Myofilament-Generated Tension Oscillations during Partial Calcium Activation and Activation Dependence of the Sarcomere Length-Tension Relation of Skinned Cardiac Cells

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ABSTRACT During partial Ca\textsuperscript{2+} activation, skinned cardiac cells with sarcoplasmic reticulum destroyed by detergent developed spontaneous tension oscillations consisting of cycles (0.1–1 Hz) of rapid decrease of tension corresponding to the yield of some sarcomeres and slow redevelopment of tension corresponding to the reshortening of these sarcomeres. Such myofilament-generated tension oscillations were never observed during the full activation induced by a saturating [free Ca\textsuperscript{2+}] or during the rigor tension induced by decreasing [MgATP] in the absence of free Ca\textsuperscript{2+} or when the mean sarcomere length (SL) of the preparation was > 3.10 μm during partial Ca\textsuperscript{2+} activation. A stiff parallel elastic element borne by a structure that could be digested by elastase hindered the study of the SL-active tension diagram in 8-13-μm-wide skinned cells from the rat ventricle, but this study was possible in 2-7-μm-wide myofibril bundles from the frog or dog ventricle. During rigor the tension decreased linearly when SL was increased from 2.35 to 3.80 μm. During full Ca\textsuperscript{2+} activation the tension decreased by < 20% when SL was increased from 2.35 to 3.10 μm. During partial Ca\textsuperscript{2+} activation the tension increased when SL was increased from 2.35 to 3.00 μm. From this observation of an apparent increase in the sensitivity of the myofilaments to Ca\textsuperscript{2+} induced by increasing SL during partial Ca\textsuperscript{2+} activation, a model was proposed that describes the tension oscillations and permits the derivation of the maximal velocity of shortening (V\textsubscript{max}). V\textsubscript{max} was increased by increasing [free Ca\textsuperscript{2+}] or decreasing [free Mg\textsuperscript{2+}] but not by increasing SL.

INTRODUCTION

We have reported in an abstract (Fabiato and Fabiato, 1974) that two types of spontaneous tension oscillations can be observed in segments of single cardiac cells from which the sarcolemma has been removed by microdissection (skinned cardiac cells). The first type of oscillations, which correspond to cyclic releases and reaccumulations of Ca\textsuperscript{2+} by the sarcoplasmic reticulum, has been extensively described (Fabiato and Fabiato, 1975 a, b, 1978). The purpose of the present article is to study the mechanism of the second type of oscillations. These consist of cycles of a rapid decrease of tension followed by a slower tension redevelopment.
ment. They are observed only during partial (submaximal) activation with Ca\(^{2+}\). They are not observed either during full activation with Ca\(^{2+}\) or when a submaximal contraction is obtained through a non-calcium activation elicited by decreasing [MgATP] in the absence of free Ca\(^{2+}\). It will be demonstrated that these oscillations are generated by the myofilaments themselves rather than by the sarcoplasmic reticulum.

Although these myofilament-generated tension oscillations have not been described, they are apparent during the plateau of tonic tension obtained with partial Ca\(^{2+}\) activation in all our previously published tracings (see, for instance, Fig. 8 A in Fabiato and Fabiato, 1975 b). But in these tracings the amplitude of the oscillations was generally < 10% of the total amplitude of the plateau of tension. Accordingly, conditions that increased the relative amplitude of the oscillations were sought in order to facilitate the study of their mechanism. The largest amplitudes were observed with the smallest preparations made of a few parallel myofibrils (Fig. 1).

The present report explains the mechanism of the myofilament-generated tension oscillations with the help of another property of the partial Ca\(^{2+}\) activation: an increase of tension induced by increasing sarcomere length from 2.20 to ~3.00 \(\mu\)m. This property of partial Ca\(^{2+}\) activation had already been reported by Endo (1972, 1973) for skinned fibers of skeletal muscle. For a long time we failed to make this observation because most of our experiments were done on skinned cardiac cells from the rat ventricle. It will be shown that these cells present a stiff parallel elastic element which is probably constituted by a high density of the elastic protein, connectin, recently described by Maruyama et al. (1976, 1977 a, b). The unloading of this parallel elastic element during the imperfectly isometric contraction caused the apparent active tension elicited by partial Ca\(^{2+}\) activation to decrease when the sarcomere length was increased above 2.20 \(\mu\)m. In contrast, skinned cardiac cells from the frog ventricle presented a compliant parallel elastic element, and their active tension increased when the sarcomere length was increased above 2.20 \(\mu\)m during partial Ca\(^{2+}\) activation. Unfortunately, the myofilament-generated tension oscillations of skinned cells from the frog ventricle were of an irregular configuration that prevented their quantitative analysis. It was not possible to reduce the stiffness of skinned cells from the rat ventricle. In contrast, it was possible to microdissect small bundles of parallel myofibrils from detergent-treated skinned cardiac cells from the dog ventricle (Fig. 1). Upon Ca\(^{2+}\) activation these preparations developed a surprisingly high active tension per cross-sectional area, whereas the resting tension was low, although not as low as in skinned cells from the frog ventricle. During partial Ca\(^{2+}\) activation these preparations presented both a positive slope of the length-tension diagram for sarcomere length increasing from 2.20 to ~2.90 \(\mu\)m and large tension oscillations of reproducible configuration, which permitted quantitative analysis.

