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DIASTOLIC SCATTERED LIGHT FLUCTUATION, RESTING FORCE AND TWITCH FORCE IN MAMMALIAN CARDIAC MUSCLE

BY E. G. LAKATTA AND D. L. LAPPÉ

From the Cardiovascular Section, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland 21224, and the Johns Hopkins Medical Institutions, Division of Cardiology, Baltimore, Maryland 21205, U.S.A.

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SUMMARY

1. When coherent light was passed through isolated isometric cardiac muscles during the diastolic or resting period, intensity fluctuations were observed in the scattered field. The frequency of these intensity fluctuations ($f_{\frac{1}{2}}$) varied with many experimental interventions known to enhance Ca^{2+} flux into the cell.

2. In rat muscles stimulated at low frequencies ($0{\cdot}1 \pm 2{\cdot}0$ min^{-1}) stepwise increases (0·4–10 mM) of [Ca^{2+}] in the bathing fluid ([Ca^{2+}]$_e$), or addition of ouabain ($10^{-6} - 6 \times 10^{-4}$ M) to the perfusate caused stepwise increases in $f_{\frac{1}{2}}$. These were paralleled by increments in resting force (RF) such that the changes in $f_{\frac{1}{2}}$ and RF were highly correlated. Substitution of K^+ for Na^+ in the perfusate resulted in parallel transients in RF and $f_{\frac{1}{2}}$.

3. In contrast to the rat, most cat muscles stimulated at low frequencies in the steady state exhibited neither diastolic intensity fluctuations nor Ca^{2+}-dependent changes in RF in [Ca^{2+}]$_e$ of 10 mM or less; when [Ca^{2+}]$_e$ was increased to 12–32 mM, however, steady-state Ca^{2+}-dependent $f_{\frac{1}{2}}$ and RF were observed. In a given [Ca^{2+}]$_e$ reduction of [Na^+]$_e$ increased $f_{\frac{1}{2}}$. In the transient state following cessation of regular stimulation at more rapid rates (12–96 min^{-1}) intensity fluctuations were present in all [Ca^{2+}]$_e$ and decayed with time (seconds to minutes); the $f_{\frac{1}{2}}$ and time course of the decay of the fluctuations were determined by the rate of prior stimulation and [Ca^{2+}]$_e$.

4. Maximum potentiation of twitch force in response to the above inotropic interventions was associated with an optimal level of $f_{\frac{1}{2}}$ which was similar in both species; when higher levels of $f_{\frac{1}{2}}$ were produced by more intense inotropic intervention, twitch force declined. Over the range of inotropic intervention up to and including that at which maximum twitch potentiation occurred, the increase in diastolic $f_{\frac{1}{2}}$ predicted the extent of twitch potentiation with a high degree of accuracy ($r > 0{\cdot}97$) both in the transient and steady states.

5. In contrast to the other inotropic interventions studied, catecholamines were unique in that neither $f_{\frac{1}{2}}$ nor RF increased over a full range of concentrations that resulted in maximum potentiation of the twitch.

6. It is concluded from these observations that $f_{\frac{1}{2}}$ reflects diastolic Ca^{2+}-dependent myofilament interaction; the increase in the extent of this interaction by inotropic interventions that do not alter the affinity of the myofilaments for Ca^{2+} probably reflects an increase in diastolic myoplasmic [Ca^{2+}], an optimal level of which is

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associated with maximal potentiation of twitch force; the difference in $f_{\frac{1}{2}}$ in rat and cat muscles under a given set of *in vitro* conditions may be related to the marked species difference in the effectiveness of excitation–contraction coupling.

INTRODUCTION

Twitch force in response to electrical excitation in cardiac muscle varies with the extent to which the myofilaments are activated by Ca^{2+}. This has commonly been referred to as inotropic state (Jewell, 1977, 1978). Recent evidence suggests that twitch force varies directly with a transient increase in myoplasmic [Ca^{2+}] that occurs subsequent to excitation (Allen & Blinks, 1978) and this appears to be a major determinant of the extent of myofilament Ca^{2+} activation. Whether the myoplasmic [Ca^{2+}] in diastole (that period from the end of a given contraction to the next excitation) is constant or might also vary with the inotropic state in cardiac muscle with intact sarcolemma is unknown. Although evidence extrapolated from chemically and mechanically skinned cardiac fibres suggests that during the diastolic period myoplasmic [Ca^{2+}] is reduced to levels of 10^{-7} M or less (Solaro, Wise, Shiner & Briggs, 1974; McClellan & Winegrad, 1978; Fabiato & Fabiato, 1978*a*), currently available direct methods of measuring [Ca^{2+}] in carciac muscle with intact sarcolemma are not capable of detecting changes in myoplasmic [Ca^{2+}] over this low range (i.e. less than 10^{-7} M). In addition, in this range of pCa, a substantial amount of Ca^{2+} can be bound to myofilaments without resulting in force production (Solaro *et al.* 1974), and relatively large changes in diastolic myoplasmic [Ca^{2+}] and Ca^{2+}-dependent myofilament interaction might occur without necessarily effecting concomitant changes in resting force (McClellan & Winegrad, 1978; Fabiato & Fabiato, 1978*a*). Measurements of quasi-elastic light-scattering properties of isolated cardiac muscle during diastole may in certain instances provide a useful non-perturbing assessment of changes in myoplasmic [Ca^{2+}] during that period (Lappé & Lakatta, 1980).

The initial application of quasi-elastic light-scattering methods to studies of striated muscle has indicated that Ca^{2+} activation of the myofilaments during a tetanus was accompanied by fluctuations in the intensity of coherent light scattered by the muscle (Bonner & Carlson, 1975). Extension of these studies to cardiac muscle demonstrated that the characteristics of these fluctuations varied with the extent of Ca^{2+} activation of the myofilaments (Lappé & Lakatta, 1980). Furthermore, fluctuations in scattered light intensity are present in rat cardiac muscle with intact sarcolemma even in the unstimulated state; they vary directly with the perfusate [Ca^{2+}] over the range of concentrations employed in studies of excitation–contraction coupling; and they cease to be detectable following removal of Ca^{2+} from the perfusate (Lappé & Lakatta, 1980). These observations led us to hypotheize that in cardiac muscle with intact sarcolemma, myoplasmic [Ca^{2+}] in diastole is not fixed, but rather varies, and might possibly be involved in the mechanisms that determine the effectiveness of the coupling of excitation to contraction and, thus, in the regulation of inotropic state.

The present studies were undertaken to determine the relationship of intensity fluctuations in the diastolic period to resting and twitch force in papillary muscles isolated from the rat and cat. These parameters were examined in response to both

optimal and excessive inotropic stimulation, effected by alterations in the rate and pattern of stimulation, by changes of the ionic milieu, and by pharmacological innervation with agents that alter Ca^{2+} flux into the cell.

METHODS

Under ether anaesthesia hearts were removed from rats (200 g) and cats (750 g) and immersed in oxygenated Krebs–Ringer solution. Right ventricular papillary muscles were removed and mounted horizontally between two metal clamps in 3 ml. perfusion chamber which permitted measurements of force and light scattering. Force, monitored on a pen recorder (Brush no. 480) was measured by a strain gauge (Statham UC-2) attached to a metal clamp that fixed one end of the preparation. In order to detect small changes in resting force the sensitivity of the force-recording apparatus was set at 200 mg full scale. Muscle length was varied by a micrometer which was connected to the other clamp. The apparatus was pneumatically isolated to reduce vibrations. Muscles were equilibrated in a solution that contained, in mM: NaCl, 119; Na_2HCO_3, 25; KCl, 3·8 $CaCl_2$, 1·0; KH_2PO_4, 1·2; $MgSO_4$, 1·2; and dextrose, 10·0; bubbling with 95% O_2–5% CO_2 at 29°C resulted in a pH of 7·4. In experiments in which the concentration of Ca^{2+} in the perfusate, $[Ca^{2+}]_e$, was varied over a wide range, Hepes buffer replaced HCO_3^- in the perfusate (Hepes 4·0 mM at pH 7·4 was bubbled with 100% O_2, and Na^+, K^+, Mg^{2+}, PO_4^{2-}, SO_4^{2-} and dextrose were present in concentrations identical to those in the Krebs solution). During the equilibration period (typically 3 hr) the muscles were stimulated to contract isometrically at a rate of 24 min^{-1} by square wave impulses 5 msec in duration and at a voltage of 20% above threshold. Following 1 hr of equilibration the muscle was stretched to L_{max} – that length at which the force developed in response to an excitation (DF), i.e. the difference between total twitch force and resting force (RF), was maximal – and remained at that length for the remainder of the equilibration period.

Light-scattering measurements

The 1 mm beam of a 5 mW He–Ne laser (Spectra Physics, $\lambda = 632{\cdot}8$ nm) was reduced to 0·25 mm by an inverted beam expander and was collimated on to the muscle (Fig. 1). Light scattered at 30° from the incident beam in the plane of the long axis of the muscle was collimated by a double pinhole system (A_1, L, A_2), and focused onto a photomuliplier tube (Hamamatsu R928). The photocurrent, unfiltered, was amplified and a.c. coupled to a Honeywell Saicor 42A analogue autocorrelator (high-frequency cut-off 1 MHz). The autocorrelation function, which represents the inverse Fourier transform of the power spectrum of the input signal, provides a measure of the component frequencies of the signal. In the present study, since the clipped a.c.-analogue method of autocorrelation was employed, the amplitude of the intensity fluctuations was not measured. The intensity fluctuations in scattered light were characterized as $f_{\frac{1}{2}} = 1/2\pi T_{\frac{1}{2}}$, where $T_{\frac{1}{2}}$ is the half-decay time of the normalized autocorrelation function. The optical system was tested by measuring the decay time of latex microspheres (Dow Chemicals) of a known size in the chamber. Measured values of $f_{\frac{1}{2}}$ agreed to within 3% of those predicted from the theoretical calculations (Bonner & Carlson, 1975), and the amplitude calculated from the probability density function suggested that roughtly two to four coherence areas were being sampled. Light scattered through the chamber and perfusate in the absence of a muscle preparation resulted in a flat autocorrelation function indicating the absence of intensity fluctuations.

Certain limitations in measuring the intensity fluctuations need be noted. While it was observed that $f_{\frac{1}{2}}$ increased dramatically during a twitch (approximately 200 Hz), gross movement in the muscle interfered with accurate quantitation of the fluctuations. Thus, the present study quantitated $f_{\frac{1}{2}}$ solely during the diastolic period. Furthermore, the relatively low levels (1–10 Hz) of $f_{\frac{1}{2}}$ encountered in these muscles during the diastolic period necessitated 15–30 sec measurement periods. This established a minimum diastolic interval that could be investigated in beating preparations. The lower limit of sensitivity of the present apparatus was 1 Hz and $f_{\frac{1}{2}}$ less than 1 Hz could not be accurately quantitated.

Experimental procedure

Immediately following the mounting of a muscle, $f_{\frac{1}{2}}$ was usually relatively high and gradually decreased thereafter during equilibration. In several experiments it was demonstrated that once

$f_{\frac{1}{2}}$ reached equilibrium under a given set of experimental conditions it did not vary further over long periods of time (Fig. 2*A*). In additional experiments, the muscle was scanned; measurements of $f_{\frac{1}{2}}$ made over different portions of the entire muscle demonstrated that the variation in $f_{\frac{1}{2}}$ across the entire muscle averaged 10 % or less (Fig. 2*B*). Following the equilibration period, the relationship of RF, $f_{\frac{1}{2}}$ measured in the non-stimulated state before excitation, and DF generated in response to electrical excitation were determined over a range of experimental conditions known to alter the inotropic state in cardiac muscle.

