Contraction and Relaxation of Isolated Cardiac Myocytes of the Frog Under Varying Mechanical Loads

Sanjay S. Parikh, Sha-zhou Zou, and Leslie Tung

The mechanics of cardiac systole and relaxation have been studied primarily at the level of the whole heart or intact muscle. End-systolic pressure–volume relations of frog hearts have been found to be load dependent, whereas those of the mammal are relatively load independent. On the other hand, myocardial relaxation as studied at the muscle level is load independent in the frog but markedly load dependent in the mammal. Interpretation of these studies is complicated because of the unknown contribution of extracellular connective tissue, neurohumoral factors, and, in the case of the heart, the complex chamber geometry. Therefore, it is valuable to study cardiac mechanics at the level of the basic unit of contractile activity—the isolated myocyte. The goal of this study was to subject isolated frog cardiomyocytes to mechanical loading paradigms that mimic those presented to the cells within the heart. In the first part of this study, the afterload and preload of contracting cells were varied to study their effects on the end-systolic force–length relation, which was consistently found to be load independent over the range of isotonic shortening tested (typically 5%). We also investigated the force–length–time response of the cells to test the concept of the heart behaving as a time-varying elastance. Our results suggest that in this regard the frog myocyte behaves like mammalian muscle, and they are consistent with the presence of a small viscosity within the cell. We conclude that the tissue structure of the frog heart may contribute to disparity in mechanical behavior at the different structural levels. In the second part of this study, we subjected isolated frog cardiomyocytes to four different loading paradigms to test the hypothesis that myocardial relaxation in the frog is independent of load. These sequences consisted of afterloaded contractions followed by conventional isotonic–isometric relaxation (ACCR) or afterloaded contractions followed by physiologically reversed isometric–isometric relaxation (ACPR). Relaxation was measured under isometric conditions using a variable afterload with either the ACCR or ACPR paradigms. The decay of force was independent of the cell length at which it occurred or the amount of shortening prior to it within the contractile cycle. Relaxation also was measured as relengthening of the cell under isotonic late-load conditions, using the ACPR paradigm either with a variable afterload or variable late load. Relengthening had a time course that was unaffected by changes in afterload (i.e., extents of shortening) or late load (equivalent to the filling pressure for the heart). Our results suggest that, within a 5% range of shortening from resting length, relaxation of frog cardiomyocytes (whether measured as a decay in isometric force with time or as isotonic relengthening of the cell) is independent of load and consequently may be rate-limited by Ca\(^{2+}\) extrusion across the plasma membrane. (Circulation Research 1993;72:297–311)

KEY WORDS • cardiac cell • myocardial contraction • muscle contraction • biomechanics • muscle relaxation

Cardiac mechanics have been extensively studied at the levels of the whole heart and intact muscle strip. As with any other mechanical pump, the performance of the heart can be assessed by quantifying the effects of load on its contractile behavior. In this study we have considered separately the two aspects of cardiac contraction: systole and relaxation.

From the Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, Md.
Supported by National Institutes of Health grant HL-40422 (L.T.).
Address for correspondence: Leslie Tung, PhD, Department of Biomedical Engineering, The Johns Hopkins University, 720 Rutland Avenue, Baltimore, MD 21205.
Received August 19, 1991; accepted October 8, 1992.

The effects of load on cardiac systolic behavior have been quantified by the use of the end-systolic pressure–volume relation (ESPVR). Although Frank and Reichel2 found a marked dependence of the ESPVR of frog hearts on loading history, subsequent mechanical studies of canine hearts by Suga and Sagawa3 suggested that the ESPVR of the mammalian heart was load independent (to a first approximation). This observation has resulted in its use as a clinical index of cardiac contractility and the modeling of the contractile apparatus of the heart as a time-varying elastance.3,4 The heart, however, has a complex three-dimensional geometry, which leads to ambiguities in interpretation of mechanical data. For example, it is difficult to establish to what extent chamber geometry or nonmuscle cells may play a role in the basic mechanical properties.

Mechanical studies of intact muscles and ventricular strips have greatly alleviated the problems associated
with the three-dimensional structure of the heart. Chamber pressure and volume can be geometrically transformed to the mechanical variables of force and length. Mammalian muscle experiments have produced results similar to those at the whole-heart level in terms of the load independence of systole and the end-systolic force–length relation (ESFLR). Analogous studies of systole on amphibian myocardium, however, have not been reported.

The effects of load on myocardial relaxation have been quantified largely in intact muscle preparations in frogs and mammals. The decay of isometric force late in the afterloaded contractions (with isotonic–isometric relaxation) for frog ventricular strips can be superimposed on the corresponding phase of an isotonic twitch, and the duration of the contractile event is unaltered. In contrast, afterloaded isotonic contractions of mammalian muscle are accelerated in their relaxation and abbreviated in duration when compared with an isometric twitch. This difference has been attributed to the contrasting Ca²⁺ transport processes in the two species. In the frog, the membrane transport processes are believed to be rate limiting. This allows the Ca²⁺ coming off from troponin to rebind, which leads to reformation of crossbridges to sustain the afterload imposed on the muscle. In mammalian myocardium, however, the Ca²⁺ coming off from troponin during relaxation is thought to be quickly taken up by the sarcoplasmic reticulum (SR). Since this inhibits further crossbridge development, the load-bearing capacity of the muscle is diminished. The external load then leads to a rapid relengthening of the muscle and a faster decay of isometric force.

While considering frog myocardial relaxation, it is important to note that, until now, the load dependence of relaxation in frog cardiac muscle has been studied only with the isotonic–isometric loading paradigm, which precludes the separation of the dependence of relaxation on the extent of shortening/lengthening from the dependence on changes in load. The extent of shortening is determined by the afterload level, which is also the external load on the muscle during lengthening. Reversing the loading sequence to the isotonic–isometric pattern allows separation of the two determinants of relaxation (extent of shortening and load during lengthening) referred to above. Although shortening is controlled by changing the afterload presented to the muscle, isotonic lengthening occurs at a late load level that can be changed independently. Also, since the cells in the ventricular wall predominantly undergo force decay during isovolumic pressure decline and lengthening during ventricular filling, the isotonic–isometric relaxation sequence serves as a reasonable approximation of the loading of muscle in situ. Under physiological conditions the cell spends more time at lengths less than its resting length, with relengthening occurring later in the course of a twitch, than does a cell undergoing the isotonic–isometric relaxation sequence. Since the active state of the muscle is greatly attenuated late in the twitch, the mediation of relaxation could become more sensitive to the external load. All these factors have been shown in mammalian muscle to affect the process of relaxation as well as steady-state levels of contractility. Similar studies of frog myocardium have not as yet been reported. Therefore, it would be useful to test for load dependence of frog myocardial relaxation under reversed isometric–isotonic conditions.