From these data a model was derived that describes the oscillations and permits calculation of the maximum (unloaded) velocity of shortening \((V_{\text{max}})\) from the rate of tension redevelopment. The effects of varying [free Ca\(^{2+}\)], [free Mg\(^{2+}\)] or sarcomere length on \(V_{\text{max}}\) and on the maximum level of tension were studied.
The data obtained with skinned cardiac cells from the three different animal species provided complementary information: the skinned cardiac cells from the rat ventricle were the most appropriate for a comparison between the tension oscillations generated by the sarcoplasmic reticulum and those generated by the myofilaments because the Ca\(^{2+}\)-induced release of Ca\(^{2+}\), which is the mechanism of the first type of oscillations, is particularly well developed in the rat ventricle; because of their low stiffness, the skinned cells from the frog ventricle were the only preparations that permitted a complete study of the length-tension diagram; finally, the small bundles of cardiac myofibrils from the dog ventricle were the only preparations used for quantitative studies because they combined tension oscillations of reproducible configuration with a moderate stiffness and because their structure was clearly visible (Fig. 1).

**METHODS**

Skinned cardiac cells were prepared by homogenization of ventricular tissue from rat, dog, and frog into broken single cells, and the remaining fragments of the sarcolemma were removed by microdissection in a relaxing solution (Fabiato and Fabiato, 1975 a, b). The skinned cells from the rat or dog ventricle were 8-13 μm wide and 35-60 μm long when they were stretched to a sarcomere length of 2.50 μm. Either 3-4-μm-wide single skinned cells or 5-7-μm-wide groups of two to four incompletely skinned cells from the frog ventricle were used. The width of the cells was always measured in relaxation, for it decreased during contraction.

The perfusion technique and the computer program used to make up the solutions were the same as previously described (Fabiato and Fabiato, 1975 a, b, 1978). In all cases the pH was adjusted to 7.00 ± 0.02 U with 20 mM (or in a few cases 50 mM) imidazole, and the solutions contained 7 mM glucose. Ethyleneglycol-bis (β-aminoethyl)ether N,N'-tetraacetic acid (EGTA) was used to buffer the [free Ca\(^{2+}\)]. The [free Mg\(^{2+}\)] was generally buffered with ATP, which binds both Mg\(^{2+}\) and Ca\(^{2+}\). Unless otherwise stated, no ADP was added to the solutions, and an ATP-regenerating system made of 14.5 mM disodium creatine phosphate (CP) and 15 U/ml creatine phosphokinase was present. When a very low [MgATP] was used, EGTA was replaced by ethylenediaminetetraacetic acid (EDTA). The free ion concentrations were expressed by their negative logarithms: pCa, pMg, pMgATP. The following apparent stability constants at pH 7.00 were used: CaEGTA, 4.9 × 10\(^6\) M\(^{-1}\) (Schwarzenbach et al., 1957; see Table I in Fabiato and Fabiato, 1978, for correspondence of the pCa values calculated with this stability constant to those that would be obtained by substituting other stability constants from the literature); Mg-EGTA, 40 M\(^{-1}\); CaATP, 5 × 10\(^3\) M\(^{-1}\); MgATP, 1.14 × 10\(^4\) M\(^{-1}\); KATP, 4.2 M\(^{-1}\) (see Fabiato and Fabiato, 1978, for reference to the original studies from which these stability constants were obtained); CaEDTA, 2.4 × 10\(^3\) M\(^{-1}\); MgEDTA, 2.3 × 10\(^3\) M\(^{-1}\) (Portzehl et al., 1964); CaADP, 3.81 × 10\(^3\) M\(^{-1}\); MgADP, 5.92 × 10\(^3\) M\(^{-1}\) (Smith and Alberty, 1956); CaCP, 19.9 M\(^{-1}\); MgCP, 39.8 M\(^{-1}\) (Perrin, 1965). The ionic strength (I/2) was adjusted to either 0.160 M or 0.240 M by the addition of a concentration of KCl that was given by the computer program.