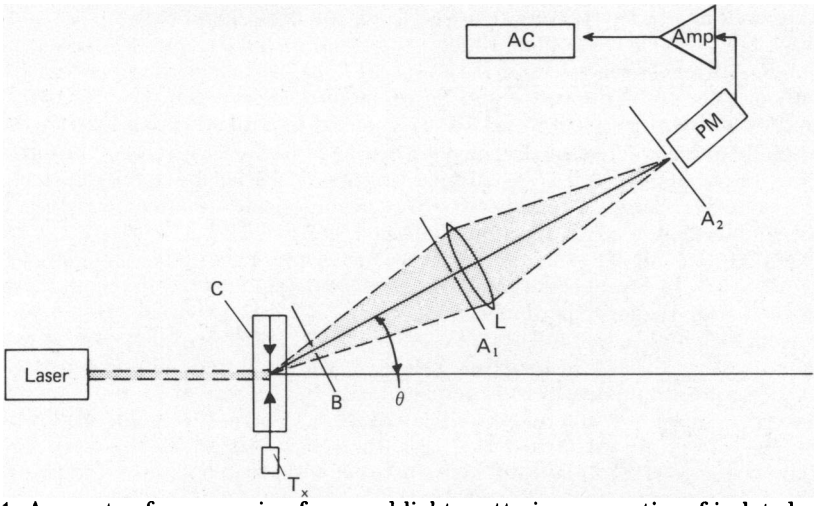

Fig. 1. Apparatus for measuring force and light-scattering properties of isolated cardiac muscle. C, plexiglass chamber in which the muscle was mounted; T_x, force transducer; B, baffle; θ, light-scattering angle; A_1 and A_2, 1 mm pinholes at 50 and 100 cm from the muscle; L, lens; PM, photomultiplier tube; Amp, operational amplifier (high-frequency cut-off 100 KHz); AC, autocorrelator.

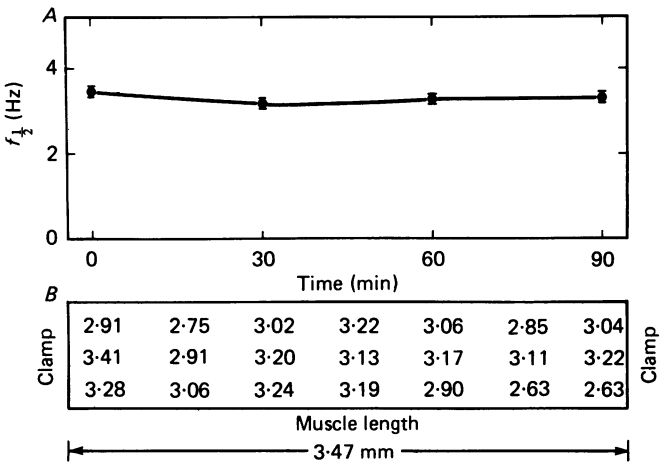

Fig. 2. Intensity fluctuations in light scattered by non-beating rat papillary muscle in a steady state. *A*, a representative example of time control measurements of intensity fluctuations (●), measured in the same area of a non-beating rat papillary muscle equilibrated in $[Ca^{2+}]_e$ of 1·0 mM. Each point represents the mean ±S.E.M. of five determinations at each point in time. Note that the variation about each point is less than 3 %. *B*, in the same muscle as in *A*, the laser beam was collimated on different areas of the muscle which encompassed essentially the entire surface area. Each number represents the mean of five determinations of $f_{\frac{1}{2}}$ for a given location of the beam. The variation about each point was as in panel *A*.

Statistical analysis

Data are expressed as mean ± standard error of the mean of n experiments. The relationship of $f_{\frac{1}{2}}$ to DF or RF was determined by a linear regression analysis (Snedecor, 1957).

RESULTS

Intensity fluctuations and force production in the steady state

In rat papillary muscles, $[Ca^{2+}]_e$ was varied from 0·4 mM to 3·0 mM, a range commonly employed in studies of excitation–contraction in this species (Gerstenblith, Spurgeon, Froehlich, Weisfeldt & Lakatta, 1979), and the relationships between steady-state $f_{\frac{1}{2}}$, RF and DF across this range of $[Ca^{2+}]_e$ examined (Fig. 3). In these experiments the muscle was stimulated at 24 min^{-1} in a given $[Ca^{2+}]_e$ until a steady

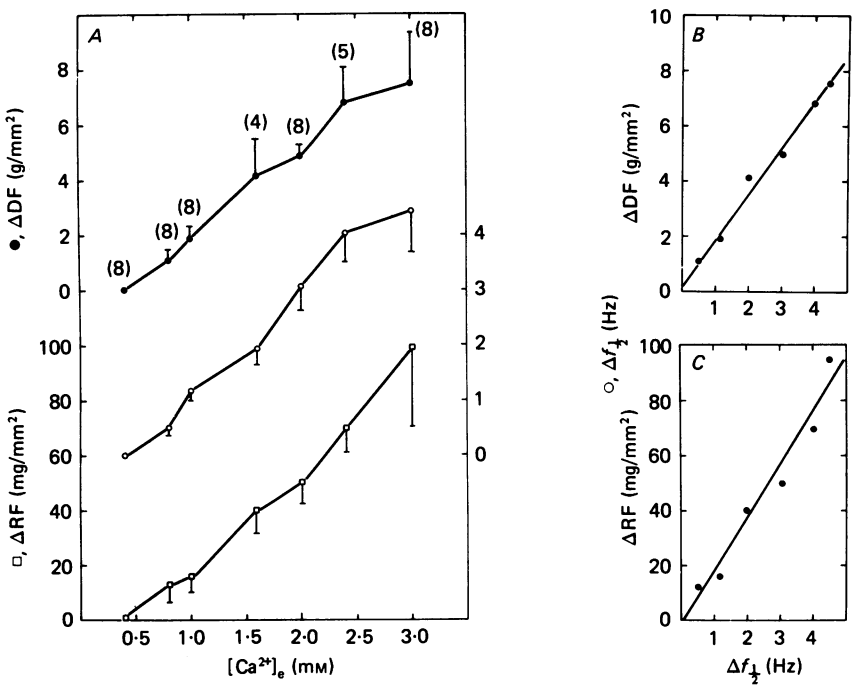

Fig. 3. The effect of $[Ca^{2+}]_e$ on $f_{\frac{1}{2}}$, RF and DF in rat papillary muscles. In *A*, $\Delta f_{\frac{1}{2}}$ (○), ΔRF (□), and ΔDF (●) represent the difference between the measurement in a given $[Ca^{2+}]_e$ and the subsequent reference control in 0·4 mM-$[Ca^{2+}]_e$. Points represent the mean ± S.E.M. of (n) muscles. In some muscles $[Ca^{2+}]_e$ was varied from high to low concentrations, while in others the sequence was reversed. Approximately half the muscles were studied in Krebs solution and half in Hepes buffer and no differences between the two groups were noted. In $[Ca^{2+}]_e$ of 0·4 mM, DF averaged 1·06 ± 0·35 g/mm², $f_{\frac{1}{2}}$ averaged 2·92 ± 0·28 Hz, and RF averaged 713 ± 62 mg/mm². Muscle cross-sectional area (CSA) was estimated by assuming a cylindrical shape and dividing muscle wet weight by length. Muscle CSA (range 0·11–0·62 mm²; mean 0·30 ± 0·05 mm²) did not influence $f_{\frac{1}{2}}$: in a given $[Ca^{2+}]_e$, $f_{\frac{1}{2}} = -1·18$ CSA + 3·25 ($r = 0·19$). *B*, the Ca^{2+}-dependence of $f_{\frac{1}{2}}$ predicts that of DF. Each co-ordinate was derived from the mean value of $\Delta f_{\frac{1}{2}}$ and ΔDF at each $[Ca^{2+}]_e$ in *A*. Over the range of $[Ca^{2+}]_e$ ΔDF = 1·7 $[\Delta f_{\frac{1}{2}}]$ + 0·19, $r = 0·99$, $P < 0·001$. *C*, the Ca^{2+}-dependence of $f_{\frac{1}{2}}$ predicts that of RF. Each co-ordinate was derived as in *B*. ΔRF = 19·4 $[\Delta f_{\frac{1}{2}}]$ − 1·2, $r = 0·98$, $P < 0·001$.

state in DF was achieved; stimulation was then discontinued for 5 min, at which time five consecutive 30 sec measurements of $f_{\frac{1}{2}}$ were made, during which RF was recorded. The muscle was then stimulated and DF was measured. Following measurements in each $[Ca^{2+}]_e$, the preparation was re-perfused with solution containing 0·4 mM-Ca^{2+} and reference measurements were taken (see below). This cycle was repeated until all $[Ca^{2+}]_e$ were studied. The difference between the measurement in a given $[Ca^{2+}]_e$ and that in the subsequent reference measurement at $[Ca^{2+}]_e$ of 0·4 mM is depicted in Fig. 3 as $\Delta f_{\frac{1}{2}}$, ΔRF, and ΔDF. Note that all three parameters exhibit a linear stepwise dependence on increasing $[Ca^{2+}]_e$ (panel *A*). Over this range of $[Ca^{2+}]_e$ ΔDF, $\Delta f_{\frac{1}{2}}$, and ΔRF could be expressed as linear functions of each other (panels *B* and *C*). Since stress relaxation (Remington, 1957 causes RF in a *given* $[Ca^{2+}]_e$ slowly to

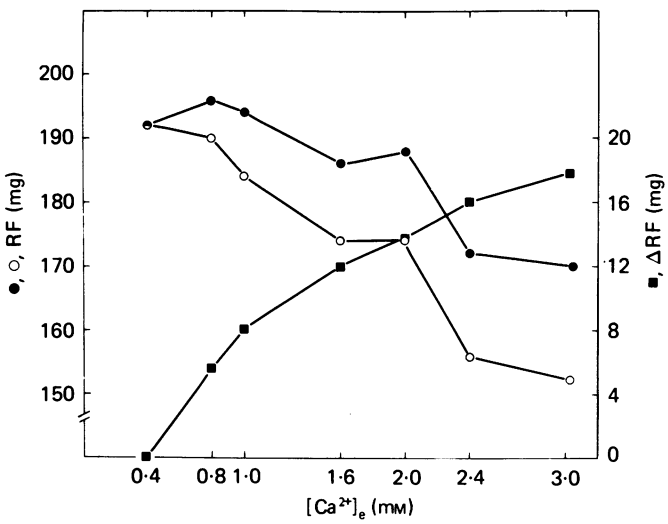

Fig. 4. A typical example of the effects of $[Ca^{2+}]_e$ and stress relaxation and their interaction on RF in papillary muscles isolated from the rat. In this experiment the sequence of variation in $[Ca^{2+}]_e$ was from low to high. RF (●), was measured at each $[Ca^{2+}]_e$, and the perfusate was switched back to a $[Ca^{2+}]_e$ of 0·4 mM for a reference control measurement of RF (○), ΔRF (■), the difference between RF in a given $[Ca^{2+}]_e$ and that in the 0·4 mM-$[Ca^{2+}]_e$ reference control, increased as a function of $[Ca^{2+}]_e$ and thus indicates a Ca^{2+}-dependent component of RF. Muscle cross-sectional area was 0·25 mm^2.

decrease over the time course of the experiment, reference measurements in $[Ca^{2+}]_e$ of 0·4 mM were of particular importance in order to detect small Ca^{2+}-dependent changes in RF. A typical example of stress relaxation is evident in the reference measurements of RF at 0·4 mM as noted in the experiment in Fig. 4. In fact, in this example in which higher values of $[Ca^{2+}]_e$ were studied later in time (typically the experiment took 150 min) RF decreased rather than increased with increasing $[Ca^{2+}]_e$. However, the reference measurements in $[Ca^{2+}]_e$ of 0·4 mM also decreased with time but to a greater extent. Hence, ΔRF, the difference between RF in a given $[Ca^{2+}]_e$ and that in subsequent reference control, was always positive and increased in a stepwise manner with increasing $[Ca^{2+}]_e$. (A similar Ca^{2+}-dependence of RF was also observed in muscles in which the effect of $[Ca^{2+}]_e$ was studied in an order of decreasing rather than increasing concentrations.) Reference control measurements thus enable

detection of small Ca^{2+}-dependent changes in RF which are otherwise marked by stress relaxation. In *non-beating* rat papillary muscles, when $[Ca^{2+}]_e$ was varied over short periods of time, similar Ca^{2+}-dependent changes in RF were observed directly without the necessity of reference control measurements (Lappé and Lakatta, 1980).

