Real-Time Kinetics of Sarcomere Relaxation by Laser Diffraction

Yves Lecarpentier, Jean-Louis Martin, Victor Claes, Jean-Paul Chambaret, Arnold Migus, André Antonetti, and Pierre-Yves Hatt

SUMMARY. Kinetics of sarcomere movement were studied in real-time by laser diffraction. Instantaneous sarcomere shortening was measured during afterloaded twitches simultaneously with instantaneous shortening and tension of the whole trabecula excised from rat right ventricle. Resting sarcomere length at optimal length was 2.20 ± 0.02 μm (mean ± SEM). Maximum amplitude of sarcomere shortening was 0.30 ± 0.01 and 0.16 ± 0.01 μm, respectively, in twitches loaded with preload only, and in "isometric" twitches. When the isotonic load (expressed as a percentage of maximum isometric force TF) increased, the maximum velocity of sarcomere relaxation max Vr (μm/sec) decreased: max Vr = -4 × 10^{-2} % TF; r = 0.95. The time course of sarcomere relaxation appeared to be progressively delayed when the total load increased from preload only up to "isometric" load. Sarcomere relaxation occurred in two successive exponential phases, a rapid phase [time constant (msec): τ1] followed by a slower one (time constant: τ2). When the total load increased, τ1 increased and τ2 decreased according to the linear relations: % TF = 0.2 τ1 + 4.8 (r = 0.83) and % TF = -0.1 τ2 + 157 (r = 0.95). The relative predominance of both the time course and the amplitude of these two phases depended upon the level of total load. The rapid process predominated at low load, the slow one at high load. The role of load and/or shortening in the time course of these two phases is discussed. (Circ Res 56: 331-339, 1985)

SARCOMERE movement in intact cardiac muscle has usually been studied under isometric conditions during the relaxation phase (Krueger and Strobeck, 1978; Krueger and Forber, 1980). Little is known about real-time sarcomere dynamics recorded simultaneously with the mechanical behavior of the whole muscle either contracting at various levels of isotonic load or after rapid modifications of loading conditions. Different mechanisms interact to control both isotonic lengthening and isometric tension decay, and their interdependence is not well established. Despite the fact that sarcoplasmic calcium concentration plays a major role in several steps of excitation-contraction coupling involved in force generation, this concentration has been shown, by aequorin technique, to be considerably lowered at the time of peak isometric tension (Ashley and Ridgway, 1970; Taylor et al., 1975; Allen and Blinks, 1978; Blinks et al., 1978; Gordon and Ridgway, 1978). Thus, uncertainties remain concerning the intracellular mechanisms governing the slow decline of isometric tension under conditions of low sarcoplasmic calcium concentration. Moreover, relaxation has recently been shown to be sensitive to the level of external load in both mammalian heart muscle (Brutsaert et al., 1978b, 1980) and in spontaneously beating single cardiac cells (Lecarpentier et al., 1979) in which the sarcoplasmic reticulum was still functional. Intracellular calcium-sequestering systems, particularly the sarcoplasmic reticulum, have been assumed to account partially for this property, which disappears in mammalian heart muscle treated with caffeine (Lecarpentier et al., 1979; Poggesi et al., 1979), and which is not observed in frog myocardium (Brutsaert et al., 1978b). The present study extends these observations by examining sarcomere kinetics during cardiac relaxation, in both "isotonic" and "isometric" contractions, and demonstrates that two mechanisms are involved during cardiac relaxation. The first, which is a rapid process, prevails at low load, whereas, the second, which is slow, prevails at high load.

Methods

Experimental System and Mounting Procedure

Adult Wistar rats (n = 10) were anesthetized with ether. The heart was quickly removed and transferred to a dissection chamber filled with a physiological oxygenated saline solution. The aorta was cannulated and perfused with the same salt solution at a flow rate of 5 ml/min. The physiological solution used during dissection and experiments consisted of the following components in mM: NaCl, 118; KCl, 4.7; NaHCO3, 24; MgSO4·7H2O, 1.2; KH2PO4, 1.1; CaCl2·6H2O, 2.5; and glucose, 4.5. This solution was in equilibrium with 95% O2 and 5% CO2 (pH = 7.4) and was maintained at 24°C.