Generally, the sarcoplasmic reticulum was destroyed by treatment of the skinned cells for one-half hour with the nonionic detergent polyoxyethylene 20 cetyl ether (Brij 58, obtained from Sigma Chemical Co., St. Louis, Mo.) in a relaxing solution with pCa 9.0 and 10 mM total EGTA. In a few control experiments the duration of the treatment with Brij 58 was increased to up to 72 h, or Brij 58 was replaced by the nonionic detergent octyl phenoxy polyethoxylethanol (Triton X-100, obtained from Sigma Chemical Co.) at a concentration of 0.5%.
FIGURE 1. Micrographs of two bundles of myofibrils from the dog ventricle during contraction. The A bands and Z lines are clear, whereas the I bands are dark because an anaptral phase contrast was used. For each myofibril bundle the white arrow points to the sarcomere that yielded during the tension oscillations. The yield phenomenon was much more easily detected during the dynamic observation than on these still photographs. In both cases the [total EGTA] was 10 mM, pMg was 3.5, and pMgATP was 2.5; Γ/2 was 0.24 M for the bundle shown in (A) and (B) and 0.16 M for the bundle shown in (C). The oscillograph tracing was obtained from the myofibril bundle that is shown in (A) and (B). Tension was induced by decreasing the pCa from 9.0 to 6.3 at the time indicated by the black arrow. The times at which the pictures were taken are indicated by the electrical signal given by the electronic system that triggered the shutter of the camera. Exposure time was 100 ms. The glass microtool attaching the cell to the transducer is visible at the left side of the pictures. Panel C corresponds to a different bundle of myofibrils, shorter than those generally used in order to show its attachment to both microtools. This myofibril bundle was activated by a higher Ca²⁺ level (pCa 5.8) than the other; in addition, Γ/2 was lower. Despite the fact that this preparation generated ~75% of its maximum tension, the sarcomere pattern is still clearly visible.
Tension was recorded from the ends of the skinned cells with a photodiode force transducer in the same way as previously indicated (Fabiato and Fabiato, 1975). Most of the experiments were done with a transducer having the following characteristics: sensitivity, 500 mV/mg; linearity, 99% for a 10-mg range; drift, 0.001 mg/min; natural frequency, 50 Hz and compliance, 2 μm/mg. Other transducers with different natural frequencies were built for some experiments to ascertain that the tension oscillations were not generated by small oscillations of a resonant lever at a critical frequency. The only modification introduced in the design of these transducers was a variation in the thickness of a sheet of brass used to make the spring to which the lever was attached (Meiss, 1974). By this means the natural frequency of the transducer varied between 100 and 10 Hz when the lever was free and between 50 and 5 Hz when the lever was attached to the preparation. The compliance of these transducers varied between 1-5 μm/mg. The compliance for the entire system, which consisted of the transducer, the microtools, and their attachment to the preparation, varied between 2-10 μm/mg. The range of forces measured was 0.001-0.600 mg, and the most compliant transducers were used only to measure the preparations with the smallest cross-sectional area. In no case did the shortening of the preparations exceed 8% of its length. The tension transducer was calibrated with a voltage-controlled force generator derived from that described by Minns (1971).

The skinned cells from the frog ventricle presented a low resting tension that was not significantly modified by Brij 58 treatment. They could be stretched to a sarcomere length of ~8 μm without breaking. However, the resting tension of this preparation was higher than that of bundles of myofibrils from the frog semitendinosus of the same size, which could also be stretched to > 10 μm sarcomere length without breaking.

Before detergent treatment, 8-13-μm-wide skinned cardiac cells from the dog ventricle presented a high resting tension that exceeded the maximum active tension when they were stretched to a sarcomere length of > 2.60 μm. This had a favorable consequence in that the structures responsible for the resting tension probably prevented the myofibrils from being overstretched during homogenization. After detergent treatment it was possible to gently microdissect these dog ventricular cells into small bundles of myofibrils without stretching them to > 30% of their length in the relaxing solution. The resulting preparations were made of bundles of myofibrils 2-7 μm wide and 20-62 μm long when the sarcomere length was 2.20 μm. These preparations presented a low resting tension: at 3.00 μm sarcomere length, the resting tension was < 25% of the maximum active tension. Finally, these preparations could be stretched to a sarcomere length of 6.0-7.0 μm without breaking.

In 8-15-μm-wide skinned cells from the rat ventricle, the resting tension was higher than in preparations of the same size from the dog ventricle. Destruction of the sarcoplasmic reticulum and the mitochondria by Brij 58 decreased the resting tension observed for sarcomere length between 2.1 and 2.4 μm but not for greater sarcomere length (Fabiato and Fabiato, 1975). The cells broke when an attempt was made to stretch them to a sarcomere length > 3.5 μm. The rat ventricular myofibrils were irregularly arranged and remained laterally connected to other myofibrils after detergent treatment. This prevented their microdissection into small bundles. Rat ventricular skinned cells were also incubated for 2-40 min at a temperature of 22-37°C with 0.05-5.00 mg/ml of elastase (type III from Sigma Chemical Co.). Each combination of a given enzyme concentration, temperature, and duration of incubation that permitted the reduction of the resting tension and the dissociation of the preparation into myofibril bundles also caused the myofibrils to be torn apart as soon as they were stretched. Maruyama et al. (1976, 1977) have provided evidence that the parallel elasticity of muscle cells is borne by an elastic protein, connectin, that is specifically digested by
elastase. Our results suggest that connectin is not only the backbone of the myofibrils but also packs the myofibrils together within the cell and that this elastic protein is not only denser but also stiffer in the rat ventricular cells than in the frog or even the dog ventricular cells.