Previous studies in cardiac muscle have demonstrated that the response of DF to inotropic intervention does not increase monotonically; rather it saturates and decays once an optimal level of inotropic stimulation is exceeded (Koch-Weser & Blinks, 1963; Dhalla & Braxton, 1968; Allen, Jewell & Wood, 1976; Gerstenblith *et al.* 1979). To determine whether $f_{\frac{1}{2}}$ saturates and decays as well, additional experiments were performed measuring $f_{\frac{1}{2}}$ and DF during excessive levels of inotropic stimulation. In additional rat papillary muscles, when $[Ca^{2+}]_e$ was increased beyond 3·0 mM, $f_{\frac{1}{2}}$ and RF continued to increase monotonically but DF reached a plateau and then decreased (Fig. 5). Thus an optimal level of $f_{\frac{1}{2}}$ is associated with maximal potentiation of DF.

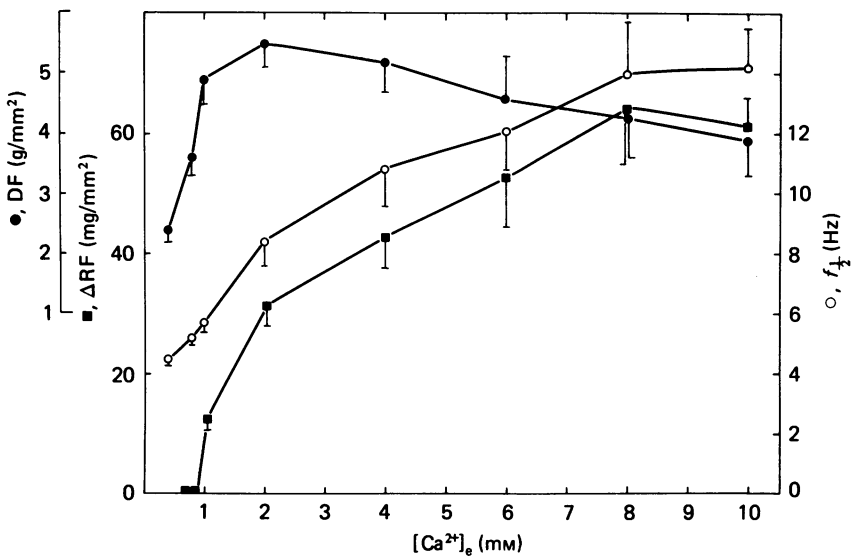

Fig. 5. The biphasic relationship of DF (●), to $f_{\frac{1}{2}}$ (○), when $f_{\frac{1}{2}}$ is raised to high levels by increasing $[Ca^{2+}]_e$ (Hepes buffer) in three rat papillary muscles. ΔRF (■), was measured as in Fig. 3. Points represent the mean ± S.E.M. of three muscles. Cross-sectional area was 0·39 ± 0·12 mm².

The Ca^{2+}-dependence of RF and the linear relationship between ΔRF and $\Delta f_{\frac{1}{2}}$ (Figs. 3 and 5) suggested that in rat cardiac muscle with intact sarcolemma the $[Ca^{2+}]$ surrounding the myofilaments in the diastolic interval was not constant but rather varied directly with $[Ca^{2+}]_e$, and that a change in $f_{\frac{1}{2}}$ might be a useful probe of a change of myoplasmic $[Ca^{2+}]$ in resting muscle. Additional evidence of the $f_{\frac{1}{2}}$–RF relationship was sought in muscles in which the sarcolemma was initially intact, as in Fig. 3, and subsequently rendered hyperpermeable to Ca^{2+} by KCl depolarization (Niedergerke, 1956*a*; Lammel, Niedergerke & Page, 1975; Chapman, 1979). Following measurements of RF and $f_{\frac{1}{2}}$ in rat papillary muscles across a range of $[Ca^{2+}]_e$, as in Fig. 3, equimolar K^+ was substituted for Na^+ in the bathing fluid ($[Ca^{2+}]_e$ = 1·0 mM). Typically, K^+ depolarization resulted in an immediate large increase in RF and $f_{\frac{1}{2}}$ while DF resulting from excitation was extinguished (Fig. 6*A*); both $f_{\frac{1}{2}}$ and RF subsequently decayed

and measurements over this portion of the transient (after 100 msec in the present example) indicated that both parameters decayed in parallel. The (●) in Fig. 6*B* indicate the average relationship of $\Delta f_{\frac{1}{2}}$ and ΔRF in three muscles across a range of $[Ca^{2+}]_e$ before KCl depolarization; the open circles indicate the average relationship of $\Delta f_{\frac{1}{2}}$ and ΔRF in the same muscle during the decay of the KCl transient. Note that the same linear function fits both sets of points ($r = 0{\cdot}99$).

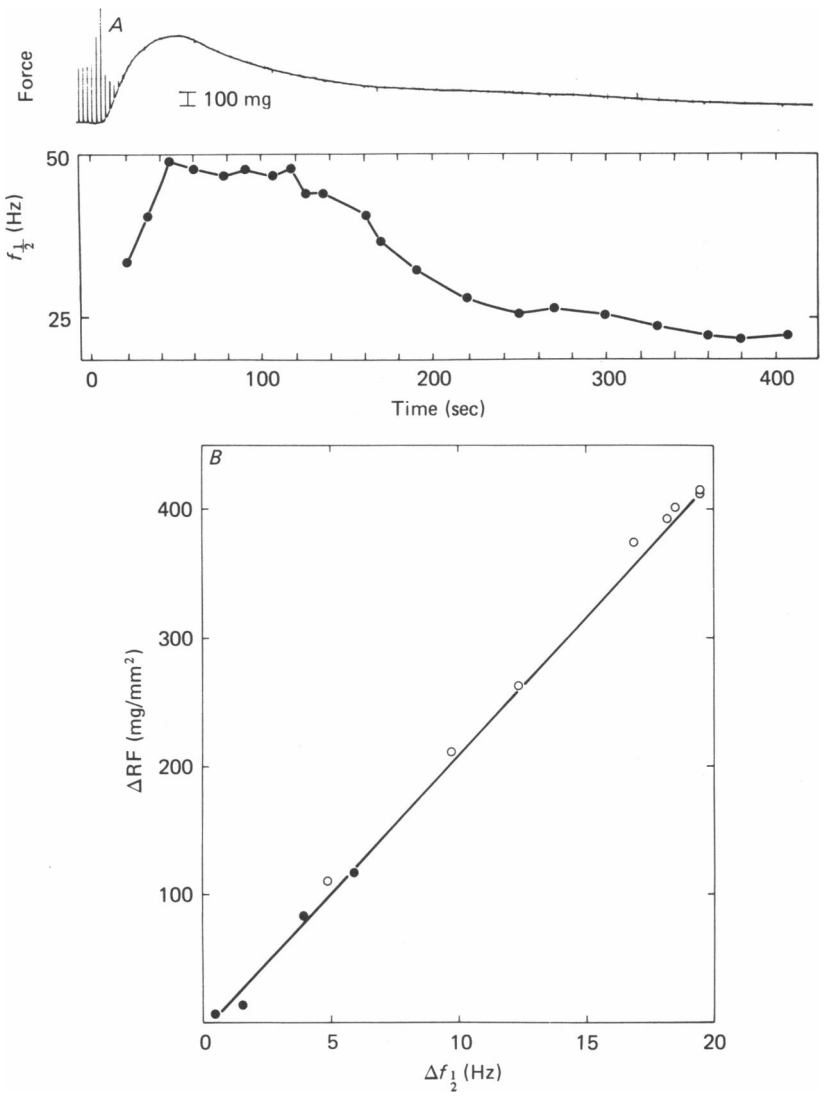

Fig. 6. A comparison of the effect of $[Ca^{2+}]_e$ and $[K^+]_e$ on RF and $f_{\frac{1}{2}}$ in rat papillary muscle. *A*, the muscle was estimated at 24 min^{-1} in $[Ca^{2+}]_e$ of 1·0 mM (Hepes buffer); at time zero, K^+ (144 mM) was substituted for Na^+ in the bathing fluid, resulting in a transient in both $f_{\frac{1}{2}}$ and RF. Over the decay portion of the transient the change in $f_{\frac{1}{2}}$ predicted that in RF. $\Delta RF = 14{\cdot}5[\Delta f_{\frac{1}{2}}] + 0{\cdot}19$, $r = 0.95$, $P < 0{\cdot}001$. *B*, the relationship between the average ΔRF and $\Delta f_{\frac{1}{2}}$ (○), measured during the K^+ transient in three muscles as in *A* and the steady level measured in the same muscles across a range of $[Ca^{2+}]_e$ before exposure to K^+ (●). Both sets of points are fitted by the same function: $\Delta RF = 21{\cdot}7[\Delta f_{\frac{1}{2}}] - 8{\cdot}4$, $r = 0{\cdot}99$, $P < 0{\cdot}001$.