Small, ribbon-shaped trabeculae or thin papillary muscles were rapidly excised from the right ventricle and
FIGURE 1. Experimental set-up. Abbreviations are: L, laser; MI, microscope; V, video camera; MC, muscle chamber; B, beam splitter; W, densitometric wedge; F, split; D1 and D2, diodes; g and h, optical signals electronically converted by D1 and D2 respectively; g/h, signal function of sarcomere length; T, electromagnetic transducer; Bj, stimulator; M, muscle tension and shortening curves vs. time (t); S, instantaneous sarcomere length curve vs. time (t).

FIGURE 2. Diffraction spectra obtained from a thin, right-ventricular rat trabecula. The two first-order diffracted lines (±1) were symmetrically spaced on either side of the central bright line of nondiffracted light (zero order). The distance between the zero-order line and the first-order lines are inversely related to sarcomere length.

mounted horizontally in a rectangular (1 X 5 cm) perfusion chamber. The glass bottom (0.2 mm thick) allowed visual observation under an inverted Reichert microscope (Fig. 1). The preparations were stimulated by means of an electrical field with two parallel silver electrode wires delivering 5-msec square pulses at a voltage slightly above threshold, and at 12 beats/min. After a one-half hour stabilization period, L*, i.e., the initial length at the apex of the length-tension curve, was determined. The remaining of the experiment was carried out with preload corresponding to L*. Dimensions of trabeculae and papillary muscles (length, width, thickness, and cross-sectional area) were measured at L*. The dimensions of specimens reported here were as follows: average length, 2.6 ± 0.2 (SEM) mm (range 2.1–3.5 mm); average width, 389 ± 34 μm (range 270–520 μm); average thickness, 167 ± 15 μm (range 90–210 μm). The cross-sectional area (s) of the muscles was calculated by considering muscle width and thickness as the major and minor axes of an ellipse (s = width X thickness X π/4); s = 0.052 ± 0.008 mm² (range 0.025–0.085 mm²). Criteria for selection of specimens were: diffraction pattern with sharp first-order line, all or no response to electrical stimulation, stable mechanical performance and the resting force/total force ratio <20%. Three of the 10 muscles in which the resting force was more than 20% of total force at L* were discarded from calculations. Mean value of resting force/total force at L* was 17 ± 2%. Average optical techniques

A 5-mW Helium-Neon laser (L) transilluminated a right ventricular rat trabecula or a thin papillary muscle horizontally mounted in the experimental chamber on the stage of an inverted microscope (Fig. 1). Thin cardiac muscle of this type acts as a tridimensional grating due to the regular array of sarcomeres, and diffracts the laser lines along several orders (at least ±1 and, in some cases, ±2). The spacing between zero and first-order of the Fraunhofer diffraction pattern was calculated according to the equation SL = λ/\sin θ where SL is the mean sarcomere length, λ = 0.6328 μm the wavelength of the laser, and θ the angular separation of the first-order diffraction line relative to the zero-order reference line. The laser beam passed through an adjustable slit and a 45-mm focal length lens, and was then collected with a 63X objective. The slit (F) was positioned in front of the lens so that its image (40 X 200 μm) was focused on the focal plane of the objective and could be superimposed onto the image of the trabecula. Both images were simultaneously observed through the microscope ocular or on the monitor of a video camera.

A region in the midlength of the specimen containing about 50–100 sarcomeres in series, was exactly superimposed onto the laser spot whose outline was determined by the adjustable slit. The diffraction pattern was then examined above the muscle chamber. A typical diffraction pattern is presented in Figure 2.

From the meridional diffraction pattern (Fig. 1), we selected the first-order line through an aperture and collected it through a lens which induced a parallel output. The beam then was split (B) into two equal parts. One beam was focused onto the PIN 10 diode D2 producing the signal h which represented the variation of intensity of the first-order line. The other beam passed through an optical density wedge (W) whose transmission was related to the lateral shift of the beam and, consequently, to the

resting force/mm² was 16.4 ± 1.4 mN/mm². The muscles developed at L* 105 ± 6 mN/mm² or 5.4 ± 0.8 mN.
grating constant due to variations in the diffraction angle. This beam was then collected and focused onto the PIN 10 diode D, giving the signal g which represented the product of the variation of intensity of the first-order diffraction line and the variation of intensity due to the beam’s lateral movement on the densitometric wedge. These signals were normalized so that initially g/h equals 1. During the twitch, the electronic system computed in real-time, the ratio g/h which is directly related to the instantaneous sarcomere length (SL) through the relationship SL = SL0/(1 + a log g/h) where SL0 is the resting sarcomere length at Lm, and a a constant characteristic of the optical set-up. In our experimental conditions, this expression can be linearized: SL = SL0 [1 + a (1 - g/h)].