Studies at the Single Cell Level

Although the use of intact muscle preparations and ventricular strips have simplified the analysis of cardiac contractile behavior, during both systole and relaxation, it is important to note that cardiac muscle strips have a well-developed extracellular connective tissue matrix. It is conceivable that viscoelastic passive properties of this matrix along with those of other noncontractile cell types are manifested in the force–length response of the muscle and obscure the mechanical properties of the myocardial contractile machinery. Therefore, it is valuable to study mechanics of cardiac contraction at the level of the basic unit of contractile activity—the isolated cardiac myocyte.

Previous mechanical studies of isolated cardiomyocytes have been limited largely to measurements of passive properties, unloaded shortening, and, in a few cases, isometric force. The active force development at different preloads and the effects of external force on relaxation kinetics have been studied previously in single frog atrial cells. However, these experiments were done under auxotonic conditions with simultaneous changes in force and length. This makes it difficult to separate the effects of changing load from those due to changes in length and extent of shortening. Until now, no studies have succeeded in measuring afterloaded contractions in isolated cardiomyocytes either with conventional or reversed (physiological) relaxation sequences.

We performed mechanical studies on isolated cardiac myocytes from the frog as a first attempt to examine the underlying cellular basis for whole-heart behavior. Previously, we have reported changes in active force development under isometric conditions due to changes in preload. In this study, we subjected myocytes to afterloaded (isometric–isotonic) contractions with a conventional (isotonic–isometric) relaxation sequence (ACCR) or a physiological (isometric–isometric or isometric–auxotonic) relaxation sequence (ACP). Afterloads and preloads were varied to mimic studies in whole heart. The results were examined in the force–length plane to determine whether the load-dependent properties of the ESPVR at the whole-heart level are reproducible for the ESFLR at the cellular level. We also reevaluated the concept of the heart behaving as a time-varying elastance from a cellular perspective.

Furthermore, the goal of this study was to explore the effects of load on frog myocyte relaxation under different loading paradigms. We subjected isolated frog myocytes to ACCRs with a variable afterload to test whether similar studies at the muscle level reflect cellular properties. The cells were also subjected to ACPRs to test for load dependence under these conditions. The afterload was varied to characterize the effects of extent of shortening/lengthening on relaxation. Finally, the effects of the late load on relaxation during isotonic lengthening were characterized.

Materials and Methods

Experimental Setup

Cardiac myocytes were isolated enzymatically (with trypsin and collagenase) from the frog (Rana pipiens) by

Downloaded from http://circres.ahajournals.org/ by guest on April 20, 2017
Langendorff retrograde perfusion. All experiments were performed on these myocytes in a 1 mM Ca\(^{2+}\) Ringer's solution containing (mM) NaCl 110, KCl 3, and HEPES 10, pH 7.2–7.4 using NaOH, along with 10 mM glucose and 0.1% bovine albumin (A-2153, Sigma Chemical Co., St. Louis, Mo.) at room temperature (23–25°C). The albumin served to improve the cells' tolerance to mechanical manipulations. The method of picking up the myocyte and mounting it onto the force transducer system is described in earlier reports. In brief, the ends of the myocyte (typically 250–400 \(\mu\)m in length and 5–10 \(\mu\)m in diameter) are drawn into the tips of two glass micropipettes by use of mouth suction (other methods using poly-l-lysine or fibrin as glues also would be appropriate). The cell is then mounted onto the tip of a glass optical-fiber force probe in an inverted U configuration (sectional view, Figure 1). A limitation of this approach is that sarcomere length cannot be measured directly. However, with the cells untethered and slack, typical sarcomere lengths are \(\approx 2.14 \, \mu\)m. A pair of platinum fibers connected to a rectangular pulse stimulator (model SD9, Grass Instrument Co., Quincy, Mass.) allows field stimulation in the chamber through which test solutions can be perfused (for all experiments reported in this study, the cells were stimulated at a frequency of 0.2 or 0.4 Hz). Cell twitch force elicited by stimulation causes bending of the force probe. Light is transmitted through the force probe and collected by a receiving optical fiber that is mounted to form an optic slit (top view, Figure 1) and whose output is proportional to the area of overlap between the two fiber ends. The light signal is therefore a measure of the differential bending between the two fiber tips.

Cell shortening can be achieved by moving the pipettes upward or the force probe downward. We have implemented the latter approach as described previously. In brief, each of the transmitting and receiving fibers is attached by dental impression compound (type 1, Kerr, Romulus, Mich.) to a tungsten wire (0.005-in. diameter), which is attached in turn with epoxy (Araldite, CIBA-GEIGY Plastics, Cambridge, England) to a piezoelectric bimorph element (part PZT-5H, Veritron, Bedford, Ohio). This arrangement forms an in-line system that can be laid within a trough intersecting the experimental chamber, thereby allowing the PZT-5H element to perturb the position of the force probe while remaining out of the solution (top view, Figure 1). However, to measure cell force during changes in cell length it is necessary to move both optical fibers equally, thereby moving the entire frame of reference. This is accomplished by finely adjusting the gains to the two bimorphs. In the absence of any cell mounted, the movements of the two fibers track one another to within 0.6% of their individual movements for any driving voltage. Therefore, we are able to continuously estimate cell force (by optical signal) and cell length (by piezoelectric bimorph voltage and correction for fiber bending). Either of these signals can then be clamped to a command level using a high-gain feedback control loop.

The force and displacement signals of the force probe were pulse code–modulated by a digital audio processor (model PCM-501 ES, Sony Corp., Japan) and recorded continuously on a videocassette recorder (model SL-HF840D, Sony) with a DC to 22-kHz bandwidth. Force-length loops were observed on-line using a digital storage oscilloscope (model 3091, Nicolet Instrument Corp., Madison, Wis.) in the X-Y mode. The recorded signals were digitized off-line at a sampling rate of 1,000 Hz and a 12-bit resolution for analysis (Lab-NB data acquisition board and LabVIEW software, National Instruments, Austin, Tex.; Macintosh Iic, Apple, Cupertino, Calif.). The digitized signals were then converted into actual units of length (microns) and force (nanonewtons), given the measured mechanical properties of the fiber cantilever. A 10-msec moving averager (zero delay) was applied to the force tracings to filter out high-frequency noise. Finally, the force \((F)\) and displacement \((x)\) of the force probe were converted into cell force \((F_c)\) and cell length \((x_c)\) by the following relations:

**Figure 1.** Diagram of the experimental chamber. Two multimode glass optical fibers (a) make up the force probe and its accompanying displacement sensor and are placed in a trough intersecting the experimental chamber. Light (thin arrow) is transmitted across the splice formed by the two fibers. Each fiber can be displaced vertically by a piezoelectric bimorph element (b) and is supported by a tungsten wire (c), which penetrates the airfluid interface at the chamber. The walls of the chamber (d) are constructed from Plexiglas, and the floor is a 25-mm square glass coverslip (e). Hypodermic tubing (f) injects and withdraws test solutions from the chamber (bold arrows). Glass pipettes (g) hold the ends of the cell (i) by suction and are moved by motorized micromanipulators (not shown). The cardiac cell is excited by field stimulation via a pair of platinum black wire electrodes (h). The sectional view shown below is taken through the center of the chamber. The two views are not drawn precisely to scale.
Figure 2. Tracings showing different phases of a physiological contraction. Panel a: Force and length tracings of a frog cardiomyocyte undergoing an afterloaded contraction with physiological relaxation and an isometric contraction at the same resting length. The different phases of the contraction are labeled as follows: phase 1, isometric contraction at the rest length (length clamp control); phase 2, isotonic shortening at the preset afterload level, held to end systole (force clamp control); phase 3, isometric relaxation at the end-systolic length (length clamp control); phase 4, isotonic relengthening at the late-load level until the end of relaxation (force clamp control); and phase 5, auxotonic restretch to the initial resting length along the passive force–length relation using a slow exponentially decaying length command (length clamp control). Force is expressed as a fraction of the peak total cell force for the isometric twitch (116 nN) at the same initial resting length (384 μm). Panel b: Contraction from panel a in the force–length plane. The arrow marks the end-systolic point.

\[ F_c = (2 \sin \theta/2)^{-1}F \]  
(1)

\[ x_c = (2 \sin \theta/2)x \]  
(2)

where \( \theta \) is the wrap angle and symmetry of the cell wrap around the force probe is assumed.22

By using this system, it is possible for us to subject the cell to different loading patterns over the course of a single twitch. In particular, we can impose afterloaded contractions with an ACCR or ACRP sequence. The different phases of the contraction will be defined later in Figure 2. However, the ACPR used in this study is nonphysiological in the sense that the afterload is constant rather than viscoelastic, as is characteristic of pressure–volume loops of the heart. We also note that the loading pattern experienced by cells in situ differs from that for the whole ventricle because of the complex three-dimensional nature of the tissue.26 Nevertheless, we have adopted this pattern in our experiments as a first approximation to the in situ conditions compared with the isometric or isotonic loading conditions.

Experimental Protocols

After being mounted onto the force transducer, the cells were made to contract isometrically at a fixed resting length (this length was always greater than slack length, as confirmed by the measurement of a passive force). Only those cells that developed a stable (within limits of beat-to-beat variability22,26) isometric level of force were used in this study. Some cells, upon repeated stimulation, showed a monotonic and rapid rise in isometric force until the force developed was sufficient to pull the cell out of one of the micropipettes within a few beats. Such cells always went into irreversible hypercontracture, perhaps because of a stress-induced rupture of the sarcolema. These cells were excluded from the study.

End-systolic force–length relation of the myocyte. Cells mounted on the force probe and contracting in a stable fashion were subjected to a series of variable ACPRs at a fixed resting length (preload). Isometric contractions were obtained by setting the afterload very high. The afterload was reduced in steps to a minimum value equal to the preload. With decreasing afterloads, the extent of systolic shortening increased. The cell’s resting length was then altered so that a new preload level was obtained, and a similar sequence of afterloaded contractions was measured. The number of contractions recorded for each preload/afterload combination was variable (from one to 10). This protocol was performed on five cells.

Relaxation of the myocyte. The effects of load on myocyte relaxation were characterized by subjecting cells to one or more of the following loading paradigms at a fixed preload.

Paradigm 1: ACCRs with varying afterload. This is the afterloaded contraction with conventional relaxation used in muscle experiments.9 The time course of force decay is studied as a function of afterload level. This protocol was performed on three cells.
PARADIGM 2: ACPRs with varying afterload and no late load. The time courses of isometric force decay at the end-systolic lengths for the variably afterloaded contractions with physiological relaxation are compared to study the effects of loading history and length on relaxation. A similar protocol has been used on mammalian muscle by other researchers.\(^8\) The rate of force decline was found to be related to the instantaneous level of force even though there was a separation in the force–time responses. This protocol was performed on three cells.

PARADIGM 3: ACPRs with varying afterload and fixed late load. The late load is the level at which isotonic relengthening for the reversed isometric–isotonic relaxation occurs and corresponds to the filling pressure of the heart during diastole. A similar protocol has been used on mammalian muscle.\(^8\) This protocol was performed on three cells.

PARADIGM 4: ACPRs with varying late load and fixed afterload. This protocol mimics that used on mammalian muscle\(^12,15\) and allows us to characterize the late-load dependence of cellular relaxation. In normal hearts the filling pressure (late load) is always less than or equal to the pressure at end diastole/preload.\(^12\) Hence, we studied relaxation only for these conditions. In paradigms 3 and 4, the parameter of interest is the rate of isotonic relengthening. This protocol was performed on two cells.

Results

Effects of Load on Systole

Figure 2a shows the force and length tracings for a typical ACPR of a cardiac myocyte of the frog. The different phases of the contraction are marked on the two tracings. Phase 1 is the isometric development of force at the resting length. When the isometric force exceeds a preset afterload level, the cell enters phase 2—the isotonic shortening to end systole. This point is determined by the instant at which shortening ceases and the cell begins to relengthen. Phase 3 is the isometric relaxation of force at the end-systolic length. When the cell force drops below a preset late-load level, the cell begins to relengthen isotonically (phase 4). Finally, in phase 5, the cell is restored to its resting/end-diastolic length along its passive length–tension relation under auxotonic loading conditions. This is accomplished under length control of the cell by providing an external exponential command signal. The ACPR sequence allows the process of relaxation to occur at lengths shorter than the resting length and is in contrast to conventional isotonic afterloaded contractions during which the isometric relaxation sequence occurs at the resting length. An isometric contraction at the resting length is also shown for comparison.

Figure 2b depicts the tracings from Figure 2a in the force–length plane. Plotting the contractions in this fashion allows us to compare results at the single-cell level with those at the whole-heart and intact-muscle level. Typical values for active force in isometric twitches lie between 25 and 150 nN, with cell-to-cell variability in the force level. For isotonic contractions at a given preload, cells may shorten from \(\approx 3\%\) to 12% of their resting length, which varies from 200 to 400 \(\mu\)m in the frog ventricle.

The end-systolic point (defined here as the point when force development or shortening reverses) for the contraction in Figure 2 is marked by an arrow. Determination of this point for variably afterloaded contractions has led to the concept of the ESFLR in the heart and, further, to its use as an index of cardiac contractility. To examine the systolic load dependence of cardiomyocyte contractility, it is necessary to determine the end-systolic points for the cellular contractions in the force–length plane.