In skeletal muscle it has been shown that measurements of non-coherent light scattering could not differentiate between Ca^{2+}-dependent activation and rigor (Katz, Sorenson & Reuben, 1978). In order to determine whether the Ca^{2+}-dependent change in $f_{\frac{1}{2}}$ resulted from a change in force *per se*, or specifically from a change in Ca^{2+}-dependent activation, it would be necessary to dissociate $f_{\frac{1}{2}}$ and force in unstimulated muscle. When muscles were perfused with hypoxic solution that did not contain glucose, large increases in resting force (rigor) occurred (Fig. 7); $f_{\frac{1}{2}}$, however, did not increase, but rather decreased. In several additional muscles large increases in RF during hypoxia were accompanied by a decrease or no change in $f_{\frac{1}{2}}$. Thus, changes in *fluctuations* in the intensity of scattered laser beam and force could be dissociated, as was the case noted in glycerinated skeletal muscle in rigor (Carlson, 1975). Thus, the increase in $f_{\frac{1}{2}}$ caused by increasing $[Ca^{2+}]_e$ or K^+ depolarization

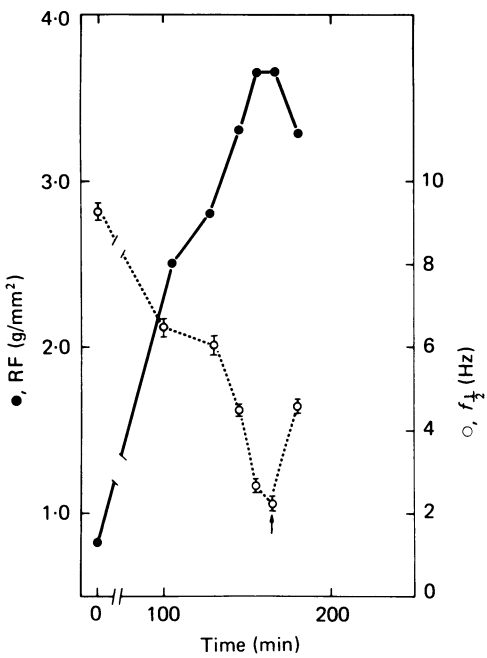

Fig. 7. An example of the effect of prolonged perfusion with hypoxic and glucose-free bathing fluid on RF and $f_{\frac{1}{2}}$. Perfusate was made hypoxic by bubbling Krebs–Ringer solution with 95 % N_2–5 % CO_2. After a significant increase in RF had occurred (100 min), stimulation at 24 min^{-1} in $[Ca^{2+}]_e$ of 2·5 mM was interrupted for 5 min intervals and RF (●), and $f_{\frac{1}{2}}$ (○), were recorded. At the time shown by the arrow, the perfusate was switched back to control for an additional measurement. Cross-sectional area was 0·35 mm^2.

cannot be attributed to the concurrent increase in resting force *per se* that accompanied these interventions, but rather must reflect a change in Ca^{2+}-dependent activation; an increase in $f_{\frac{1}{2}}$ when $[Ca^{2+}]_e$ was increased, or during K^+ depolarization therefore provides an indirect estimate of a relative increase in $[Ca^{2+}]$ in the space surrounding the myofilaments in response to these interventions.

Cardiac glycosides act to increase Ca^{2+} flux into the cell (Langer, Serena & Nudd, 1974). Earlier experiments (Hoffman, Bartelstone & Bassett, 1967; Gerstenblith *et al.* 1979) have indicated that in cardiac muscle with intact sarcolemma, small

increases in resting force accompanied the twitch potentiation in response to glycosides and that at toxic concentration large increases in resting force accompany a diminution in twitch force. Ouabain ($10^{-6}-10^{-3}$ M) was added to the perfusate bathing rat muscles that were stimulated at 24 min^{-1} (Fig. 8). When the response in DF to a given concentration was constant (approximately 10 min), stimulation was stopped for 5 min and $f_{\frac{1}{2}}$, RF, and DF in response to a test excitation were measured as in Fig. 3; the sequence was repeated at each ouabain concentration as the latter was progressively increased. Note that both the level of $f_{\frac{1}{2}}$ and RF increased in a parallel manner as ouabain concentration was increased. The stepwise increase in DF in response to increasing ouabain up to and including the concentration at which DF was maximal (10^{-4} M) was predicted by the increase in $f_{\frac{1}{2}}$: $\Delta DF = 0{\cdot}3\ [\Delta f_{\frac{1}{2}}] - 0{\cdot}13$, $r = 0.99$, $P < 0{\cdot}001$. At ouabain concentrations greater than 10^{-4} M a depression in DF was observed.

In these experiments it was not possible to return to the pre-ouabain base line between concentrations of ouabain for measurements of RF, and considering the effect of stress relaxation to reduce RF with time (Fig. 4) the actual increase in RF would be expected to be slightly greater than that measured.

Recent evidence has suggested that in a given $[Ca^{2+}]_e$, changes in resting muscle length over the ascending portion of the length–DF relationship result in changes in inotropic state (Jewell, 1977, 1978; Lakatta & Henderson, 1977; Lakatta & Spurgeon,

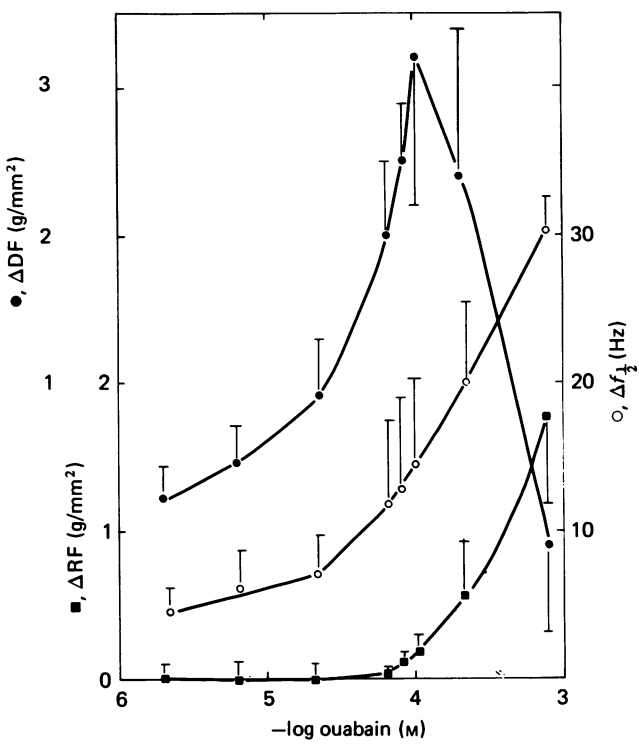

Fig. 8. The effect of ouabain on $f_{\frac{1}{2}}$, DF and RF in rat papillary muscles. $\Delta f_{\frac{1}{2}}$ (○), ΔRF (■), and ΔDF (●), represent the change from control level in 0·25 mM-$[Ca^{2+}]_e$. The points represent the mean ± S.E.M. of three muscles. At control, DF was 0·67 ± 0·31 g/mm², $f_{\frac{1}{2}}$ was 3·61 ± 0·92 Hz, and RF was 712·0 ± 118·0 mg/mm². Cross-sectional area was 0·40 ± 0·10 mm².

1980). Diastolic RF, $f_{\frac{1}{2}}$, mean sarcomere length, and DF in a test excitation were measured across a range of muscle lengths (Fig. 9). The muscle was stimulated at 24 min^{-1} in a $[Ca^{2+}]_e$ of 1·0 mM, and when steady-state DF was achieved at each length, stimulation was stopped for 5 min; five consecutive 30 sec measurements of $f_{\frac{1}{2}}$ were then made, and in the same region of the muscle, sarcomere length was estimated from the diffraction pattern of the scattered light (Carlson, 1975; Krueger & Pollack, 1975); the muscle was stimulated and DF measured. The muscle was

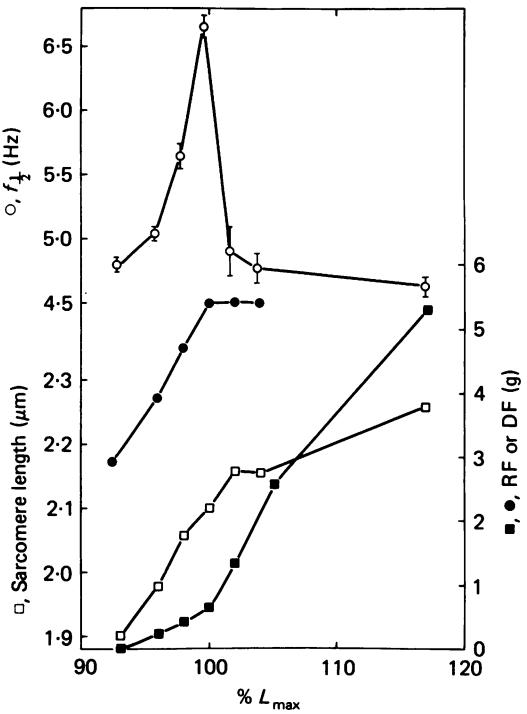

Fig. 9. A typical example of the effect of resting muscle length on $f_{\frac{1}{2}}$ (○), (mean ± S.E.M. of five determinations), RF (■), sarcomere length, (□) and DF in a test beat (●), in a rat papillary muscle. At lengths greater than 104 % L_{max} the muscle did not develop force in response to an excitation. Over the ascending portion of the length–DF curve (L_{max} and below) length-dependence of DF was predicted by that in $f_{\frac{1}{2}}$: $\Delta DF = 1{\cdot}5[\Delta f_{\frac{1}{2}}] - 4{\cdot}07$, $r = 0{\cdot}97$, $P < 0{\cdot}01$. Cross-sectional area was 0·23 mm^2.

stretched to the next length, and the sequence repeated at each length. RF, DF, $f_{\frac{1}{2}}$ and sarcomere length increased in a linear fashion with increasing muscle length until L_{max} was reached; further stretch, which resulted in an exponential increase in RF, was accompanied by a sharp decline in $f_{\frac{1}{2}}$. Strikingly similar results were observed in the two additional muscles studied in this protocol, and suggest that $f_{\frac{1}{2}}$ is retarded in muscles stretched to lengths greater than L_{max} which result in high levels of passive force. It is noteworthy that the large increases in RF were not accompanied by proportionate increases in sarcomere length, suggesting that the ends of the muscle were stretched to a greater extent than the region which was illuminated by the laser beam (Krueger & Pollack, 1975; Pollack & Krueger, 1976). Over the ascending limb of the length–DF relationship (i.e. lengths of L_{max} and below), $f_{\frac{1}{2}}$ measured before a test excitation was closely correlated with DF resulting from that excitation

($r = 0{\cdot}95$ in this example and $r = 0{\cdot}98$ in each of the two additional muscles studied). Furthermore, the average slope of the function relating $f_{\frac{1}{2}}$ to DF as length was varied in the three muscles was 2·7 g/mm^2·Hz^{-1} and is of the same order of magnitude as that observed when the inotropic state was altered by changing $[Ca^{2+}]_e$ (Fig. 3*B*).