The stage of the Biovert inverted-microscope (Reichert, Vienna, Austria, American Optical, Buffalo, N.Y.) was replaced by a thermoelectrically controlled heating and cooling stage (Cambion, Cambridge Thermionic Corp., Cambridge, Mass.). Unless otherwise stated, temperature was maintained at 22 ± 0.1°C. The microscope was equipped with differential interference or anoptral phase contrast. Photography and cinematography were done only with anoptral phase contrast. This optical system, which is unique to the Reichert microscope, is a differential phase contrast that eliminates the halo and produces a highly contrasted image on a dark background (Wilska, 1954). With this type of contrast, the I bands appear dark and the A bands and Z lines appear clear (Fig. 1).

To measure the mean sarcomere length and to observe the sarcomeres of the entire preparation, a dry objective with a x63 magnification and a 0.70 numerical aperture was used. For detailed studies of the sarcomere kinetics during the tension oscillations, an oil immersion objective with a x160 magnification and a 1.25 numerical aperture that was corrected for a 170-μm-thick coverslip was used. This objective was covered with oil and placed in contact with the lower surface of the bottom of the perfusion chamber, which was made of glass 140–150 μm thick (i.e., thinner than the glass of a normal coverslip). The two ends of the 8-13-μm-wide skinned cells were impaled with glass microtools to which the myofibrils developed strong adherence. This adherence was sufficient to attach the small bundles of myofibrils without the requirement of an impalement by the microtools. One of the microtools was attached to the lever of the transducer and maintained strictly perpendicular to the longitudinal axis of the myofibrils. The preparation was parallel to and 20–50 μm above the bottom of the chamber. The condenser had a 0.70 numerical aperture, giving a 3-mm working distance above the preparation. The useful resolution of this optical system was 0.3 μm (Fig. 1).

The length of the preparation was generally adjusted by hand-controlled movements of the micromanipulator. However, for stiffness measurements and for changing the length during contraction, the microtool that was not attached to the transducer was connected to a piezoelectric micromanipulator (Burleigh Instruments Inc., E. Rochester, N. Y.) controlled with a specially built unit. Then the length change was effected in either 0.1 or 0.2 s.

During the experiment, the sarcomere length was estimated with a Unitron filar eyepiece micrometer (Unitron Instruments Inc., EPOI, Inc., Woodbury, N.Y.). Precise sarcomere measurements were made on cinematographic or photographic pictures that were projected on a screen with reference to a scale obtained from the photographic picture of a stage micrometer. The sarcomere lengths were monitored continuously during contraction with a 16-mm ciné-camera (1-P, Photo-Sonics, Inc., Burbank, Calif.) at 50 frames per second. To maintain a clear sarcomere pattern during contraction it was necessary to activate all the myofibrils of the preparation as nearly simultaneously as possible. The rapid diffusion of externally applied solutions in the 2-7-μm preparations permitted the sarcomeres to remain visible even during high levels of activation (Fig. 1 C; see Fig. 2 E in Julian et al., 1978 a, for comparison). However, the sarcomere pattern was lost and only the mean sarcomere length was measured during high levels of activation of the 8-13-μm-wide skinned cells from the rat ventricle. To obtain a sufficient illumination for the cinematography, the light source of the Reichert Biovert microscope was replaced by a xenon high-intensity light source (XBO 150 W, Carl Zeiss, Inc., New York) with appropriate heat absorption filters. The high-intensity light source and the
ciné-camera were attached to the top of the Faraday cage to avoid any introduction of vibrations. A square wave that was displayed on the oscillograph triggered an optical signal that was printed on the edge of the ciné-film to permit correlation between tension and sarcomere motion. The average sarcomere length of the preparation was obtained by measuring the length of several (5-20) sarcomeres and dividing the measurement by the number of sarcomeres. The measurements were made in the different areas of the preparation so that the lengths of all the sarcomeres visible in the preparation were used to calculate the mean sarcomere length. In some experiments a still 35-mm camera (Carl Zeiss, Inc.) was used instead of the ciné-camera to obtain a better resolution. The shutter of the camera was triggered electronically, and the triggering system produced an electrical signal that was displayed on the oscillograph to permit comparison between changes in structure and tension (Fig. 1). The exposure time was 100 ms.