Calibration of the sarcomere length at rest was checked by replacing the trabecula with gratings of known spacing positioned in the muscle plane. The accuracy of the apparatus was 1%, which corresponds to a change in sarcomere length of 200 Å. When the laser beam illuminated a different region in the midlength of the trabecula, the intensity variations (h) were modified but not the ratio g/h, i.e., the variations in sarcomere length during contraction. The ratio g/h, which is a function of the mean length value of about 50–100 sarcomeres illuminated by the laser beam, was independent of the position of the slit on the central segment of the muscle. The noise in the SL vs. time trace was minimized by diminishing background light. g/h was recorded with a low-pass filter (cut-off frequency: 2 kHz).

Computation was used to determine the function which best fitted sarcomere relaxation. The biphasic time course of sarcomere relaxation could not be fitted by a single exponential function. Figure 3 shows the time course of a twitch loaded with moderate afterload. Two exponential functions, whose time constants were respectively τ1 and τ2, were found to fit the whole time course of sarcomere relaxation of the experimental curves. A linear fit might also be used for both the two phases of sarcomere relaxation, especially at low load, where τ1 appeared to be very short and τ2 very long. However, the exponential fit accounted for most of the curves, particularly at middle and high loads, and often at low load. The onset (t0) of the first exponential fit occurred at the onset of rapid sarcomere shortening, soon after the time-to-peak sarcomere shortening (see Fig. 3: arrow 1 and t0). The onset of the second exponential fit corresponded approximately to the isotonic-isometric transition where the slope of sarcomere relaxation suddenly decreased (see Fig. 3: arrow 2). Peak velocities of sarcomere shortening and lengthening were expressed graphically as the positive and negative maximum slopes of the SL vs. time curve. Maximum velocity of sarcomere lengthening (max. Vl) was also calculated from the equation of the first exponential fit; this calculated value is:

\[
\frac{\Delta SL}{\tau_1} \exp \left( -\frac{t}{\tau_1} \right) = \frac{\Delta SL}{\tau_1}
\]

where ΔSL is the amplitude of sarcomere elongation at t0, t the time at the onset of the first exponential fit, i.e., t0 = 0, and τ1 the time constant of the first exponential fit (Fig. 3).

**Electromagnetic Lever System**

A miniature panel meter (Eagle-Picher Industries, Inc.; sensitivity = 100 μA; internal resistance = 600 ohms) with center zero was adapted by replacing the pointer with a stainless steel tube and two small aluminum supports. This part was attached to the coil by epoxy cement. The rod was bent into the shape of a hook. The equivalent moving mass of the whole system (lever and coil) was 40 mg. The displacement of the lever was measured by means of an opto-electronic system mounted in the panel meter. The light beam of a miniature infra-red light-emitting diode (LS 600) was modulated by a small shutter on the lever and captured by a small photodiode (H 35). The resulting signal was amplified and linearized by operational amplifier circuits. The linear range was 1 mm with a linearity of 1%. The displacement signal was filtered with a third-order Rayneter low pass-filter (optimal transient response cut-off frequency = 100 Hz). The filtered signal was differentiated using an active differentiator circuit. The RC constant was set to 0.1 second.

**Force Generation and Measurement**

The coil of the lever system was suspended within the field of a permanent magnet. The torque on the lever and, hence, the load imposed on the preparation, was proportional to the current through the coil when the lever was within the linear displacement range. The current was generated by a current source and set by means of decade switches in calibrated steps of 0.05 and 1 mN up to a total of 10 mN. Load alterations were performed by switching the current to a second level controlled by a second set of decade switches.