Catecholamines represent a unique class of inotropic agents in their 'dual effect' of potentiating the twitch and enhancing relaxation (Rolett, 1974). Catecholamine-induced cAMP–protein-kinase-mediated phosphorylation results in an enhanced rate and extent of Ca^{2+} removal from the myoplasm by sarcoplasmic reticulum (Fabiato & Fabiato, 1977; Katz, 1979). It might be expected that this would minimize an

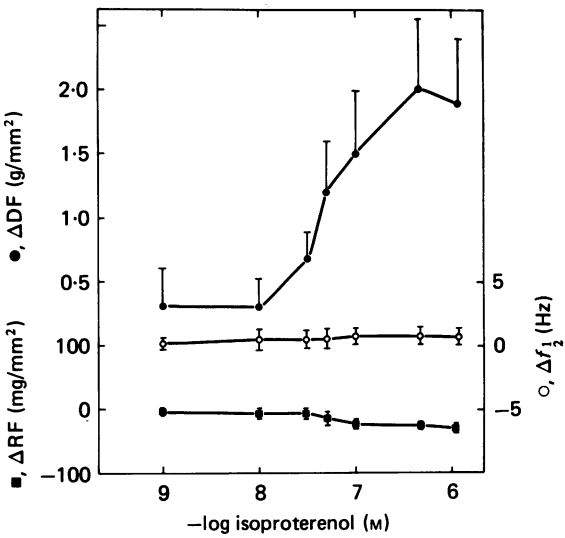

Fig. 10. The effect of increasing concentrations of isoproterenol on $f_{\frac{1}{2}}$, RF and DF in rat papillary muscle. Points represent the mean ± S.E.M. of three muscles. $\Delta f_{\frac{1}{2}}$ (○), ΔRF (■), and ΔDF (●), represent the change from control level in $[Ca^{2+}]_e$ of 0·4 mM. At control, $f_{\frac{1}{2}}$ was 5·16 ± 1·04 Hz, DF was 2·12 ± 1·02 g/mm^2, and RF was 1·24 ± 0·33 gm/mm^2. Cross-sectional area was 0·19 ± 0·04 mm^2.

increase in $f_{\frac{1}{2}}$ when inotropic state was increased in response to catecholamines. Isoproterenol in incremental concentrations (10^{-9}–10^{-6} M) was added to the perfusate of rat papillar muscles (Fig. 10), and DF, $f_{\frac{1}{2}}$ and RF were measured at each concentration as in Fig. 8. Note that the full inotropic response of DF to isoproterenol was achieved with no increase in $f_{\frac{1}{2}}$ or RF. Thus, catecholamines enhance inotropic state without increasing diastolic $f_{\frac{1}{2}}$ and in this manner differ from the other inotropic interventions studied.

Apparent differences between the responses of DF to inotropic interventions in rat cardiac muscle and that isolated from other species such as the cat under given *in vitro* conditions have been well documented previously (Henderson, Brutsaert, Parmley & Sonnenblick, 1969). Of particular note is the species difference in response to $\Delta[Ca^{2+}]_e$ and a change in the rate of stimulation (Lakatta, 1980). Experiments in cat muscle were undertaken to determine whether the response in $f_{\frac{1}{2}}$ to these inotropic interventions also differed from that observed in the rat. The effect of $[Ca^{2+}]_e$ on the steady-state level of diastolic $f_{\frac{1}{2}}$ in three cat papillary muscles stimulated at 2 min^{-1}

is depicted in Fig. 11*A*. Note that when $[Ca^{2+}]_e$ was less than 12·0 mM, the average $f_{\frac{1}{2}}$ in these muscles was less than 1 Hz (dotted line) and could not be accurately quantitated. This is in marked contrast to the situation in the rat (Fig. 3*A*), in which $f_{\frac{1}{2}}$ of 3–6 Hz was observed over a range of 0·4–3·0 mM. It is also noteworthy that in cat muscles bathed in $[Ca^{2+}]_e$ of less than 12·0 mM, a Ca^{2+}-dependence of resting force was also not detected. In order for $f_{\frac{1}{2}}$ to reach measurable levels in the steady state at this low frequency of stimulation, $[Ca^{2+}]_e$ had to be increased to higher levels, over which DF and $f_{\frac{1}{2}}$ increased in parallel. At this frequency of stimulation DF usually increased over the entire range of $[Ca^{2+}]_e$. In $[Ca^{2+}]_e$ in which $f_{\frac{1}{2}}$ could be accurately measured, DF was highly correlated with $f_{\frac{1}{2}}$ (Fig. 11*B*) and it is noteworthy that the slope of the function relating $f_{\frac{1}{2}}$ and DF across the range of $[Ca^{2+}]_e$ in the cat was similar in magnitude to that in the rat (Fig. 3*B*). Thus, except for a shift to higher $[Ca^{2+}]_e$ in the cat, the $f_{\frac{1}{2}}$–DF relationships in the cat and rat (Fig. 3*B*) are remarkably similar. In addition, at higher levels of $[Ca^{2+}]_e$, increases in $f_{\frac{1}{2}}$ were accompanied by increases in RF in cat muscle.

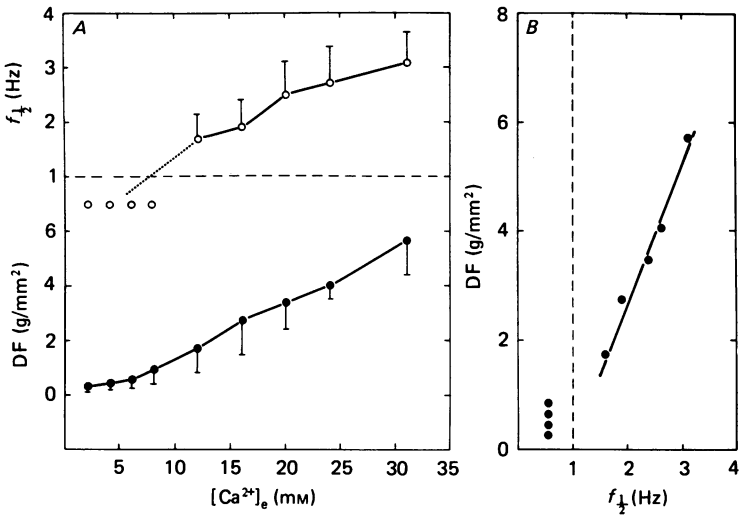

Fig. 11. The dependence of steady-state $f_{\frac{1}{2}}$ (○), and DF, (●), on $[Ca^{2+}]_e$ in cat papillary muscles which were stimulated regularly at 2 min^{-1}. *A*, the points represent the mean ±S.E.M. of three muscles. At levels of less than 1 Hz (dotted line) $f_{\frac{1}{2}}$ could not be accurately quantitated and the symbols have been arbitrarily placed in the Figure at the same level below 1 Hz. Cross-sectional area was 0·21 ± 0·05 mm^2. *B*, the Ca^{2+}-dependence of $f_{\frac{1}{2}}$ predicts that of DF. Co-ordinates are derived from mean $f_{\frac{1}{2}}$ and from *A*. $DF = 2{\cdot}6[f_{\frac{1}{2}}] - 2{\cdot}51$, $r = 0{\cdot}97$, $P < 0{\cdot}001$.

In additional cat muscles stimulated at 2 min^{-1}, $[Na^+]$ in the bathing solution, $[Na^+]_e$, was reduced to 50 % of the control level. This has been demonstrated to result in a net gain of cellular Ca^{2+} (Langer, 1965). The steady levels of $f_{\frac{1}{2}}$ and DF before, during and following a reduction of $[Na^+]_e$ are presented in Table 1. Note that a reduction in $[Na^+]_e$ caused an increase in diastolic $f_{\frac{1}{2}}$ and DF in the ensuing test beat and that these changes were reversed upon return to the control $[Na^+]_e$.

TABLE 1. The effect of $[Na^+]_e$ on intensity fluctuations in the diastolic period and force development in response to excitation

	$f_{\frac{1}{2}}$ (Hz)			DF (g/mm²)		
Expt. no.	Control 1 ($[Na^+]_e$ = 144 mM	Low $[Na^+]_e$† (72 mM)	Control 2 ($[Na^+]_e$ = 144 mM	Control 1 ($[Na^+]_e$ = 144 mM	Low $[Na^+]_e$ (72 mM)	Control 2 ($[Na^+]_e$ = 144 mM
23	< 1·0	1·99	1·0	1·36	4·39	1·04
31	2·18	4·55	1·72	1·68	5·95	1·72
32	1·66	3·06	2·04	0·30	4·12	0·25

In all solutions $[Ca^{2+}]_e$ was 4·0 mM and muscles were stimulated at 2 min^{-1}.
† 72 mM-sucrose replaced Na^+.

Transient state measurements of intensity fluctuations

In a given $[Ca^{2+}]_e$ the capacity to develop force in cardiac muscle varies with the rate and pattern of stimulation, and when abrupt changes are made in the rate of stimulation a staircase that occurs in DF reflects a staircase in inotropic state (Niedergerke, 1956*b*; Wood, Heppner & Weidmann, 1969; Allen *et al.* 1976). Experiments were undertaken to determine whether transients in DF were paralleled by transients in $f_{\frac{1}{2}}$. While the present methods precluded measurements of the ascending staircase which results from increasing the rate of stimulation, the transient that follows a change in stimulation frequencies from higher frequencies to those 2 min^{-1} or less could be examined. When regular stimulation of cat papillary muscle at 60 min^{-1} was abruptly stopped, an exponential decay in $f_{\frac{1}{2}}$ was observed, and the time course of this decay varied directly with the $[Ca^{2+}]_e$ (Fig. 12). Note that the steady level of $f_{\frac{1}{2}}$ was also dependent on $[Ca^{2+}]_e$, and that the steady-state levels in the higher $[Ca^{2+}]_e$ are comparable to those in the rat in $[Ca^{2+}]_e$ of 3·0 mM or less (Fig. 3*A*). In additional experiments the decay of $f_{\frac{1}{2}}$ was measured following periods of differing rates of stimulation while the muscle was bathed in constant $[Ca^{2+}]_e$ (Fig. 13). Note that the level of $f_{\frac{1}{2}}$ during the initial measurement period was greater for greater rates of stimulation, and that all the curves eventually converged to the same steady level (dependent upon $[Ca^{2+}]_e$). The dependence of the decay of $f_{\frac{1}{2}}$ on $[Ca^{2+}]_e$ and the rate of prior stimulation is similar to the decay characteristics of the DF following a period of regular stimulation (Allen *et al.* 1976). To establish the relationship of $f_{\frac{1}{2}}$ to DF during this decay period, in additional muscles a test beat was delivered after each measurement of $f_{\frac{1}{2}}$ during the decay period following stimulation at 60 min^{-1} in $[Ca^{2+}]_e$ of 4·0 mM (Fig. 14). Note that following regular stimulation, the decay in $f_{\frac{1}{2}}$ measured at 30 sec intervals (*A*) is similar to that of DF in a test beat (*B*); indeed, over the time course of the transient, $f_{\frac{1}{2}}$ and DF were linearly related (*C*). The slope of the linear function relating $f_{\frac{1}{2}}$ and DF during this transient state was essentially the same as that relating the two in the steady state across a range of $[Ca^{2+}]_e$ (Fig. 11*B*).

During the period following regular stimulation under conditions of excessive inotropic stimulation, a biphasic relationship between $f_{\frac{1}{2}}$ and DF could be demonstrated in cat muscles (Fig. 15). Panel *A* depicts the $f_{\frac{1}{2}}$ in cat muscle measured in the 30 sec interval following stimulation at different rates and DF measured in a test excitation after each measurment of $f_{\frac{1}{2}}$. Note that initially $f_{\frac{1}{2}}$ and DF increased in parallel as a function of the prior stimulation rate. However, following stimulation

at 60 and 96 min^{-1}, as $f_{\frac{1}{2}}$ continued its linear increase, DF reached a plateau and then declined. Fig. 15*B*, depicts the decay in $f_{\frac{1}{2}}$ and DF following regular stimulation in high $[Ca^{2+}]_e$. As $f_{\frac{1}{2}}$ progressively decreased, DF in a test excitation first increased (rest potentiation) and then, as $f_{\frac{1}{2}}$ approached 4 Hz, decreased in parallel with $f_{\frac{1}{2}}$, thus establishing a biphasic relation between the two parameters.