Figure 3 shows a series of ACPRs for a cell starting from the same resting/end-diastolic length (preload fixed). The afterload presented to the myocyte is varied. Figure 3a shows the force and length tracings; Figure 3b shows the same data in the force–length plane. When the afterload is sufficiently high, the cell contracts isometrically. As the afterload is decreased, there is a greater amount of systolic shortening (corresponding to an increased stroke volume). Also shown is a contraction during which the cell force is held isotonic at its resting/preload level and shortening occurs. The end-systolic points for all these contractions are joined by the dashed line, which shows a slight upward curvature (Figure 3b). This corresponds to the ESFLR for the cell in this contractile state. An isometric contraction in the middle of the range of lengths spanned by the ACPRs is also shown, and the peak force lies only slightly above the ESFLR. The resting force/preload of the lower length isometric contraction is determined by the passive force–length relation and is lower in this case than the late-load level chosen for the other contractions at the higher preload.

It is important to note, however, that for a given load presented to the cell there is some beat-to-beat variability in the end-systolic point, as observed previously for isometric contractions of single cardiomyocytes.\(^22,29\) We characterized this variability over the course of 21 successive isotonic contractions with afterload equal to the preload level (to maximize the extent of shortening) in a cell holding preload constant. There was no systematic change in the extent of shortening from one beat to the next, suggesting the lack of any "short-term" memory in this preparation. The mean extent of shortening during phase 2 of the 21 contractions was 10.03% of the resting length (229 \(\mu\)m) with a standard deviation of 0.98%. Such beat-to-beat variability makes it difficult to determine unambiguously the ESFLR of a cell from a single contraction under each different loading condition. Therefore, the end-systolic points are plotted for all contractions measured. By doing so, we obtain a cluster of points in the force–length plane for each loading condition. This eliminates the potential for arbitrariness associated with the selection of a single contraction under a specific load to extract the cell's ESFLR.

Figure 4a shows a series of ACPRs for two different preloads (resting lengths). Here, phase 3 of the contractile cycle is allowed to proceed all the way to the end of relaxation (i.e., there is no phase 4). Phase 5 of the cycle occurs under passive conditions at an exponentially decaying strain rate. In this experiment, three contractions were recorded for each combination of preload and afterload. Although the end-systolic points for all the contractions in the series are plotted in the figure, only one force–length trajectory for each combination
of preload and afterload (dashed lines in Figure 4a) is shown for the sake of clarity. All the end-systolic points cluster around the same concave ESFLR, irrespective of preload or afterload. The force–length trajectories during phase 5 for the different contractions superimpose regardless of the extent of shortening and trace out the passive force–length relation.

Figures 4b and 4c illustrate the load independence of the ESFLR of two other frog myocytes. The end-systolic points for all contractions are plotted in the force–length plane. They include points from isometric and isotonic as well as afterloaded contractions. As in Figure 4a, we find that for each cell the clusters of end-systolic points lie around the same ESFLR, regardless of the type of load.

Force–length isochrons were obtained by joining points that occurred at the same instant after the start of the contractile cycle for contractions with the same preload but varying afterloads. Similar pressure–volume isochrons of the heart have been extracted\(^3,4\) and used to characterize the contractile state. We investigated the behavior of the force–length isochrons for a single frog myocyte under different loading patterns to compare cellular contractility with that of whole heart and muscle. First, we examined purely isometric contractions. Data were obtained for isometric twitches at different resting lengths. Figure 5a illustrates the active components of force in the force–length plane along with the corresponding isochrons. The time to peak appears to depend slightly on the resting length (in this case, time to peak contraction fell by \(\approx 15\%\) for a 9\% drop in resting length). Nevertheless, the isochrons still move in a fanlike fashion with time and share a common intercept on the length axis (89±0.16\% of highest resting length). Next, we examined the force–length isochrons for a series of twitches with a fixed preload and varying afterload (Figure 5b). The force represented in this figure is the total force, active plus passive, on the cell. Early in the contractile cycle, twitches with higher values of afterload are still in the isometric phase (shortening has not begun), leading, therefore, to overlapping points at the right ends of the isochrons. Later into the cycle, contractions enter into phase 2 (isotonic shortening), forming a series of isochrons that shift in a parallel fashion to the left, unlike those of Figure 5a. Note that the time from stimulus to end systole changes by less than 1\% for all the afterloaded contractions and is slightly delayed when compared with the isometric contraction. To compare our results with those of frog ventricular strips, we extracted force–length isochrons for variably afterloaded contractions from data published by Brutsaert et al.\(^9\) The results\(^8\) resemble those for the cell in Figure 5b.

Although cell force during the shortening phase of an afterloaded contraction is clamped to the afterload level, it does not reflect the true load presented to the contractile machinery of the myocyte. As the cell shortens, a portion of the afterload is transferred from the passive elements to the contractile apparatus (assuming that the two structures are in parallel\(^30,33\)). Therefore, the true load on the contractile apparatus increases with time by an amount equal to the drop in passive force from its resting level. The ESFLR for the contractile apparatus can be determined by subtracting the passive

---

**Figure 3.** Afterloaded contractions with physiological relaxation (ACPRs) with preload fixed and afterload varied. A series of ACPRs with variable afterload are shown in the force–length plane. Also shown are an isotonic contraction at the higher preload and an isometric contraction at a lower preload. Cell force is normalized to peak total isotropic force (116 nN) at the higher resting length (384 μm), to which cell length is normalized. Panel a: Time tracings for force (top tracings) and length (bottom tracings). Panel b: Same data in the force–length plane. For the loop contractions at the higher preload, the late-load level is lower than preload. The dashed line connects the end-systolic points (o) and represents the end-systolic force–length relation. An isometric twitch (darker tracing) at a lower resting length (373 μm) is also shown (●, peak force).
end of phase 3 (×) represent the passive force of the myocyte at shortened cell lengths. Panel c: ESFLR for another cell. Cell length has been normalized to the highest resting length measured (386 μm), and forces are normalized to the peak total isometric force (96 nN) at the highest rest length. The contractions measured in this cell were obtained at a stimulus frequency of 0.4 Hz. In all panels, the loading condition for each point are provided in the table inset. The upper line in each panel represents the best second-order polynomial fit to all the end-systolic points for that cell. The lower line through the crosses (×) represents the best exponential fit to points along each cell’s passive length–tension relation.