RESULTS

Myofilament-Generated Tension Oscillations

In skinned cardiac cells from the rat ventricle, phasic contractions (the first type of tension oscillations) were observed in the presence of a low [total EGTA] (Fabiato and Fabiato, 1975 a). An increase of the [total EGTA] in the buffer resulted in a decrease in the amplitude of these phasic contractions that disappeared when the [total EGTA] was > 0.20-0.40 mM. This finding suggested that these phasic contractions were caused by a cellular sink that competed with the EGTA buffer for Ca²⁺. This Ca²⁺ sink was the sarcoplasmic reticulum inasmuch as the oscillations were not observed when the cells had been treated with high concentrations of caffeine or when the sarcoplasmic reticulum had been destroyed by a detergent. It was also demonstrated that mitochondria had no role in the genesis of these phasic contractions. Thus, these tension oscillations were attributed to cyclic releases from and reaccumulations of Ca²⁺ in the sarcoplasmic reticulum and were used to gather arguments in favor of a Ca²⁺-induced release of Ca²⁺ in skinned cardiac cells (Fabiato and Fabiato, 1975 a).

The major characteristics of these sarcoplasmic reticulum-generated tension oscillations are shown in Fig. 2 A. (a) The phasic contractions constituting these tension oscillations began with a relatively rapid increase of tension above the level of resting tension and ended with a slower relaxation, as shown on the tension-derivative tracing. (b) These phasic contractions were observed in a broad range of pCa, which was even broader than shown in Fig. 2 A. (c) Decreasing the pCa resulted in an increase in the frequency and amplitude of these oscillations.

The second type of tension oscillations, the description of which is the purpose of the present study, were observed in the presence of a high [total EGTA] (Fig. 2 B). The major characteristics of these oscillations were the following: (a) They consisted of the cyclic repetition of a rapid decrease of tension followed by a 10-20-times slower redevelopment of tension, as emphasized by the recording of the tension derivative in which the negative phase was much larger than the positive phase. (b) They were observed only in a narrow range of pCa corresponding to a partial activation of the myofilaments.
FIGURE 2. Tension oscillations generated by the sarcoplasmic reticulum vs. the myofilaments in an 11-μm-wide skinned cardiac cell from the rat ventricle. Tension (T) and its first derivative (dT/dt) were recorded in the presence of pMg 3.5, pMgATP 2.5, and Γ/2 0.16 M. Continuous variation of pCa from 7.2 to 5.7 were obtained by simultaneous perfusions with two solutions of pCa 7.2 and 5.7. The rates of the two perfusions were progressively varied, so that the output of one perfusion was initially zero and the output of the other maximum, whereas the reverse was achieved at the end of the recording. In (A) the myoplasmic [free Ca²⁺] was slightly buffered and the oscillations observed were attributed to cyclic Ca²⁺ releases from and Ca²⁺ reaccumulations by the sarcoplasmic reticulum. In (B) the myoplasmic [free Ca²⁺] was strongly buffered and the tension oscillations were observed for a limited period of time during the decrease of pCa (i.e., they were obtained in a narrow range of pCa). These tension oscillations were deemed generated by the myofilaments themselves.

Consequently, they were seen only for a limited period of time during the progressive decrease of pCa used in the experiment shown in Fig. 2 B.

The tension oscillations of this second type were not generated by the mitochondria, because they were observed in skinned cells treated with 0.005 mM ruthenium red and 10 mM azide, which should inhibit Ca²⁺ transport and binding by the mitochondria.

These tension oscillations were not generated by the sarcoplasmic reticulum, because they were observed in the presence of a [total EGTA] as high as 50 mM, as well as in the presence of 20 mM caffeine or after destruction of the sarcoplasmic reticulum by Brij 58 or Triton X-100. These oscillations were observed in skinned cells that had been treated for up to 72 h with 0.5% Brij 58.
or 0.5% Triton X-100. Except for the data documented in Figs. 2 and 3 D, all the experiments documented in this article were done in the presence of 10 mM total EGTA and on skinned cells that had been treated for one-half hour with 0.5% Brij 58, which is sufficient to destroy irreversibly the capacity of the sarcoplasmic reticulum to accumulate actively and release Ca$^{2+}$ (Orentlicher et al., 1974; Fabiato and Fabiato, 1975 a).

These tension oscillations were not related to an imperfect pH buffering within the preparations, inasmuch as they were not modified by increasing the concentration of imidazole to 50 mM, from the usual concentration of 20 mM.

These tension oscillations were not generated by the transducer because their frequency was not modified by varying the natural frequency of the transducer system from 5 to 50 Hz, whereas their frequency varied considerably when the ionic environment of the preparation was modified. In addition, sarcomeric oscillations were observed under microscope and on ciné-films in skinned cells that were held against the bottom of the perfusion chamber, stretched, and completely immobilized by two noncompliant microtools, neither of which was attached to a transducer. These localized sarcomeric oscillations were similar to those observed during the tension oscillations. However, the ends of the skinned cells had to be attached to microtools in order for the oscillations to occur; no oscillations of this type were observed on unrestrained (freely floating) skinned cells.