In several cat muscles RF was examined at high sensitivity of the force-recording apparatus during the period immediately following cessation of stimulation (Fig. 16). Note that when stimulation was stopped (arrow indicates last stimulus) a transient elevation in RF typically occurred following completion of the last twitch. This transient in RF, previously referred to as an after-contraction (Feigl, 1967), has

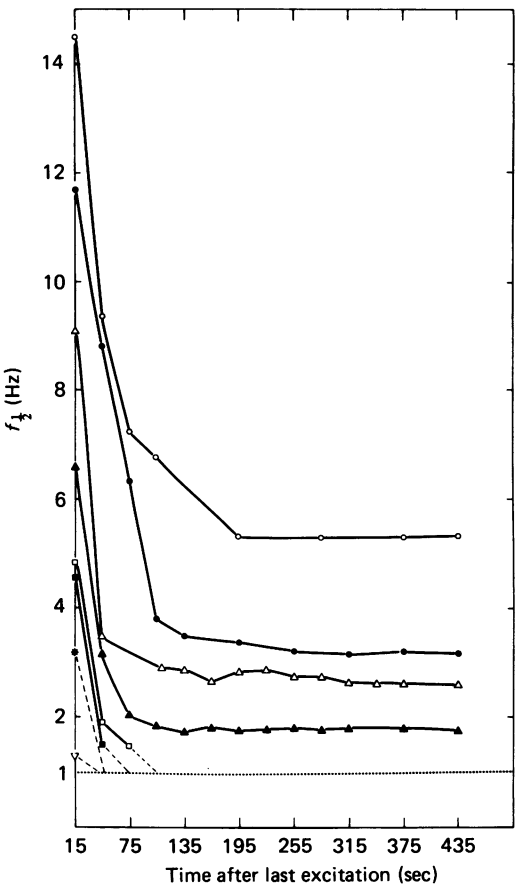

Fig. 12. A typical example of the change in $f_{\frac{1}{2}}$ with time following a period of regular stimulation in cat papillary muscle. The muscle was stimulated regularly at 60 min^{-1} in $[Ca^{2+}]_e$ of 2·0 (▽), 4·0 (*), 6·0 (■), 8·0 (□), 12·0, (▲), 24·0, (△), 32·0 (●) and 48·0 (○) mM. Stimulation was discontinued and after a 5 sec period $f_{\frac{1}{2}}$ was measured at regular intervals following the termination of stimulation. Each measurement was made over a time period of approximately 20 sec and each point in the Figure represents the mean time of the measurement period. At times following regular stimulation equal to or greater than those where extrapolated lines cross the dotted line, $f_{\frac{1}{2}}$ was less than 1 Hz (dotted line) and could not be accurately measured. In preliminary experiments it was determined by the addition of sucrose that $f_{\frac{1}{2}}$ was not altered by increases in osmolality to those levels that occurred with the addition of $[Ca^{2+}]_e$.

recently been attributed to a secondary release of Ca^{2+} within the cell (Fabiato & Fabiato, 1978*b*; Allen & Kurihara, 1980). Careful examination of the records in Fig. 16 during the period of regular stimulation suggests that these RF transients were initiated following each twitch but were obscured by the contraction in response to the subsequent excitation. Measurements of $f_{\frac{1}{2}}$ during the period following each after-contraction are indicated by the number to the right of the force tracing at each $[Ca^{2+}]_e$. Note that both the height and relaxation time of the after-concentration and

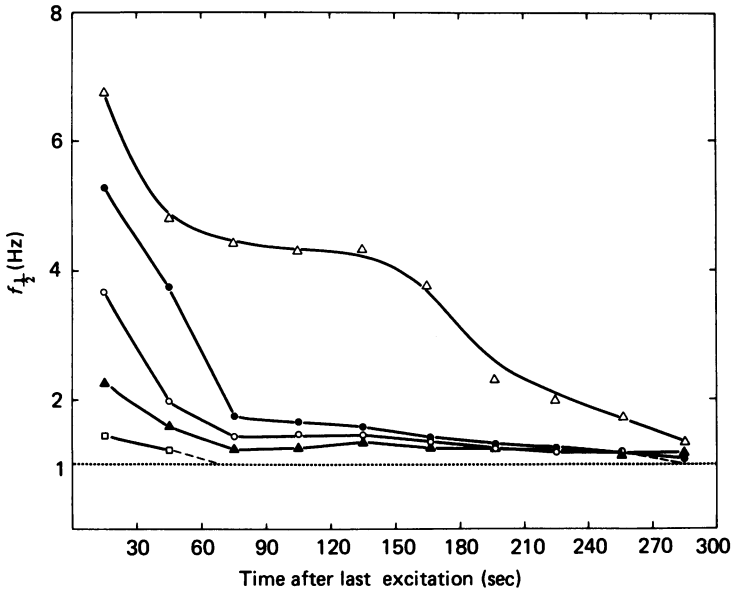

Fig. 13. A typical example of the effect of the rate of prior stimulation on the decay in $f_{\frac{1}{2}}$ that follows termination of that stimulation in a cat papillary muscle. Stimulation rate was 12 (□), 24 (▲), 36, (○), 60, (●) and 96 (△) min^{-1}; $[Ca^{2+}]_e$ was 4·0 mM.

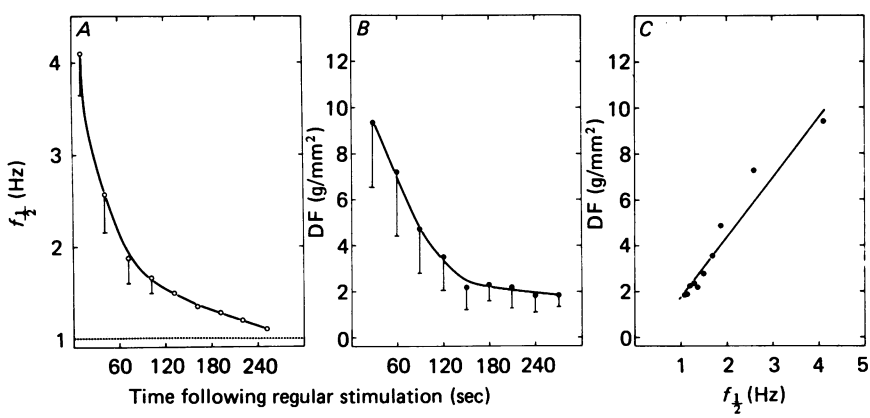

Fig. 14. The decay of $f_{\frac{1}{2}}$ (*A*) and DF in a test beat (*B*), following regular stimulation at 60 min^{-1} in $[Ca^{2+}]_e$ of 4·0 mM. Points represent mean ± S.E.M. of four muscles; cross-sectional area was 0·37 ± 0·16 mm^2. *C*, the decay in $f_{\frac{1}{2}}$ predicts that in DF. The co-ordinates were derived from the mean DF and $f_{\frac{1}{2}}$ in *A* and *B*. Over the time period of measurement, $DF = 2·7[f_{\frac{1}{2}}] - 1·0$, $r = 0·97$, $P < 0·001$. Three of the four muscles were those used in the protocol illustrated in Fig. 10.

$f_{\frac{1}{2}}$ vary in a stepwise manner with $[Ca^{2+}]_e$. In muscles in which $f_{\frac{1}{2}}$ following the after-contraction was relatively high (i.e. in high $[Ca^{2+}]_e$) the relaxation time of the after-contraction was markedly prolonged; in some instances RF continued to decay with time for several minutes before returning to the base line level (Fig. 17). When this occurred the decay in RF paralleled the decay in $f_{\frac{1}{2}}$.

In rat cardiac muscle, in contrast to that from the cat, $f_{\frac{1}{2}}$ did not decay from the initial level (first 30 sec measurement) following stimulation (Fig. 18*A*) but persisted into the steady state, the level being determined by the $[Ca^{2+}]_e$. Note that in the rat

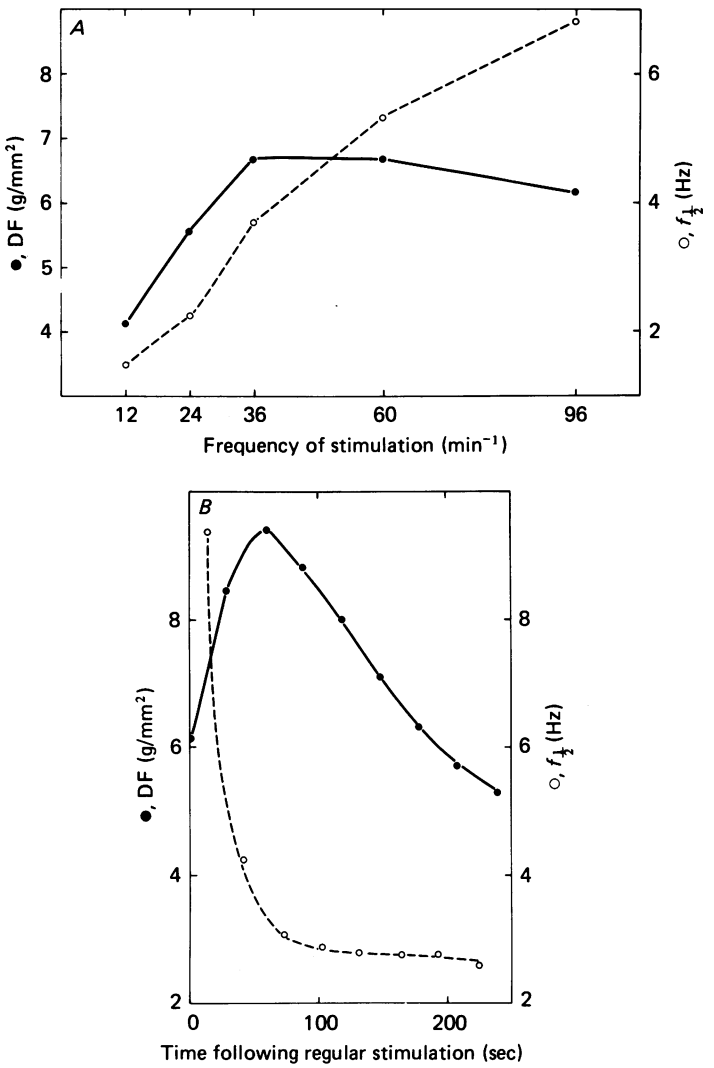

Fig. 15. The biphasic relationship of DF and $f_{\frac{1}{2}}$ in the transient state following regular stimulation in a cat muscle. *A*, in this example $f_{\frac{1}{2}}$ (○), was measured during the first 30 sec following stimulation at various frequencies in $[Ca^{2+}]_e$ of 4·0 mM, and DF, (●), was measured in response to a test excitation following measurement of $f_{\frac{1}{2}}$. *B*, $f_{\frac{1}{2}}$, (○), and DF in a subsequent test beat, (●), following regular stimulation at 60 min^{-1} in a $[Ca^{2+}]_e$ of 24·0 mM. DF at time zero is that recorded in the last beat during regular stimulation.