A unilateral force-sensing feedback circuit controlled the current source. This method is described elsewhere (De Clerck et al., 1978), and only a short overview is given here. The unfiltered displacement signal was compared to a present reference level, representing the position of an "electronic stop." If the length signal was higher than this level, no feedback occurred, and the preparation carried the load set by means of the decade switches. When it became lower, the lever was held in that position by the

![Figure 3. MF, muscle force (top trace); ML, muscle shortening length (middle trace); SL, sarcomere shortening length (bottom trace). MF, ML, and SL were plotted as a function of time. Loading characteristics of the twitch: preload = 1 mN; afterload = 0.5 mN. A vertical dashed line separated the isotonic relaxation phase from the isometric relaxation phase of the whole muscle. Sarcomere relaxation occurred in two experimental phases. The onset t0 of the first exponential phase (arrow 1) occurred soon after the peak sarcomere shortening. The onset of the second exponential phase (arrow 2) corresponded to the isotonic-isometric transition of muscle relaxation.](image-url)
feedback mechanism, and the current through the coil was proportionally decreased and represented the load carried by the preparation. By adjusting this reference level, resting length and preload could be set. If the preparation developed a higher force than the load set with the decade switches, isotonic shortening occurred. In the course of an isometric contraction, the lever moved a very small amount (proportional to the force developed). This static compliance amounted to 1.2 \( \mu m/N \), and the dynamic compliance was negligible. The force signal was filtered and differentiated with the same kind of circuits as those of the length signal to minimize relative phase errors.

**Results**

**Mechanical Parameters of Sarcomere Kinetics**

Sarcomere relaxation behavior was tested by either loading the muscle at various levels, or modifying the total load with load clamp steps during isotonic shortening. Real-time sarcomere shortening (SL) was recorded simultaneously with muscle shortening (ML) and with muscle force (MF). Figure 4 shows SL, ML, and MF in a typical muscle, contracting at three increasing levels of total load. Sarcomere kinetics clearly depended upon the loading conditions. When the load was increased from preload only (twitch a) up to isometric load (twitch c); there was a progressive decrease in the maximum amplitude of sarcomere shortening and in the peak velocities of shortening and of lengthening. Hereafter, the term "isometric" will refer exclusively to the mechanical behavior of the whole trabecula: indeed, when the total load became high enough, no external shortening of the whole muscle oc-

![FIGURE 4. Real-time kinetics of sarcomere and whole cardiac trabecula. Muscle force MF (top trace), muscle shortening length ML (middle trace), and sarcomere shortening length SL (bottom trace) were plotted as a function of time. A series of three superimposed contractions (twitches a, b, and c) can be seen against various loads, i.e., from isotonic twitch a with preload only (1 mN), then with various total loads (twitch b: 2 mN); up to a full "isometric" twitch c. These three contractions were similarly preloaded at \( L_u^u \) with 1 mN. During "isometric" twitch c, ML did not shorten, although SL decreased 7% of its resting length value at \( U^u \). When the afterload increased in twitches b and c, the mechanical parameters of sarcomere (i.e., maximum amplitude of sarcomere shortening, peak velocities of shortening and of lengthening) decreased, and further time courses of sarcomere relaxation appeared progressively delayed. Mechanical characteristics of this typical trabecula at \( L_u \) and at rest: MF = \( 1 \) mN; ML, 180 \( \mu m \); SLo = 2.15 \( \mu m \); width, 360 \( \mu m \); thickness, 180 \( \mu m \); cross-sectional area, 0.05 \( mm^2 \).]

![TABLE 1

<table>
<thead>
<tr>
<th>Twitch with preload only</th>
<th>&quot;Isometric&quot; twitch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcomere length at rest (SLa) ( (\mu m) )</td>
<td>2.20 ± 0.02</td>
</tr>
<tr>
<td>Final sarcomere length at peak shortening (( \mu m ))</td>
<td>1.90 ± 0.02</td>
</tr>
<tr>
<td>Maximum amplitude of sarcomere shortening (( \mu m ))</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Percentage of maximum sarcomere shortening amplitude (% SLa)</td>
<td>13.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM. This table shows the mechanics of sarcomere during the twitch loaded with preload only and during "isometric" twitch.]