Under direct or cinematographic observation, the sarcomere motion during the tension oscillations was clearly visible despite the fact that the amplitude of the sarcomeric oscillations was generally below the theoretical limit of the resolution of the microscopic system. The sarcomeres that moved were easily noticed and localized on account of their rapid movement. Printed individual frames from ciné-film or still photographs taken at different phases in the cycle of tension oscillation confirmed the variations in the length of individual sarcomeres (Fig. 1).

Upon Ca$^{2+}$ activation the sarcomeres contracted homogeneously during the phase of tension development from base line to about the maximum level of tension. Then a few sarcomeres in one or several areas of the preparation rapidly lengthened (yield phenomenon). This corresponded to the phase of rapid decrease of tension. Subsequently, the sarcomeres that had yielded returned slowly to the initial length while the tension slowly redeveloped. The contrast between the abruptness of the yield phenomenon and the slowness of the return to the initial sarcomere length was striking. Correlations between oscillographic and cinematographic or photographic recordings confirmed that the yield phenomenon indeed corresponded to the abrupt decrease of tension (Fig. 1 A). The sarcomeres that yielded were generally the same during subsequent cycles, but, during repeated Ca$^{2+}$ activations of the same preparation, various sarcomeres in various areas of the preparation yielded in an apparently random manner.

Although the qualitative description of the cinematographic observations is derived from several hundreds of films, 44 recordings obtained from 2-7-μm-wide bundles of myofibrils from the dog ventricle were reviewed to provide some statistical information relative to the number and distribution of the
sarcomeres that yielded. The length of each cell was segmented for analysis in two central and two peripheral (i.e., close to the microtools) quarters. The yield occurred in 24 cases within the two central quarters (Fig. 1 C), in 18 cases in one of the two peripheral quarters (Fig. 1 A and B), and in two cases in both one of the central and one of the peripheral quarters. In no case were the sarcomeres that yielded during the tension oscillations longer than the others in the relaxed preparation. In three preparations that were at little more than slack length, one segment had sarcomeres 0.2-0.3 μm shorter than any other sarcomere in the relaxed preparation, and this segment was precisely that which yielded during contraction. When the yield phenomenon occurred in the central quarters of the preparation, it was often extended to several sarcomeres with a maximum in one or two sarcomeres, while the adjacent sarcomeres had lengths intermediary between this maximum and the sarcomere length of the nonyielding segments of the preparation. Thus, it was difficult to decide how many sarcomeres were yielding. In contrast, when the yield occurred in one of the peripheral quarters it was often limited to one or two sarcomeres (Fig. 1 A and B). Then the lengths of the other sarcomeres of the preparation did not change significantly during the tension oscillation because the yield was limited to 5 to 10% of the length of the preparation. This facilitated the quantitative analysis used for testing the model that will be proposed for the tension oscillations.

In 2-7-μm-wide bundles of myofibrils from the dog ventricle, the entire cross section of the preparation lengthened at the level of the segments that yielded. These preparations presented tension oscillations of an amplitude of 30-50% of the total amplitude of the tension developed. In contrast, in 8-13-μm-wide skinned cells from the rat ventricle, the yield occurred in several locations in the preparation, each of them being limited to a small fraction of the cross section. The tension oscillations of these latter preparations were only ~10% of the total amplitude of the tension developed. The tension oscillations of the rat ventricular skinned cells were also about five times faster than those of dog ventricular preparations of the same size. Finally, the tension oscillations had the same characteristics in the skinned cells from the frog ventricle as in the two other tissues, but in the frog ventricular cells the sarcomere yielding was generally multifocal. This resulted in tension oscillations of irregular configuration. Accordingly, only the small bundles of dog cardiac myofibrils were used for quantitative analysis of the tension oscillations.

The myofilament-generated tension oscillations were observed only during a partial activation with Ca²⁺. In the presence of a pMg 3.5, pMgATP 2.5, and Γ/2 0.16 M, tension oscillations were obtained only when the pCa was between 6.4 and 5.7; they were never observed in the presence of a full Ca²⁺ activation with pCa 5.0 (Fig. 3 A and C). Tension oscillations were not observed, either, when a submaximal tension was induced by decreasing [MgATP] to pMgATP 5.5 in the virtual absence of free Ca²⁺ (Fig. 3 D). These conditions are known to produce a rigor tension (Weber and Murray, 1973; Fabiato and Fabiato, 1975 b). Finally, the tension oscillations were never observed when the mean sarcomere length of the preparations was >3.00-3.10 μm.