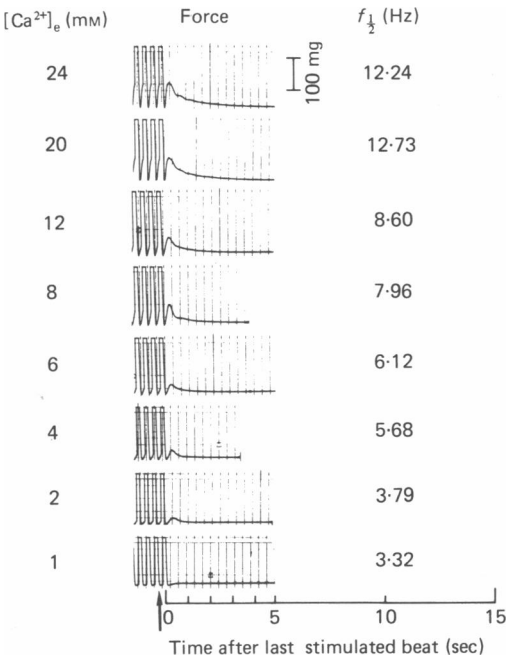

Fig. 16. Transients in RF (after-contractions) and $f_{\frac{1}{2}}$ following periods of regular stimulation in a typical cat muscle. Each panel represents an actual record of the force at high sensitivity of the force-recording apparatus. Arrow indicates termination of regular stimulation at 60 min^{-1} in a given $[Ca^{2+}]_e$. The number to right of each panel is $f_{\frac{1}{2}}$ measured over the 20 sec period following the transient in RF.

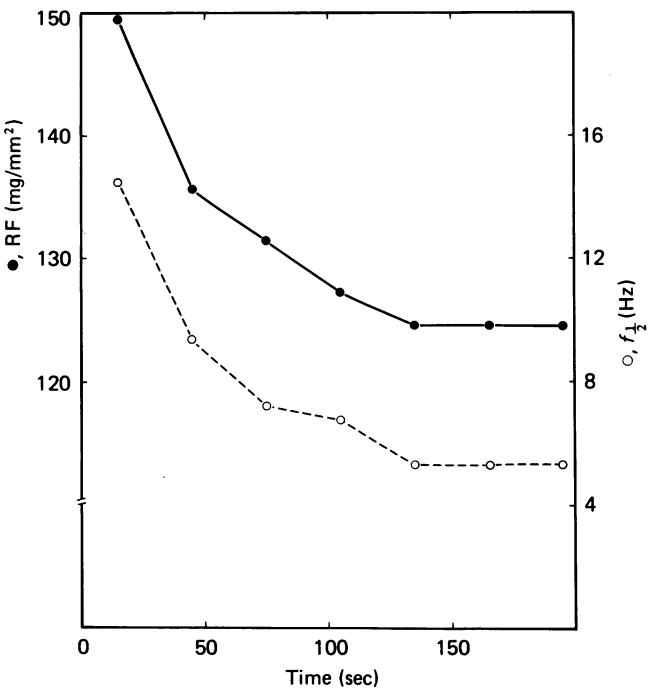

Fig. 17. The decay in $f_{\frac{1}{2}}$ (○), and RF (●), in a cat papillary muscle following regular stimulation at 60 min^{-1} in $[Ca^{2+}]_e$ of 48·0 mM. Cross-sectional area was 0·29 mm^2.

in $[Ca^{2+}]_e$ of 1·0–4·0 mM $f_{\frac{1}{2}}$ is comparable with those of the *initial* measurement following stimulation in the cat (Figs. 12 and 13). In the rat, DF in a test excitation did not decay from the initial measurement following a period of stimulation, and thus paralleled $f_{\frac{1}{2}}$ (Fig. 18*B*).

Intensity fluctuations and the sarcomere diffraction pattern

In many instances the sarcomere diffraction pattern and the intensity fluctuations were measured simultaneously and the effect of inotropic interventions on each was examined. In Fig. 19 photographs of the zero-order (bright light) and first-order (arrows) sarcomere diffraction patterns are presented with simultaneous measurements of $f_{\frac{1}{2}}$ and RF as a function of $[Ca^{2+}]_e$ in a typical 'resting' rat papillary muscle. Note that when $[Ca^{2+}]_e$ was increased from 0·4 to 4·0 mM the increase in $f_{\frac{1}{2}}$ and RF was accompanied by a small increase in the distance between the zero- and first-order

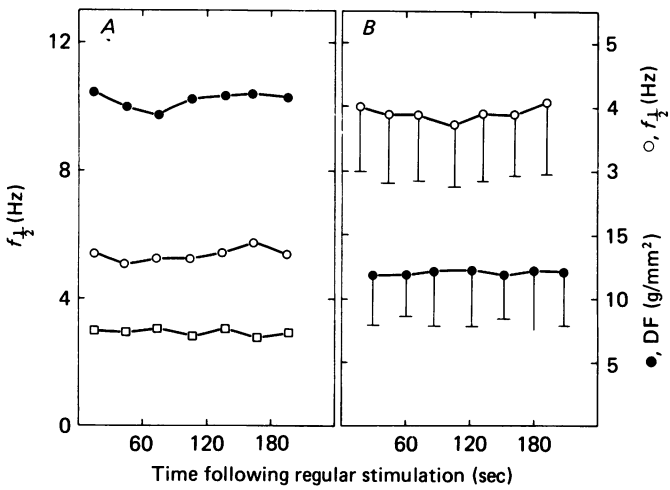

Fig. 18. *A*, $f_{\frac{1}{2}}$ following a period of regular stimulation at 60 min^{-1} in a $[Ca^{2+}]_e$ of 0·4 mM (□); 1·0 mM (○); and 4·0 mM (●), in a typical rat papillary muscle. *B*, measurement of $f_{\frac{1}{2}}$ (○), and DF in a test beat (●), during the period following stimulation at 60 min^{-1} in a $[Ca^{2+}]_e$ of 1·0 mM. Points represents the mean ± S.E.M. of three muscles; cross-sectional area was 0·17 ± 0·03 mm^2. During regular stimulation DF averaged 5·76 ± 2·24 g/mm^2, which was significantly less than that in the first beat (30 sec) following regular stimulation.

diffraction bands, suggesting a decrease in average sarcomere length (Krueger & Pollack, 1975). Note, however, that as $[Ca^{2+}]_e$ increased further, the first order of diffraction faded and disappeared; thus average sarcomere length could no longer be estimated. This fading of the first-order diffraction band, which suggests inhomogeneity of the striation pattern within the muscle, was clearly evident in $[Ca^{2+}]_e$ of 2·0 and 4·0 mM when the diffraction patterns (first and second photographs) are compared with that of 0·4 mM $[Ca^{2+}]_e$. Maximum DF (5·81 g/mm^2) occurred at $[Ca^{2+}]_e$ of 4·0 mM; further increases in $[Ca^{2+}]_e$ were accompanied by a progressive decline in DF (1·85 g/mm^2 in $[Ca^{2+}]$ of 24 mM). Between each measurement in differing values of $[Ca^{2+}]_e$, the preparation was perfused with $[Ca^{2+}]_e$ of 0·4 mM, at which time $f_{\frac{1}{2}}$ and RF decreased, DF increased, and the first-order diffraction pattern 'reappeared' (e.g. photograph on the extreme right of the Figure). The same phenomenon – gradual

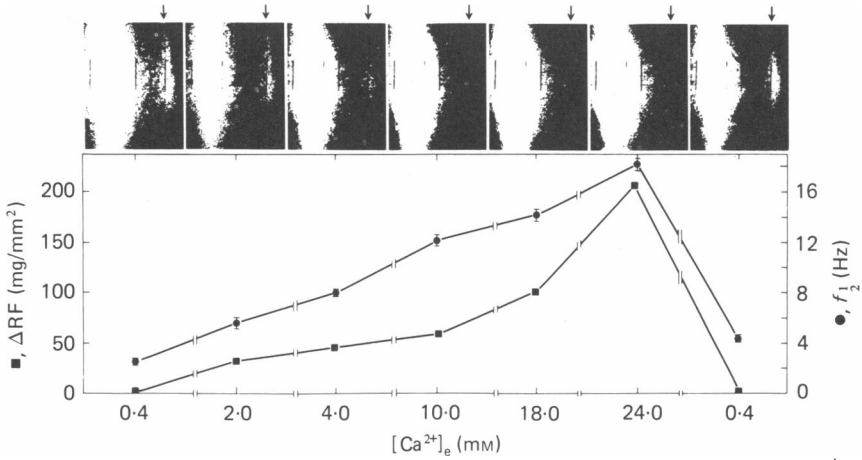

Fig. 19. A typical example of the sarcomere diffraction pattern (top panels), $f_{\frac{1}{2}}$ (○), and RF (■), measured over a wide range of $[Ca^{2+}]_e$ (Hepes buffer) in a rat papillary muscle. Photographs of the diffraction pattern were made in the same area of the muscle at the same camera setting in each $[Ca^{2+}]_e$. Arrow indicates the first order of diffraction. ΔRF is the difference between RF in a given $[Ca^{2+}]_e$ and that in a reference control in $[Ca^{2+}]_e$ of 0·4 mM. Cross-sectional area was 0.24 mm².

progressive fading and eventual disappearance of the diffraction pattern while $f_{\frac{1}{2}}$ increased – was also observed in other muscles in response to toxic concentrations of ouabain (Fig. 8).

DISCUSSION

The present results indicate that light-scattering properties of cardiac muscle during the diastolic interval are not constant but vary in response to many interventions known to enhance Ca^{2+} flux into the cell. In addition, changes in light-scattering properties in response to most of these inotropic interventions are accompanied by small but measurable changes in diastolic or resting force. It has already been demonstrated that intensity fluctuations in cardiac muscle made hyperpermeable by 'chemical skinning' vary directly with Ca^{2+}-dependent force and it appears likely in that preparation that the frequency of intensity fluctuations ($f_{\frac{1}{2}}$) varies directly with the extent of Ca^{2+}-myofilament interaction (Lappé & Lakatta, 1980). The Ca^{2+}-dependence of both $f_{\frac{1}{2}}$ and RF in the present study suggests to us that Ca^{2+}-dependent interaction of the myofilaments is present during diastole in cardiac muscle with *intact* sarcolemma as well, and that $f_{\frac{1}{2}}$ indicates the extent of this interaction. This concept is supported by the demonstration that the function describing the $f_{\frac{1}{2}}$–RF relation in muscles in which the sarcolemma was made hyperpermeable to Ca^{2+} by KCl depolarization was the same as that which described the two parameters when $[Ca^{2+}]_e$ was varied in the muscles with the sarcolemma intact (Fig. 6). In response to inotropic interventions in which the affinity of the contractile proteins for Ca^{2+} is unaltered, i.e. variation in $[Ca^{2+}]_e$, an increase in $f_{\frac{1}{2}}$ and RF implies an increase in the *average* $[Ca^{2+}]$ in the myoplasmic compartment over the period in which the measurements were made. A similar case could be made for the increase in $f_{\frac{1}{2}}$ in response to ouabain, and reduction of $[Na^+]_e$. Since these

interventions have a dramatic effect on the potentiation of DF it becomes important to question whether the Ca^{2+}-dependent change in $f_{\frac{1}{2}}$ might in some way be related to the coupling of excitation to contraction, i.e. the regulation of the inotropic state in cardiac muscle.