![FIGURE 5. Relationship between total load and maximum sarcomere peak velocity during contraction (max Vc) and relaxation (max Vr). Maximum sarcomere velocity was graphically determined by the maximum slope of the SL vs. time curve. Total load was expressed in terms of percentage of maximum isometric force at \( L_u \) (TF). Mean values of sarcomere velocity were calculated at five increasing levels of % TF: 0–20% TF, 21–40% TF, 41–60% TF, 61–80% TF, and 81–100% TF. Maximum velocity of sarcomere (\( \mu m/sec \)) exponentially decreased when % TF increased according to the equations max Vc = 2.2 exp (–1.4 \times 10^{-2} \% TF) \( (r = 0.99) \) during the contraction phase and max Vr = –4 exp (–2.5 \times 10^{-2} \% TF) \( (r = 0.95) \) during the relaxation phase.]
was observed at "isometric" load (Table 2: D/C ratio = 0.62 ± 0.13).

The time course of sarcomere shortening differed strikingly from that of sarcomere lengthening according to the loading conditions. In a series of afterloaded twitches with similar preload (Fig. 4), sarcomere shortening initially presented a common pathway whose duration increased with total load, although the sarcomere vs. time curves appeared to be distinct from each other during the overall time course of relaxation phase (Fig. 4, twitches a, b, and c). When total load increased from twitch a with preload only up to isometric twitch c (Fig. 4), time-to-peak sarcomere shortening barely increased, and further time course of sarcomere relaxation appeared to be progressively delayed. The higher the total load, the later a given degree of sarcomere lengthening occurred. Twitch a with preload only rapidly reached its initial resting length. Sarcomere relaxation of the afterloaded twitches (twitch b in Fig. 4 and the twitch in Fig. 3) displayed two distinct and successive phases of auxotonic relaxation. First, a short and rapid phase, during which the main part of sarcomere lengthening was achieved (from arrow 1 to arrow 2 in Fig. 3). Second, a slow phase occurred, during which sarcomeres lengthened and reached their initial resting length (after arrow 2 in Fig. 3). In afterloaded twitches, the rapid phase of sarcomere lengthening corresponded in time to the isotonic relaxation phase of the whole muscle and the slow phase to the isometric relaxation phase. The bending from the rapid phase to the slow phase occurred approximately at the transition between the successive isotonic and isometric relaxation-phases of muscle force (MF) (arrow 2, Fig. 3). When the load increased, the amplitude of sarcomere lengthening decreased during the rapid phase and increased during the slow phase. In Figure 4, at any given moment of the rapid phase of sarcomere relaxation, the lower the load, the higher the instantaneous speed of sarcomere lengthening. Inversely, at any given moment in the slow phase, the higher the load, the higher the instantaneous velocity of sarcomere lengthening.

Modeling of sarcomere lengthening vs. time was fitted with two exponential functions. The time constants \( r_1 \) and \( r_2 \) of exponential fits of the fast and slow phases, respectively, were expressed as percentages of the maximum isometric force (MF) at \( L_{max} \) (Fig. 6). \( r_1 \) and \( r_2 \) were linearly related to the total load according to the equations: \( \% MF = 0.2 \cdot r_1 + 4.8 \) (coefficient of linear regression \( r = 0.83 \) and \( \% MF = -0.1 \cdot r_2 + 157 \) (coefficient of linear regression \( r = 0.95 \). When total load increased, the linear increase in \( r_1 \) appeared to be associated with a linear decrease in \( r_2 \). This load-dependent balance between the two phases \( r_1 \) and \( r_2 \) depended upon the level of load. At low load, the rapid phase predominated, and at high load the slower phase predominated. In the twitch with only the preload, only the fast phase was observed (Fig. 4, twitch a). In the
“isometric” twitch (Fig. 4, twitch c), the rapid phase disappeared and only the slow phase was observed. The relaxation time course of the “isometric” twitch c was markedly delayed, compared with that of twitches a and b.

Maximum velocity of sarcomere lengthening was also calculated from the maximum value of the exponential fit: \( \max \dot{V}_r \) (calculated) = \(-\Delta SL/\tau_1 \). This was then compared with \( \max \dot{V}_r \) (graphic): \( \max \dot{V}_r \) (calculated) = 1.08 \( \max \dot{V}_r \) (graphic) - 0.02 (coefficient correlation \( r = 0.95 \)). Statistical analysis shows that the slope of this linear relationship did not differ significantly from 1.