Once initiated, the oscillations could continue for as long as 20 min. In some cases, however, they stopped after a decrease in both frequency and amplitude
FIGURE 3. Requirement of a partial Ca\(^{2+}\) activation for the occurrence of the myofilament-generated tension oscillations. In this and in all the subsequent figures, the cells had been treated for 1 h with 0.5% Brij 58 and, unless otherwise stated, the [total EGTA] was 10 mM; arrows correspond to the solution changes. All the data shown in this particular figure were obtained from skinned cardiac cells from the rat ventricle in the presence of an I/2 of 0.16 M. In (A–C) pMg was 3.5 and pMgATP was 2.5. (A) Effects of varying the pCa of the activating solution on the level of tension and on the occurrence of myofilament-generated tension oscillations in a 12-μm-wide cell. (B) Spontaneous interruption of the tension oscillations in an 11-μm-wide cell. (C) Effect of varying pCa on the level of tension (continuous curve) and on the frequency of observation of tension oscillations (dotted curve) in 8–13-μm-wide cells. The tension developed by a given cell at each pCa was expressed as a percentage of the tension developed by the same cell at pCa 5.0. The frequency of observations of tension oscillations at a given pCa was expressed as a percentage of the total number of observations made in all the cells at this pCa. For the tension-pCa curve, each point represents the mean and each vertical bar represents 1 SD, which is shown on only one side for clarity. (D) Absence of tension oscillations when a submaximal tension was elicited by increasing pMgATP in the virtual absence of free Ca\(^{2+}\) in a 9-μm-wide cell. Initially pCa 6.0 was obtained in the presence of 10 mM total EGTA and pMg 3.5. After the solution change, pMgATP 5.5 was obtained with 1.0 mM total ATP, 10.0 mM total EDTA, and 0.90 mM total magnesium; the pMg of this solution was 6.4, and it contained 0.21 mM total calcium.
(Fig. 3 B). In all cases in which a spontaneous interruption of the oscillations was observed, they stopped at or slightly below the level of the maximum tension reached during the oscillations. Thus, it was decided that the maximum level of tension reached during the oscillations at a given pCa was most likely to correspond to the isometric tension that would have been developed in the absence of oscillations ($P_o$). This was confirmed by comparing, in the same cells, $P_o$ defined by this criterion when the cells presented tension oscillations to $P_o$ obtained when these cells happened to lack tension oscillations despite the fact that they were activated with the same pCa. Accordingly, the maximum level of tension reached during the oscillations was used as $P_o$ for plotting tension-pCa curves (Figs. 3 C and 5) and length-tension diagrams (Figs. 4, 6, and 7) in the presence of tension oscillations.

In conclusion, the occurrence of myofilament-generated tension oscillations required three conditions: (a) that the activation was induced by Ca$^{2+}$ rather than by rigor, (b) that this Ca$^{2+}$ activation was partial, and (c) that the sarcomere length was shorter than 3.00-3.10 $\mu$m. The observation of tension oscillations in 90% of the cases under appropriate conditions (Fig. 3 C) renders it unlikely that they were caused by some physical damage to the preparations.

Sarcomere Length-Tension Diagrams

To obtain length-tension diagrams, the data from preparations of different widths and thicknesses were normalized by expressing the resting or active tension developed by a given preparation as a percentage of the maximum tension developed by the same preparation in the presence of a saturating [free Ca$^{2+}$] (pCa 5.0) and at the optimal sarcomere length. Preliminary experiments showed that the optimal sarcomere length was $\sim$2.20 $\mu$m when measured during the plateau of contraction. Accordingly, the resting sarcomere length was set at $\sim$2.25-2.30 $\mu$m with the filar eyepiece micrometer so that the sarcomere length could be expected to reach 2.20 $\mu$m during the plateau of contraction. This measurement, however, was imprecise and had to be corrected by precise measurements made on the frames of the ciné-film. Consequently, the optimal sarcomere length that was reached during the plateau of contraction was not exactly 2.20 $\mu$m but varied between 2.10 and 2.30 $\mu$m, as shown by the bold solid bar in Figs. 4, 6, and 7 for each tissue. Experiments showed that the tension induced by full Ca$^{2+}$ activation of a given cell did not change significantly when the active sarcomere length varied in this range. Thus, the maximum tension was defined as the tension developed by a given cell in the presence of pCa 5.0, pMg 3.5, and pMgATP 2.5 at the sarcomere length defined by the bold solid bar for each tissue. The maximum tension was measured before and after any change of the sarcomere length or of the activating solution and, when the two measurements differed, the mean of these two determinations of maximum tension was used as a reference. The measurements were discarded, however, when the maximum tension had decreased by >10% of its original value.

In the skinned cells from the frog ventricle, the resting tension increased slowly and at a nearly constant rate when the sarcomere length was increased from 2.20 to 3.80 $\mu$m (Fig. 4, ▲). This low stiffness is consistent with Winegrad's
hypothesis that the high resting stiffness of the intact frog cardiac muscle is caused by extracellular connective tissue (see Fig. 6 in Winegrad, 1974).