Effects of Load on Relaxation

Figure 6 shows force and length tracings for a cell contracting from the same resting length but with the ACCR paradigm. The duration of the contractile process is independent of the load against which shortening occurs, a result similar to that obtained using the same loading sequence in studies of ventricular strips from the frog.9 In addition, the decay of isometric force late in the contractile cycle is essentially superimposable, both in time and magnitude, on the corresponding phase of an isometric twitch at the same resting length. This result contrasts with that for mammalian muscle, in which the duration of the contractile cycle decreases and the rate of isometric force decay accelerates with decreasing afterload.9 A quantitative analysis of the load dependence of relaxation under this paradigm has been described by Sys et al.32 It is schematized in Figure 7A: The time during which the cell can bear a given load is compared for an isometric twitch (d) and an afterloaded twitch (c). The ratio c/d is plotted as a function of the afterload (normalized to the peak isometric active force, i.e., a/b). The more load dependent the muscle, the greater the disparity between c and d for afterloaded contractions. A c/d value of 1 represents load independence. The result is shown in Figure 7B. The ratio of c/d for the frog myocyte is very close to 1, indicating near load independence.

Relaxation was examined under the physiological paradigm (ACPR), in which relengthening occurs at a shorter cell length, at a lower load level, and later in the twitch cycle than in the contractions in Figure 6. Since relaxation consists of an isometric decay (phase 3) of force followed by an isotonic relengthening phase (phase 4), we explored the load dependences of both phases. In Figure 8 we show a series of ACPRs with variable afterloads: Here, phase 3 was allowed to proceed all the way to the end of relaxation (i.e., there was no phase 4). One contraction is isotonic at the resting length (tracing A), whereas the others (tracings B–D) occur with progressively lower values of afterload and
corresponding greater extents of shortening. The isometric decay of force during phase 3 for each contraction is normalized to its end-systolic active force level.

The resulting normalized tracings are superimposable to within 0.027 (normalized force) of the mean tracing. Figure 9 illustrates the effects of load on the relengthening process (phase 4) of the ACPR paradigm, in which the late load was held constant. Mammalian muscle subjected to ACPRs with varying afterload shows a decrease in duration of the contractile cycle with lower afterloads. Also, the peak isometric relaxation velocity is a function of the extent of lengthening, which is determined by the level of afterload at which systolic shortening occurs. This loading paradigm is represented in Figure 3. As afterload decreases, the extent of systolic shortening increases. We quantified the duration of contraction as a function of afterload by performing the analysis schematized in Figure 9a: The time between stimulus and onset of isotonic shortening (phase 4) was measured for each afterloaded contraction (k). In addition, we measured for each contraction the time after the stimulus at which the length tracing of the twitch with afterload equal to preload (i.e., when the cell shortens isotonically throughout systole) passes through the end-systolic length of the contraction in question (l). We plotted the ratio k/l versus the extent of systolic shortening for each contraction (Figure 9b). The extent of shortening (x) was normalized to the peak shortening recorded for the isotonic contraction at the same preload (y). Analogous to the analysis for afterloaded contractions with the isotonic–isometric relaxation sequence (Figure 7), load independence is repre-
presented by k/l values of 1. For the frog myocyte, the ratio k/l is close to 1 regardless of extent of shortening (x/y), suggesting that the duration of contraction is essentially independent of afterload (i.e., extent of shortening). We note, however, that the cell lengths during phase 4 are not exactly superimposable and that the time course of relengthening for the lowest level of afterload appears to be slightly abbreviated with respect to those for the higher afterloads. Thus, there may be some secondary dependence of the time course of reextension on the extent of shortening. To compare our results with those from mammalian muscle, we performed a similar analysis on data reported by Brutsaert and Sys.8 The results are shown in Figure 9c. The difference between mammalian results and those from the frog is striking. There is a sizable disparity between parameters k and l for each level of afterload in the case of mammalian myocardium, causing the ratio k/l to rise significantly as the extent of afterloaded shortening decreases.

Alternatively, it is possible to alter the late-load level while maintaining the extent of shortening (i.e., afterload) constant, thereby simulating variations in the filling pressure of the intact heart. This late-load level has been shown to be the major factor controlling load-mediated relaxation in mammalian muscle. Contractions of the same cell under constant preload and afterload conditions but varying late loads are shown in Figure 10a. The late load was lowered in steps from the preload value until phase 3 proceeded all the way to the end of relaxation, mimicking the protocol used in whole-muscle experiments.12 The cell relengthens isotonically during phase 4 until all contractile activity has ceased (phase 5; the auxotonic stretch back to initial resting length begins in this cell at 1,265 msec), and the load is borne solely by the passive structures associated with the cell. Therefore, the cell length at the end of phase 4 depends on the late-load level and is determined by the passive force–length relation. However, when the length tracings are shifted so as to equalize the final resting length (i.e., the passive length–tension relation is subtracted), they have similar time courses (Figure 10b). This shows that the time course of relengthening and the duration of the active process in the frog myocyte are independent of load level.

**Discussion**

Isolated cardiac myocytes have been used as a physiological model for many aspects of cardiac function,18 such as electrophysiology and biochemistry. We have found the isolated cardiac myocyte to be a viable preparation for the study of cardiac mechanics as well, allowing us the potential to probe myocardial behavior by using small tissue samples should whole hearts or muscles be unavailable. The isolated cardiac myocyte is devoid of the external connective tissue matrix that complicates the study of the basic myocardial contractile machinery in

---

**Figure 7.** Quantitative analysis of the load dependence of relaxation. Panel A: Schematic of analysis technique (similar to that used by Sys et al.12). For a given afterloaded contraction, the duration for which the cell can sustain the afterload is measured (c). The duration for an isometric twitch during which its load bearing capacity exceeds the afterload value is also measured for comparison (d). The afterload value (a) is normalized to the peak active isometric force (b) developed by the cell. Panel B: Plot of c/d vs. a/b for the experiment in Figure 6. A drop in the value of c/d at lower afterloads, as in mammalian myocardium,42 would indicate greater load dependence but is essentially absent in the frog cardiomyocyte.

**Figure 8.** Relaxation rates plotted under different loading histories and at different lengths. Tracing A shows the relaxation in force for the isometric twitch (inset). Tracings B–D show the relaxation at the end-systolic length for a series of variably afterloaded contractions with physiological relaxation that start from the same resting length. Afterload is decreased from tracing B to tracing D. Note that the switch from phase 2 to phase 3 in these contractions was controlled by a timer, leading to a slight discontinuity in the force tracing. Therefore, we normalized each tracing to the force level immediately after the discontinuity, which is slightly less than its end-systolic active force level, minus the level of passive force. The inset shows the contractions in the force–length plane. Cell force is normalized to peak active isometric force (93 nN) at the resting length (385 μm).
Figure 9. Quantitation of the effects of extent of shortening (afterload) on the duration of the contraction for afterloaded contractions with physiological relaxation. All contractions re-lengthen during phase 4 under the same late load. Panel a: Schematic diagram of the analysis technique. k, Time from stimulus to the onset of isotonic lengthening (phase 4) for a given contraction (as the afterload drops [extent of shortening increases], k decreases); l, time between stimulus and the instant at which the contraction in which afterload equals preload (lower tracing) re-lengthens to the end-systolic length of the contraction in question; x, extent of shortening for the afterloaded contraction being considered; y, extent of shortening for the twitch where afterload equals preload (i.e., maximum isotonic shortening for that preload). Data are from a single frog ventricular myocyte. Panel b: Graph of k/l vs. x/y for data such as in Figure 3, but for another frog ventricular myocyte where peak total isometric force was 85 nN at a resting length of 404 μm. The peak shortening of an isotonic twitch at the preload (y) was 5.8% of resting length. Panel c: Graph of k/l vs. x/y for a series of variably afterloaded mammalian muscle contractions with the physiological relaxation sequence. The values are extracted from data published by Brutsaert and Sys. Peak shortening for an isotonic twitch at the same preload (i.e., y) was 18.7% of resting length.