**FIGURE 6.** Relationship between total load and time constants \( \tau_1 \) (rapid phase) and \( \tau_2 \) (slow phase). Total load was expressed in terms of the percentage of the maximum isometric force at \( L_{ma} \) (% TF); \( \tau_1 \) and \( \tau_2 \) were linearly related to the % TF by the following equations: % TF = 0.2 \( \tau_1 + 4.8 \) (\( r = 0.83 \)) and % TF = -0.1 \( \tau_2 + 157 \) (\( r = 0.95 \)). The rapid process predominated at low load, the slow one at high load. The transition between the two phases was more precisely determined at low load than at high load, where \( \tau_1 \) and \( \tau_2 \) were not strikingly different (\( \tau_1/\tau_2 = 0.5 \) at 70% TF).

**FIGURE 7.** Muscle force MF (top trace), muscle shortening length ML (middle trace), and sarcomere shortening length SL (bottom trace) were plotted as a function of time. Three twitches, a, b, and c, were similarly preloaded at \( L_{ma} \) with 1 mN. Isotonic total load of control twitch a was maintained at 2 mN during the overall contraction. Total load of twitches b and c was 1 mN at the beginning of the contraction phase. An abrupt load clamp step of 1 mN was imposed during twitches b and c, at two different times during the last third of the contraction phase, in such a way that their final total load was similar to that of control twitch a (2 mN). The subsequent time course of twitches b and c was strikingly abbreviated. Mechanical characteristics of the trabecula were similar to those of Figure 4.

**FIGURE 8.** Muscle force MF (upper trace), muscle shortening length ML (middle trace), and sarcomere shortening length SL (lower trace) were plotted as a function of time. Three twitches, a, b, and c, were similarly preloaded at \( L_{ma} \). Isotonic total load of control twitch a was maintained at 2 mN during the overall duration of the twitch. Two load clamp steps of various amplitude (0.5 mN in twitch b and 1 mN in twitch c, respectively) were imposed at the same time in such a way that the final total load was similar to that of control twitch a (2 mN). These load clamps were triggered at the same time as that imposed during twitch c in Figure 7. The higher the load step, the shorter the overall duration of the contraction. Mechanical characteristics of the trabecula: similar to those of Figure 4.

**Load Sensitivity of Sarcomere Relaxation**

The load-bearing capacity of sarcomeres was also tested during isotonic contractions by triggering small, abrupt modifications in load (load clamp steps) of various amplitude and at different times during the twitch. In Figure 7, two afterload twitches b and c were rapidly load-clamped from 1 mN to a final total load of 2 mN, which was the total load of the control twitch a. When the load step occurred during the last third of the shortening phase as in twitches b and c (Fig. 7), the time course of sarcomere relaxation became markedly abbreviated. For a given isotonic load, the onset of isometric relaxation

...
of the whole trabecula depended upon the timing of load clamp steps. In the load-clamped twitches b and c, sarcomere relaxation occurred in two successive rapid and slow phases, as was observed in control twitch a. For the three twitches a, b, and c (Fig. 7), the sarcomere relengthening level which was measured at the MF isotonic-isometric transition corresponded to the end of the rapid phase of lengthening and appeared scarcely influenced by the time at which the load clamp was triggered. Thus, the onset and the end of the rapid phase of sarcomere relaxation clearly depended either on the level of total load (Figs. 3 and 4), or on the moment at which the final level of total load was reached after the load clamp steps (Fig. 7). Later, the slow phase of sarcomere relaxation of twitches a, b, and c (Fig. 7) exhibited a common pathway when returning to the resting length; this pathway was independent of the time at which the load clamps were imposed.

These results, which test the influence of the load on the time course of sarcomere relaxation, were also corroborated by those shown in Figure 8. Load clamp steps of various amplitude were imposed at the same moment during the last third of the shortening phase. These modified the load-bearing capacity of the muscle and the time course of sarcomere relaxation. As in Figure 7, sarcomere relaxation presented a rapid phase followed by a slow one. The higher the amplitude of the load clamp step, the sooner the rapid phase occurred. The end of the rapid phase corresponded to the MF isotonic-isometric transition. The slow phases of sarcomere relaxation of twitches a, b, and c (Fig. 8) presented a similar time course. The sarcomere resting length was reached at the end of the common pathway of the slow phase.

