force–velocity curve) need to be planned with great care. Parmley *et al.* (1969) drew attention to this problem, and pointed out the hazards of the usual procedure of stepwise changes in the force opposing shortening. However, in many papers that have appeared since then (e.g. Brutsaert & Sonnenblick, 1971; Yeatman, Parmley, Urschel & Sonnenblick, 1971; Meiss & Sonnenblick, 1972) there are no explicit statements to indicate that the authors have taken heed of this warning. The nature of the problem is illustrated in Fig. 13, which shows the procedure required to obtain a force–velocity curve from a muscle stabilized under isometric conditions. The velocity of shortening has been determined at this level of contractility by suddenly changing the mechanical conditions from isometric to isotonic and then measuring the velocity of shortening in the first isotonic beat ($\dot{S}_i$ in the inset diagram). If the muscle is allowed to stabilize at that load, its velocity of shortening will increase to a stable value, $\dot{S}_s$. After each period of isotonic beating, the muscle must be returned to isometric conditions and allowed to stabilize there before the next isotonic measurement. For each measurement, then, the muscle will move around a 'loop' as shown in the inset diagram. The line joining all the

open circles in Fig. 13 is the force–velocity curve at the level of contractility present under isometric conditions. The filled circles fall on force–velocity curves representing seven different levels of contractility.

An ideal procedure therefore would be to have the muscle beating under isometric conditions, except for every tenth beat, which would be an isotonic beat (afterloaded or quick release) against a different load. The velocity of shortening would be measured in that beat, and the ten intervening beats before the next velocity measurement would allow the muscle time to re-stabilize at the isometric level of contractility. This is a ritual

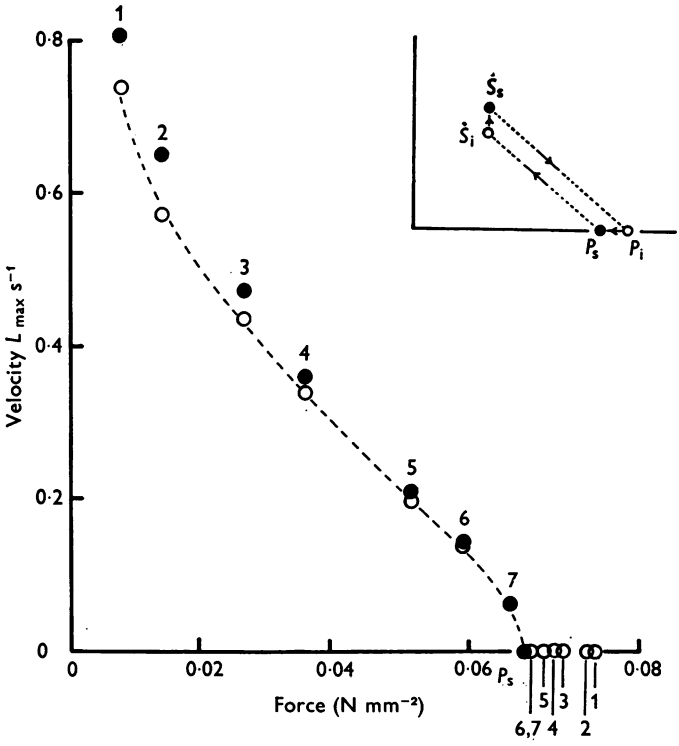

Fig. 13. Influence of load-dependent changes of contractility on force-velocity data. The inset schematic diagram shows points on a force-velocity plot that correspond with an isometric-isotonic-isometric sequence of the type shown in Fig. 4*a*. The graph shows force-velocity data obtained from afterloaded isotonic contractions against various loads. The solid circles show the stable performance of the muscle, and the open circles show the performance in the first beat after a change in the conditions of contraction. The muscle was returned to isometric conditions to stabilize after measurements had been made against each isotonic load. The dashed line links points that are presumed to show the performance of the muscle when its state of contractility is the same as it is under isometric conditions. *Preparation B*: frequency of stimulation 24 min^{-1}.

that cries out for automation, and our colleague Dr David Allen has produced a programme that allows such force–velocity curves to be obtained under computer control with the aid of a LINC 8 computer.

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