Figure 10. Effects of late load on re-lengthening during afterloaded contractions with physiological relaxation. Panel a: Force (top) and length (bottom) tracings for a cell with fixed preload and afterload but varying late load. The late load was reduced in steps from the preload value until phase 3 extended to the end of relaxation. Force tracings are normalized to peak total isometric force (45 nN) at the resting length (286 μm). Panel b: Expanded length tracings during the isotonic re-lengthening process. Tracings are shifted so as to superimpose final resting length.

Multicellular (whole-heart and intact-muscle) preparations. It is also geometrically simple and homogeneous, allowing a straightforward interpretation of the results. In addition, all the processes responsible for the exchange of ions with the environment and for excitation-contraction coupling are believed to be preserved. This study tested the systolic and relaxation behavior of isolated myocytes under different controlled loading patterns to reexamine, at the cellular level, concepts derived from the whole heart and intact muscle.

End-Diastolic Force-Length Relation

In the typical ACPR contraction, cell length recovers during phase 4 to a value that is determined by the
passive force–length relation. As the late load is decreased, the level of resting length during phase 4 is diminished below the starting preload value (Figure 10); phase 5 then restores length back to its starting length. In the limit of low late load, phase 3 continues all the way to the end of relaxation (i.e., there is no phase 4), and the force–length trajectory during phase 5 traces out the passive force–length relation of the cell (Figure 4). We find that the passive forces in these myocytes can be a significant fraction of the total cell force. This suggests that, although much of the surrounding connective tissue has been enzymatically removed, passive structures, either intracellular or membrane related, are capable of bearing substantial loads at the cellular level. Studies aimed at the identification of cellular structures responsible for high levels of resting force have found that the major component of resting stiffness of the myocyte is associated with intracellular longitudinal structures (e.g., connectin–titin filaments) that help to maintain the integrity of the A band.

**End-Systolic Force–Length Relation**

The data for single myocytes in the frog suggest that there is virtually no difference in ESFLRs for isometric, variably afterloaded, or variably preloaded contractions (Figures 3 and 4). This observation contradicts the observations of Frank and Reichel on whole amphibian hearts, in which the ESPVR for afterloaded and isotonic contractions from different preloads always lay to the right of the isovolumic pressure–volume relation. Although we observed some beat-to-beat variation in the end-systolic points for successive contractions, we found that the cluster of points in the force–length plane resulting from each afterloaded condition lay close to a common force–length relation (Figure 4), suggesting that the cellular ESFLR is essentially independent of afterload or preload. In this respect, frog myocardium is similar to that of the mammal. Although few frog muscle studies have been published with which we can compare our results, we would expect frog myocardial strips to show this behavior. It is possible then, that although the single myocyte demonstrates load/history independence in its ESFLR, the complex three-dimensional geometry of the heart along with its spongy and porous nature in the frog causes the ESPVR to be load dependent.

The ESFLR for these myocytes is frequently concave upward to the force axis (Figures 3 and 4), especially in the low range of lengths, much like that of intact mammalian papillary muscles. An upward concavity of the ESFLR was shown to be consistent with a linear ESPVR by geometrically relating single-fiber behavior to that of the ventricular chamber.

**Does the Cell Behave as a Time-Varying Elastance/Force Generator?**

The dynamic stiffness of isolated cardiac muscle and cardiac myocytes has been measured under isometric conditions using high-frequency length perturbations and is linearly related to the level of active force. Therefore, it can be considered to be a measure of the number of attached crossbridges. On the other hand, Suga and Sagawa plotted pressure–volume isochrons for the heart under different loading conditions. The increasing slopes of the isochrons from the onset to the end of systole result from an increase in the amount of activator Ca$^{2+}$ available to the myofilaments. The instantaneous ratio of ventricular pressure to volume (i.e., slope of the isochron, or elastance) was found to be purely a function of time, independent of loading history or pattern, leading to the notion that the ventricle behaves as an elastic bag that stiffens (increasing elastance) during systole. Later experiments on excised mammalian muscles, however, showed a different behavior. The force–length isochrons moved in a parallel fashion, as opposed to a fanlike movement. This observation along with later studies on the whole heart necessitated a modification in the time-varying elastance concept to incorporate a viscous element into the model for cardiac contractility. A similar analysis on frog ventricular strips has not been reported. We extracted force–length isochrons for variably afterloaded contractions of frog myocardium from data published by Brutsaert and coworkers and found that they moved in a parallel fashion much like those for mammalian muscle. To confirm that whole heart and muscle behave according to their inherent contractile machinery during systole and are not greatly influenced by their connective tissue components, it is essential to characterize the time-dependent behavior of force–length isochrons for a single myocyte under different loading conditions.

Figure 5a shows that as long as there is no shortening of the cell (isometric), the isochrons move in a fanlike behavior. The instantaneous active isometric force, $F(t)$, at a given resting length, $l(t)$, can be described as follows:

$$F(t) = E(t) \cdot [l(t) - l_0]$$

where $E(t)$ is the slope of the isochron and $l_0$ is the length-axis intercept of the isochron. Note that since isometric contractions at lower lengths appear to peak slightly earlier than those at higher lengths, the above expression holds true only up to the earliest time to peak, after which the value of $l_0$ increases slightly. This phenomenon is observed in mammalian heart experiments as well. Therefore, during systole the cell appears to behave as a time-varying length-dependent elasticity/force generator and seems to be consistent with the time-varying elastance concept.