In summary, real-time kinetics of sarcomere during relaxation lead to the following conclusions. Sarcomere lengthening occurred in two successive exponential phases. The time constants \( T_1 \) and \( T_2 \) of these two phases were linearly related to the total force imposed on the muscle. The rapid and the slow phases can be modulated independently: for a given final level of total load, the onset and the end of the rapid phase depended on loading changes previously imposed during the twitch, i.e., the timing (Fig. 7) and/or the amplitude (Fig. 8) of load clamp steps. On the contrary, the time course of the common pathway of the slow phase appeared to be independent of these loading modifications.

**Discussion**

**Dynamics of Sarcomere Shortening and Lengthening**

This paper focuses on the mechanical behavior of sarcomeres during relaxation at various levels of load. These results reveal the complex influence that the level of instantaneous load has upon intracellular mechanisms involved in sarcomere contraction and relaxation processes. Dimensions of the trabeculae and the maximum isometric force at \( L_{max} \) per cross-sectional area were quite similar to those observed by Pollack and Huntsman (1974), Krueger and Pollack (1975), ter Keurs et al. (1980), and Gordon and Pollack (1980). Sarcomere velocity and sarcomere shortening shown in Tables 1 and 2 appeared to be of a comparable order of magnitude to those observed by other investigators. In twitches where the load represented a small fraction of isometric load, Pollack and Krueger (1976) observed that the maximum unloaded sarcomere velocity reaches 10 \( \mu \text{m/sec} \) in rat cardiac trabecula. Similar values have been found by Krueger et al. (1980) in isolated cardiac cells during unloaded contraction. In our study, however, the maximum speed of sarcomere shortening was measured in the preloaded twitch which represented 17 ± 2% of maximum "isometric" tension. This preloaded twitch differs somewhat from unloaded conditions and accounts for the fact that we obtained lower values as compared with those of Pollack and Krueger (1976). This also explains why the average sarcomere length at peak shortening of the preloaded twitch was 1.90 ± 0.02 \( \mu \text{m} \) (Table 1) rather than the 1.6 \( \mu \text{m} \) found in unloaded contraction by Pollack and Krueger (1976), ter Keurs (1978) in intact papillary muscle, and Krueger et al. (1980) in single cardiac cell. During the isometric twitch, we found the same internal sarcomere shortening (7% of the resting sarcomere length at \( L_{max} \)) as previously measured by Krueger and Pollack (1975). This internal sarcomere shortening observed during "isometric" contraction has been shown to be due to high compliance in the extremities of the preparation (Krueger and Pollack, 1975).

**Time Course of Sarcomere Relaxation Occurring in Two Phases**

Sarcomere relaxation was characterized by two successive exponential phases whose relative importance was "balanced" by the general loading conditions (Fig. 6). The rapid phase corresponding to the isotonic phase of the whole muscle lengthening contributed to the major part of relengthening. The slow phase terminated sarcomere lengthening and corresponded to the slow final decay of isometric tension. The onset and duration of the first rapid phase of sarcomere relaxation appeared to be modulated by the level of load, the amplitude of load variations, and the time at which a given level of final total load was triggered (Figs. 4, 7, and 8). In twitches load-clamped to the same final total load, the relengthening level of the sarcomere at the end of the rapid phase was slightly modified as compared with the varying times at which the MF isometric" relaxation began (Figs. 7 and 8). In these twitches, the slow phase presented a common trajectory slowly reaching the initial resting sarcomere length. The relative role of the two phases during...
sarcomere relaxation appeared to be a function of the level of load. At low load, the rapid phase was predominant, while the second phase became predominant at heavy load. The "transition" between these two phases was quite evident, particularly at low load, when their time constants $t_1$ and $t_2$ were very different (Fig. 6). At heavy load, this transition appeared less precise because the difference between $t_1$ and $t_2$ was much smaller than at low load (Fig. 6). The fact that these two phases could not be fitted with a single exponential function argues in favor of two different mechanisms. This was corroborated by the fact that the time course of the rapid phase could be modified by changing the loading conditions without any change in the time constant of the slow phase (Figs. 7 and 8). It was also substantiated by the observation of the rapid phase only, during the preloaded twitch, and of the slow phase only, during the "isometric" twitch (Fig. 4). Other investigators have shown that sarcomere relaxation in mammalian muscle could be characterized by two distinct phases. In unloaded single cardiac cells, Krueger et al. (1980) have found that sarcomere lengthening successively presents a rapid relaxation phase followed by a very slow one. Moreover, a biphasic curve has been observed in intact cardiac muscle in which relaxation is terminated by a slow phase of isometric tension decline (Krueger and Farber, 1980). These phases have been assumed to be governed by distinct mechanisms (Krueger and Strobeck, 1978) as suggested by the opposite effects of calcium on isometric and isotonic relaxation (Strobeck et al., 1975). Various hypotheses have been proposed to explain rapid sarcomere lengthening. The afterload pulling on the muscle contributes to rapid shortening. Restoring forces occur at sarcomere length below the slack length (1.90 $\mu$m), where one might expect a significant effect on sarcomere relaxation. In our study, sarcomere length at peak shortening in twitch with preload reached only 1.90 ± 0.02 $\mu$m (Table 1), and internal restoring forces could hardly be involved in the rapid phase of sarcomere relaxation.