The present results indicate that over the extremes of an inotropic intervention such as a change in $[Ca^{2+}]_e$, an optimum $f_{\frac{1}{2}}$ is associated with maximum potentiation of DF, i.e. the relationship of DF to the diastolic parameters of $f_{\frac{1}{2}}$ and RF is biphasic. Over a range of $[Ca^{2+}]_e$ up to and including that at which DF is maximally potentiated, the increase in $f_{\frac{1}{2}}$ measured in diastole, i.e. before excitation, predicted the extent of twitch potentiation with a high degree of accuracy. There are several plausible mechanisms that may explain this relationship. First, diastolic myoplasmic $[Ca^{2+}]$ could reflect the extent of Ca^{2+} loading of other intracellular compartments, i.e. sarcoplasmic reticulum, from which Ca^{2+} is released following excitation (Wood *et al.* 1969; Allen *et al.* 1976). In this case, $f_{\frac{1}{2}}$ need not be directly involved in modulation of the inotropic state but may be a diastolic indicator of the extent to which these other compartment(s) are Ca^{2+}-loaded. It is noteworthy, however, that $f_{\frac{1}{2}}$ did not increase but rather decreased when an increase in Ca^{2+} loading of the sarcoplasmic reticulum would be anticipated during the decay of the KCl transient (Chapman, 1979).

Changes in diastolic myoplasmic $[Ca^{2+}]$ would be expected to result in changes in Ca^{2+} bound to high-affinity Ca^{2+} binding sites on the contractile proteins (Potter & Gergely, 1975) in the diastolic period. Changes in the extent to which these sites are 'primed' with Ca^{2+} before excitation might alter the response of the contractile proteins to additional Ca^{2+} released into the myoplasm subsequent to excitation. This represents a second possible mechanism through which a change in diastolic properties could relate to a change in DF.

A third possible mechanism relating an increase in $f_{\frac{1}{2}}$ to an increase in DF is Ca^{2+}-triggered release from the sarcoplasmic reticulum. Evidence for this is derived from studies in mechanically skinned cardiac cells in which the amount of Ca^{2+} release for a given extent of Ca^{2+} loading of the sarcoplasmic reticulum varied both with concentration of the 'trigger $[Ca^{2+}]$' and the rate at which this $[Ca^{2+}]$ was achieved (Fabiato & Fabiato, 1975*a*, 1978*a*, 1979). In intact muscle, diastolic myoplasmic $[Ca^{2+}]$ might possibly represent a component of the trigger for Ca^{2+} release. Additional Ca^{2+} entering the myoplasm via the slow inward current during excitation and Ca^{2+} already present in the myoplasm during diastole could sum to constitute the trigger for Ca^{2+} release from the storage site. This would be an efficient mechanism for the modulation of inotropic state in cardiac muscle in that relatively small changes in diastolic myoplasmic $[Ca^{2+}]$ (a fraction of 1 % of the Ca^{2+} required to activate fully the myofilaments: Solaro *et al.* 1974) could result in large changes in the amount of Ca^{2+} released into the myoplasm subsequent to excitation. This model does not preclude changes in Ca^{2+} loading of an intracellular release site by interventions such as a change in $[Ca^{2+}]_e$, but such would not be required to explain the observed inotropic effects. It is noteworthy that changes in $[Ca^{2+}]_e$ or perfusion with trivalent cations (Sanborn & Langer, 1970; Philipson & Langer, 1979), both of which serve to alter the $[Ca^{2+}]$ bound to sarcolemmal receptors (Bers & Langer, 1979), could produce the observed rapid staircase in twitch force solely by modulation of either

or both components of the trigger for Ca^{2+} release, thus obviating a model in which this site is localized at the sarcolemma (Langer, 1978).

The decline in DF that is associated with supra-optimal inotropic stimulation reflects what previously has been referred to as 'negative aspects of activation' (Koch-Weser & Blinks, 1963). There are several known mechanisms through which higher levels of diastolic myoplasmic [Ca^{2+}] or cellular 'Ca^{2+} overload' might lead to a decline in DF, which include negative effects on: sarcolemmal slow inward current (Niedergerke & Orkand, 1966; Boyett & Jewell, 1978); intercellular electrical communication (De Mello, 1975); energy production by mitochondria (Chance, 1965); and Ca^{2+} release from the sarcoplasmic reticulum (Fabiato & Fabiato, 1974*a*). The present results indicate that altered diastolic sarcomere spatial relations, as evidenced by the fading of the first-order diffraction pattern, occur when diastolic myoplasmic [Ca^{2+}] reaches excessive levels (Fig. 19). This must be considered as an additional possible mechanism effecting a reduction in DF at excessive levels of inotropic stimulation in papillary muscle preparations.

Like a change in $[Ca^{2+}]_e$, variation of the interstimulus interval is associated with a change in the extent of Ca^{2+} release into the myoplasm with excitation and results in changes in DF (Allen & Blinks, 1978). Following a train of beating at 60 min^{-1} (Fig. 14), $f_{\frac{1}{2}}$ and DF decayed monotonically and the decay in DF response to a test excitation was proportional to the level to which $f_{\frac{1}{2}}$ decayed before that excitation. Assuming that the affinity of contractile proteins for Ca^{2+} is unaltered with changes in the rate or pattern of stimulation, this result suggests that a change in myoplasmic Ca^{2+} in the interstimulus interval is in itself a factor, or varies directly with other factors that determine the 'Bowditch phenomenon' or 'memory' (Johnson, 1979) of this type of muscle. It is noteworthy that in the first few seconds following regular stimulation in canine ventricular muscle *in vivo*, a decay in the X-ray diffraction pattern was observed and has been interpreted as indicating that a fraction of cross-bridges do not disengage immediately upon cessation of the last twitch but rather decay with time (Matsubara, 1979). In addition, the extent of this residual cross-bridge interaction varied directly with the rate and pattern of previous stimulation (Matsubara, Yagi & Endoh, 1978, 1979; Matsubara, 1979).

In the present study, following the cessation of more intense inotropic stimulation that resulted in relatively high $f_{\frac{1}{2}}$ in cat muscle, DF exhibited a biphasic transient, i.e. rest potentiation followed by rest decay, while $f_{\frac{1}{2}}$ decayed monotonically. Potentiation of DF with an increase in the interstimulus interval, i.e. the 'Woodworth effect' (Koch-Weser & Blinks, 1963), may in part be a manifestation of the biphasic relation of $f_{\frac{1}{2}}$ to DF that could be demonstrated in the steady state in high $[Ca^{2+}]_e$ even at low frequencies of stimulation.

The extent to which each of the studied species exhibited a 'Woodworth' or 'Bowditch' effect could be predicted on the basis of the species difference in $f_{\frac{1}{2}}$ following an increase in the interstimulus interval. In cat muscle, rest potentiation in DF was lost as $f_{\frac{1}{2}}$ decayed, and this muscle subsequently exhibited a 'rest decay' in DF. In rat muscle, rest potentiation in DF (Fig. 18) persisted into the steady state and was accompanied by the persistence rather than a decay of $f_{\frac{1}{2}}$. Thus, in the steady state at long interstimulus intervals, $f_{\frac{1}{2}}$ could easily be detected in rat under conditions in which it could not be detected in cat (unless $[Ca^{2+}]_e$ was raised to extremely high

levels or $[Na^+]_e$ was reduced). Since the affinity of the myofilaments for Ca^{2+} appears to be identical in the rat and cat (Fabiato & Fabiato, 1978*b*), the marked species difference in $f_{\frac{1}{2}}$ across a range of $[Ca^{2+}]_e$ in the steady state at low frequencies of stimulation (Fig. 5 *versus* Fig. 11) suggests that the regulation of diastolic myoplasmic $[Ca^{2+}]$ is markedly different in the two species, and that in a given set of conditions the average diastolic myoplasmic $[Ca^{2+}]$ is higher in the rat. This may explain several other differences noted between the two types of muscles, including a twofold increase in resting heat, marked K^+ efflux, and a narrow action potential in rat *versus* cat (Coraboeuf, Delahayes & Sjöstrand, 1969; Spear & Moore, 1971; Loiselle & Gibbs, 1979). The biphasic relationship of $f_{\frac{1}{2}}$ and DF, and the relatively high levels of $f_{\frac{1}{2}}$ in rat muscle stimulated at low frequencies and bathed in $[Ca^{2+}]_e$ of 2·5 mM may also explain why inotropic interventions at this $[Ca^{2+}]_e$ in rat muscle fail to potentiate or depress DF (Koch-Weser & Blinks, 1963; Dhalla & Braxton, 1968; Hadju, 1969; Gerstenblith *et al.* 1979). To observe potentiation by inotropic interventions in the rat it is first necessary to lower $[Ca^{2+}]_e$ or add agents that result in Ca^{2+} efflux from the cell (Forester & Mainwood, 1974; Gerstenblith *et al.* 1979; Sutko & Willerson, 1980).

Unlike the other inotropic interventions studied, there is some evidence to suggest that catecholamines and a change in resting muscle length alter the affinity of the contractile proteins for Ca^{2+}. While a close correlation between $f_{\frac{1}{2}}$ and DF was observed after increasing muscle length, the affinity of the contractile proteins for Ca^{2+} appears to be increased at longer lengths (Hibberd & Jewell, 1979) and the increase in $f_{\frac{1}{2}}$ with stretch, while not precluding an increase in diastolic $[Ca^{2+}]$, can only be interpreted as a change in Ca^{2+}–myofilament interaction. This may explain in part the length dependence of resting and activation heat in cardiac muscle (Gibbs, Mommaerts & Ricchiuti, 1967; Cooper, 1979; Coulson, Houser & Breisch, 1979).

Catecholamine superfusion was the only inotropic intervention employed in which $f_{\frac{1}{2}}$ did not increase when DF was potentiated. This might readily be explained on the basis of two specific actions of catecholamines that have not been demonstrated for the other inotropic interventions examined: (1) cAMP-dependent phosphorylation of sarcoplasmic reticulum (Fabiato & Fabiato, 1975*b*; Katz, 1979) leads to enhanced Ca^{2+} accumulation by this organelle, an effect which would be expected to minimize the Ca^{2+} loading of the myoplasmic compartment in diastole in the presence of increased Ca^{2+} flux into the cell; (2) cAMP-dependent phosphorylation of troponin causes an apparent decrease in the affinity for Ca^{2+} (McClelland & Winegrad, 1978) and would tend to minimize an increase in $f_{\frac{1}{2}}$ for a given increase in myoplasmic$[Ca^{2+}]$. Thus, no conclusion regarding the effect of catecholamines on diastolic myoplasmic $[Ca^{2+}]$ can be made on the basis of the present results.

The failure of $f_{\frac{1}{2}}$ to increase during potentiation of DF by catecholamines or during the decline of the K^+ contracture suggests that Ca^{2+} loading of the sarcoplasmic reticulum does not of itself increase $f_{\frac{1}{2}}$, and that increases in $f_{\frac{1}{2}}$ with inotropic interventions therefore cannot be explained by changes in the optical properties of the sarcoplasmic reticulum itself. That the fluctuations require the presence of Ca^{2+}, vary directly with Ca^{2+}-dependent force, and affect the sarcomere diffraction pattern (Fig. 19) suggests that they reflect, at least in part, a Ca^{2+}-dependent myofilament interaction. Two types of Ca^{2+}-dependent sarcomere oscillations have been demon-

strated in mechanically skinned cardiac cell fragments (Fabiato & Fabiato, 1978*a*) and sarcomere oscillations have been observed in unstimulated trabeculae and papillary muscles when Ca^{2+} was present in the bathing fluid. While Ca^{2+}-dependent motion of the myofilaments is one possible source of the scattered-light fluctuations, Ca^{2+}-dependent motion or changes in polarizability of other structures within the muscle may also occur, and therefore the precise physical cause of the intensity fluctuations cannot be determined from the present results.

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