However, once the cell shortens isotonically against a fixed afterload, the force–length isochrons depart from their fanlike behavior and move in a parallel fashion. This behavior more closely approximates the results from similar studies of mammalian muscle, later studies of whole mammalian heart, and earlier frog ventricular strip experiments. Figure 5c also suggests that the parallel movement of the isochrons for variably afterloaded contractions reflects a basic property of the contractile machinery and is not a consequence of the load transfer during shortening from the passive to the active structures. Therefore, the cellular contractile machinery cannot, in general, be modeled as a simple time-varying force generator, which would demand a fanlike movement of the isochrons regardless of loading history. The shift of the isochrons for afterloaded contractions from a fanlike to a parallel movement appears to be velocity dependent, implicating perhaps the contribution of an inherent cellular viscosity to the
Relaxation

One focal point of cardiac mechanics is the period of relaxation, since it is intimately tied with the filling of the heart, which in turn mediates contractility via the Frank-Starling relation. Myocardial relaxation begins when force development or shortening reverses and begins to decline. In the case of an isometric twitch, the decay in force during relaxation depends on the instantaneous number of attached crossbridges, which declines as Ca\(^{2+}\) is removed from the myoplasm.

Muscle shortening during afterloaded contractions causes a loss in myofilament sensitivity to Ca\(^{2+}\) and results in an augmented dissociation of Ca\(^{2+}\) from troponin. If this Ca\(^{2+}\) is not taken up quickly, it could reassociate with troponin and cause the reformation of load-bearing crossbridges. Muscle experiments have shown that the duration of contraction in frog ventricle muscle is independent of afterload and that the decay in isometric force for an afterloaded contraction can be superimposed on the corresponding phase of an isometric twitch. Frog myocardium has a poorly developed SR and, hence, depends primarily on the Na\(^{+}\)-Ca\(^{2+}\) exchanger and ATP-dependent Ca\(^{2+}\) pump in the sarcolemma for the extrusion of intracellular Ca\(^{2+}\). Together, these are believed to be the rate-limiting steps governing the decline of contractile activity in this tissue.

The contractile event in mammalian muscle, however, shortens in duration as the afterload is lowered. The decay of isometric force after lengthening is accelerated when compared with an isometric contraction. These observations have been attributed to the presence of a well-developed SR in mammalian myocardium, which results in the rapid uptake of Ca\(^{2+}\) from the myoplasm and prevents redevelopment of load-bearing crossbridges. The muscle is therefore unable to sustain the load and relengthens rapidly, leading to further detachment of crossbridges and a rapid decay in isometric force. The role of the SR in mammalian relaxation has been further confirmed by subjecting skinned mammalian myocytes to variably afterloaded contractions. Brij-58 detergent digestion of the SR causes these myocytes to relax in a load-independent manner.

The effects of external load on frog and mammalian myocardial relaxation, as discussed above, have been investigated almost exclusively on muscle strips. However, these preparations are abundant in passive extracellular connective tissue and other cell types. The viscoelastic passive properties of this connective matrix may contribute to the overall force–length–time response of the muscle. This complication serves as motivation for mechanical studies of relaxation at the cellular level. In this study, we characterized the effects of varying load under different paradigms on the relaxation of frog cardiac myocytes. The results reflect the interplay between the contractile properties of the myofilaments (actin–myosin interaction), the degree of activation of the myofilaments, and the load imposed on the cell.

The crossbridges linking thick and thin filaments bear external load. Hence, their number determines the load-bearing capacity (LBC) of the cell. Relaxation corresponds to a loss in LBC, i.e., a decay in the number of attached crossbridges. Therefore, the effects of load on relaxation, in essence, reflect the load dependence of the decline in LBC.

For the purpose of this discussion, we wish to consider whether we can treat the contractile machinery of the frog ventricle as a time-varying length-dependent force generator and express the LBC as

\[
\text{LBC} = \alpha(L) \cdot \beta(t)
\]

where \(\alpha(L)\) represents the well-known Frank-Starling relation and \(\beta(t)\) describes the time course of Ca\(^{2+}\) bound to troponin leading to actin–myosin interaction. Although we have found the variable \(\beta\) to have a small length dependence (Figure 5a), the change in \(\beta\) is negligible when the length changes are modest. Since all the results presented on relaxation in this study show peak length changes of less than 5% of the resting length, we have neglected any length dependence of \(\beta\) in the above expression for LBC. Similar representations of the contractility of cardiac muscle have been used by other researchers to model myocardial mechanical behavior. This notion differs somewhat from the conclusions drawn earlier on the force–length–time behavior of a cell during systole, in which we explained our results by invoking the contributions of a cellular viscosity to the mechanical response of the contractile machinery. A similar contribution can also be incorporated in the simplified model of LBC presented here. In that case, the viscous component of the SR would oppose the force generated by the attached crossbridges in a manner dependent on the velocity of shortening. However, since the length changes in the myocytes for this part of the study are slow (peak velocities are usually less than 0.15 cell length/sec), the viscous component of the overall mechanical response is small and would lead primarily to a slight delay in the length response and not an alteration in time course. Therefore, we shall ignore it for the purpose of this discussion.

The results from muscle experiments suggest that whereas the \(\beta\) component of the LBC in mammalian muscle appears to be load and history dependent, the variable \(\beta\) in frog myocardium is independent of load or history within the contraction and can be represented purely as an instantaneous function of time. The experiments in this study allow us to test this hypothesis and to determine whether \(\beta\) is load independent at the cellular level regardless of loading paradigm.

When we examined the effects of varying afterload under the ACCR paradigm, the data (Figures 6 and 7) show that the duration of the contraction and the decay of isometric force are independent of afterload. This result confirms the observations from similar studies of frog muscle strips and suggests that in this tissue the afterloaded shortening and subsequent relengthening
have no effect on either the variable \( \alpha \) (evidenced by the superimposed isometric force decays for the different afterloads) or \( \beta \) (no change in contractile duration). Hence, the load independence of relaxation in frog myocardium for the isotonic–isometric paradigm appears to be an inherent cellular property.

The ACR paradigm closely mimics the loading conditions of in vivo myocardial preparations only during the systolic phase. The ACR paradigm serves as a better approximation to the loading of muscle in the heart during isovolumic relaxation and subsequent filling to end diastole, and, hence, has been termed “physiological” relaxation. The shortening of muscle occurs later in the contractile cycle and under a lower external load. In addition, the cell spends substantially more time at lengths less than its resting length. Although the effects of changing afterloads and late loads during physiological relaxations have been studied on mammalian muscle, similar studies on frog myocardium have not been reported. We investigated the effects of load and history on both phases of physiological relaxation, isometric relaxation (phase 3) and isotonic shortening (phase 4), to test further the hypothesis that the decline of LBC in frog myocardium is insensitive to load/history within the contraction.