Biochemical and mechanical arguments have previously emphasized the role of the sarcoplasmic reticulum on intracellular calcium movements during both contraction and relaxation. The $Ca^{++}$-induced release of $Ca^{++}$ from the sarcoplasmic reticulum is well-developed in adult rat ventricle and absent in frog myocardium and in newborn rat ventricle (Fabiato and Fabiato, 1978). Moreover, cardiac relaxation has been shown to be sensitive to the loading conditions (Brutsaert et al., 1978a, 1978b; Lecarpentier et al., 1979). This mechanical property expresses the possibility of modifying the timing of the isotonic-isometric transition according to the level of total load and requires the presence of an efficient sarcoplasmic reticulum. Thus, it disappears in mammalian single cardiac cells after pretreatment with the detergent BRIJ-58 (Brutsaert et al., 1978a), which destroys both sarcolemma and sarcoplasmic reticulum (Orentlicher et al., 1974; Fabiato and Fabiato, 1975). Load sensitivity also disappears in a caffeine medium (Lecarpentier et al., 1979; Poggesi et al., 1979) which partially inhibits the sarcoplasmic reticulum (Weber and Herz, 1968). Furthermore, load sensitivity of relaxation is not observed in the frog ventricular strip (Brutsaert et al., 1978b) where the sarcoplasmic reticulum is scarcely developed (Staley and Benson, 1968), nor in newborn rat myocardium (personal observation) in which the sarcoplasmic reticulum is not yet functional (Fabiato and Fabiato, 1978). Influence of the load on relaxation appears to be of physiological and pathological relevance because it can be observed in intact heart muscle (Raff and Glantz, 1981) and because it disappears after hypoxia (Chuck et al., 1981) but persists during cardiac hypertrophy (Lecarpentier et al., 1982). All these arguments suggest a partial involvement of the sarcoplasmic reticulum in the time course regulation of the rapid phase of sarcomere relaxation.

On the other hand, intracellular mechanisms governing cardiac relaxation at high load are not yet clearly understood. As previously discussed by Krueger and Farber (1980), relaxation terminated by a slow phase during isotonic tension decline. The slow final lengthening could be due to the force in the series elastic elements being relieved gradually. The free calcium concentration, studied with the aequorin technique, has been shown to return almost to its level at rest, long before isotonic tension decay (Taylor et al., 1975; Gordon and Ridgway, 1978). The molecular mechanism of this isotonic force decline at low calcium concentration remains uncertain, but might be explained by subtle interactions between actin and myosin filaments or by a cooperative effect modifying myofilament calcium affinity (Bremel et al., 1973; Taylor et al., 1975). Moreover, additional evidence supports a link between the calcium binding properties of troponin C and load and/or length changes (Endo, 1973; Allen and Kurihara, 1982; Housmans et al., 1983). Our results appear to be compatible with such a hypothesis: at high load or slight amplitude of sarcomere shortening, the calcium affinity of troponin C would seem to be higher than at low load, and vice versa. This may be confirmed by further experiments. In conclusion, sarcomere relaxation seems to occur in two successive phases, a rapid phase followed by a slower one, the respective time courses of which depend upon the general loading conditions.

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