Earlier, we concluded that to a first approximation the ESFLR for the frog cardiomycocyte is insensitive to preload and afterload. The protocol in Figure 8 was designed to investigate the effects of extent of shortening during phase 2 on the subsequent decay of isometric force in phase 3. Contraction A is isotropic throughout (at resting length), whereas the decay in force for contractions B–D occurs at shorter lengths (end-systolic lengths). However, when the decay in force for each contraction is normalized to its end-systolic active force level, the tracings during phase 3 are superimposable. These findings further support the notion that, in frog myocardium, the LBC is a separable function of time after the onset of contraction, \( \beta(t) \), and of the instantaneous length, \( \alpha(L) \), and that the time course of isometric force decay in the frog myocyte is independent of the length at which relaxation occurs and of the extent of systolic shortening before the decay within the contractile cycle. Note, however, that the changes in length imposed on the cells in this study are relatively slow. In a previous study we have shown that high-frequency (100-Hz) sinusoidal length perturbations reduce the amount of contractile force and dynamic stiffness of isolated frog myocytes. Such rapid length changes as well as quick stretches and releases could cause detachment of a large number of crossbridges, all of which may not reattach fast enough when compared with the time course of contractile activation of the myofilaments (\( \beta \)). Therefore, the muscle would not be able to sustain the same level of load as predicted by the simple \( \alpha \cdot \beta \) formulation and would in effect have an altered LBC.

Isotonic shortening constitutes the final phase of physiological relaxation (phase 4). In the physiological sequence, there are two factors that can affect the decline of LBC during shortening—the extent of shortening and the external load under which shortening occurs. The use of an ACR paradigm allows us to probe each of these two factors separately.

The effects of varying afterload (i.e., extent of shortening) on isotonic lengthening of mammalian muscle ACPR sequences have been reported. The duration of the twitch cycle decreases with decreasing afterload, suggesting that the \( \beta \) component of the LBC in mammalian myocardium is altered by the extent of shortening. In frog myocytes, however, the extent of shortening of the cell increases with decreasing afterload but has little effect on the lengthening time course under constant late load (Figure 3), suggesting that the decline of LBC is unaffected. This conclusion is supported further by the analysis presented in Figure 9.

The external (late) load has two effects on isotonic shortening in mammalian myocardium. As the late load increases, it increases the rate of shortening from the end-systolic length to the resting diastolic value. This is commensurate with an increased driving force (filling pressure in the intact heart), causing a rapid stretch of the muscle. Simultaneously, however, the duration of the contractile event in mammalian muscle shortens with increasing late load, suggesting that the \( \beta \) component of the LBC is load dependent and perhaps mediated by rapid SR uptake. Similar studies on frog ventricular strips have not yet been reported; however, on the basis of our results, we would expect to see little late-load dependence of the LBC. The protocol in Figure 10 allows us to determine whether the external load during lengthening affects the decay of LBC in frog cardiomycocytes while the extent of shortening is maintained constant. The data show that the rate of isotonic lengthening during phase 4 is determined by the instantaneous balance between the LBC and the external load on the cell (or, alternatively, late load minus passive force). Whereas the late load determines the amount of impending lengthening by altering both the instant at which phase 4 (isotonic lengthening) begins and the final resting value of length (in accordance with the passive length–tension relation), the time-dependent decay of LBC (\( \beta \)) is largely unaffected under different late loads.

The results from Figures 6–10 confirm that under different loading paradigms the LBC declines with a time course that is independent of load or history. Therefore, in the case of frog myocardium, the function \( \beta \) appears to be length and load independent. Since \( \beta \) represents the availability of activator \( Ca^{2+} \) to the myofilaments, our results implicate \( Ca^{2+} \) uptake/removal as the rate-limiting step in frog myocardial relaxation.

**Potential Limitations**

Several limitations of our study should be noted. The apparent load insensitivity of the ESFLR and time course of relaxation could be due to insufficient shortening of the cells, which is often only \( \approx 5\% \) of their resting lengths under unloaded or preloaded isotonic conditions. This would correspond to an ejection fraction of \( \approx 0.1 \) (assuming the ventricle to be a thin-walled cylinder with the myocytes oriented only circumferentially) and is much lower than typical ejection fractions (0.4–0.5) measured in the frog heart. More realistic thick-walled models of the ventricle would predict larger ejection fractions from our cell data, but they would still be significantly lower than those measured in the heart. The modest shortening in these cells could be explained by the low levels of activator \( Ca^{2+} \) that result.
from long periods of inactivity (1–24 hours) during storage between the isolation procedure and the actual experiment or from the isolated state of the preparation. Low activator Ca\(^{2+}\) may also account, in part, for the upward concavity of the ESFLR, as has been shown in muscle and whole-heart studies. Another factor is that most studies by other researchers have probed myocardial mechanics with the muscle at the length at which maximum active isometric force develops. However, we were limited to lower cell lengths, for the reason that increases in cell length beyond those shown in this study invariably led to irreversible hypercontracture of the cell that was perhaps due to mechanical rupture of the sarcomera. Finally, mammalian muscle exhibits a “transient beat” phenomenon. By this phenomenon, the first isometric beat of the muscle after the transition from steady-state afterloaded contractions develops a higher level of force than the ensuing isometric beats. The force level drops monotonically thereafter until it reaches a new steady state. If one were to examine the first beat, it would lead to a greater measured load dependence of the ESFLR. In our study, we did not examine this issue specifically; however, in the frog myocytes, there was never a systematic change in the isometric force level after a series of afterloaded or isometric contractions. Hence, we regard it unlikely that the apparent load insensitivity of the frog cell ESFLR would disappear if we were to measure only the first isometric beat.

**Summary**

This study establishes the single cell as a viable preparation for studies of cardiac mechanics. Although single-cell studies cannot completely replace whole-heart experiments, they may help to distinguish between inherent contractile properties that are intact at every level of organization and those that are a consequence of the complex geometry of the heart, the connective tissue, and neurohumoral control factors.

Our results suggest that within the limitations described above, frog myocardium has an ESFLR that demonstrates no load dependence at the cellular level. In this respect, our findings contradict studies of the ESFVR from experiments on frog heart but are in accord with experiments on mammalian heart and muscle strips. Furthermore, within a range of cell shortening of 5%, the duration of the twitch contraction and the time course of relaxation remain invariant despite the many loading conditions tested, suggesting that the decline of load-bearing capacity in frog myocardial cells is independent of loading paradigm or history within the contraction. Our results confirm the limited muscle experiments in the same tissue and strongly suggest that the relaxation properties of the tissue are mediated at the cellular level, with Ca\(^{2+}\) extrusion being the rate-limiting step in decline of load-bearing capacity.

**References**

34. Brady AJ, Farnsworth SP: Cardiac myocyte stiffness following extraction with detergent and high salt solutions. *Am J Physiol* 1986;250:H932–H943
51. Tung L, Parikh S: Reduction of contraction and dynamic stiffness of single frog ventricular cells by high frequency length oscillations. (abstract) *Biophys J* 1990;57:551a
Contraction and relaxation of isolated cardiac myocytes of the frog under varying mechanical loads.
S S Parikh, S Z Zou and L Tung

Circ Res. 1993;72:297-311
doi: 10.1161/01.RES.72.2.297

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/72/2/297

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/