The curve of tension induced by decreasing the [MgATP] to pMgATP 5.5 in the virtual absence of free Ca$^{2+}$ showed a plateau for sarcomere length varying

![Diagram](image-url)

**Figure 4.** Effect of varying the mean sarcomere length on the resting tension measured at pCa 9.0 (▲), the active tension obtained for a partial Ca$^{2+}$ activation with pCa 6.0 (△), for rigor activation by pMgATP 5.5 with pCa 9.2 (●), and for a full Ca$^{2+}$ activation (□) with pCa 5.0 in 3-7 µm wide either completely or partly skinned cells from the frog ventricle. In all cases the tension developed by a given cell at a given sarcomere length and pCa was expressed as a percentage of the tension developed by the same cell at pCa 5.0 and at a sarcomere length between 2.13 and 2.25 µm (bold solid bar). The active tension was plotted as a function of the sarcomere length reached during the plateau of contraction, which was 0.03-0.12 µm less than the resting sarcomere length. During partial Ca$^{2+}$ activation, the amplitude of tension ($P_o$) was measured by the difference between the maximum level of the tension reached during the oscillations and the resting tension. The curves for $P_o$ at pCa 6.0/3.0 and for rigor activation do not pass exactly through the mean of the measurements. The composition of the solution used to obtain pMgATP 5.5 is given in Fig. 3. The application of this solution was always preceded by a perfusion in a relaxing solution at pCa 9.0 and pMgATP 2.5. In the other solutions, the total EGTA was 10 mM, pMg was 3.5, pMgATP was 2.5, and $\Gamma/2$ was 0.16 M.
between 2.00 and 2.35 μm (Fig. 4, ●). An increase of sarcomere length above 2.35 μm resulted in an almost linear decrease of this rigor tension (Weber and Murray, 1973; Fabiato and Fabiato, 1975 b) that was annihilated at a sarcomere length of 3.80 μm. This curve represents the first demonstration of a descending limb of the length-tension diagram in cardiac muscle. The high resting tension observed at a supraoptimal sarcomere length in intact multicellular preparations of cardiac muscle had prevented the demonstration of a descending limb of the length-tension diagram in these preparations (Krueger and Pollack, 1975; Julian and Sollins, 1975; Jewell, 1977). The only difference between this curve of rigor tension as a function of sarcomere length obtained in skinned frog cardiac cells and the curve reported by Gordon et al. (1966) for the tetanic tension of the intact skeletal muscle fibers is that the sarcomere lengths corresponding respectively to the end of the plateau and to the annihilation of the tension were both 0.15 μm greater in the former than in the latter preparation. This may be explained by a difference in the length of the thin filaments in the two tissues (Page, 1974; Robinson and Winegrad, 1977).

The curve of maximum Ca²⁺-activated tension induced in skinned cells from the frog ventricle by pCa 5.0 in the presence of a pMgATP 2.5 differed from the curve reported by Gordon et al. (1966) in that it presented an abrupt change of slope at a sarcomere length of ~3.10 μm (Fig. 4, □). The tension decreased by <20% when sarcomere length increased from 2.35 to 3.10 μm but decreased steeply at sarcomere lengths of >3.10 μm. The only statement that can be made about the homogeneity of the sarcomere length is that no visible sarcomere was shorter than 2.70 μm when the mean sarcomere length was 3.00 μm. Obvious unhomogeneity with differences in sarcomere lengths of >0.3 μm was observed only when the mean sarcomere length of the preparation was >3.00 μm, and the degree of unhomogeneity increased with the sarcomere length. Although the entire preparation was observed, it was not possible to see the structure of the sarcomere in which each microtool was impaled and that of the next sarcomere because of the light diffraction by the microtool. Yet it is unlikely that these hidden sarcomeres could be responsible for the large tension still developed at 3.00-μm mean sarcomere length during full Ca²⁺ activation, especially because no similar finding was observed in the same preparations during rigor tension.

Finally, the most important finding for the present study was that the tension induced by partial Ca²⁺ activation of skinned cells from the frog ventricle with pCa 6.0 increased as the sarcomere length was increased from 2.20 to about 3.00 μm (Fig. 4, ◄ and ----). The ratio between the tension developed at pCa 6.0 at a given sarcomere length and the tension developed at pCa 5.0 at the same sarcomere length increased linearly when the sarcomere length was increased from 2.10 to ~3.10 μm (Fig. 4, continuous curve). When the sarcomere length was increased above 3.10 μm, P_{o,pCa 6.0} decreased steeply while the ratio P_{o,pCa 6.0}/P_{o,pCa 5.0} remained constant (~91%).

The increase in relative tension by increasing sarcomere length from 2.20 to 3.00 μm was not limited to an activation with pCa 6.0. An increase in the sarcomere length from 2.20-2.30 μm to 2.60-2.70 μm and from 2.60-2.70 μm to 3.00-3.10 μm produced a shift to the left of the force-pCa curve (Fig. 5). Thus,
increasing sarcomere length produced an apparent increase in the sensitivity of the myofilaments to Ca\(^{2+}\) (Fig. 5).

Although these results are in general agreement with those first reported by Endo (1972, 1973) in skinned fibers from frog skeletal muscle, we did not observe in skinned cardiac cells the following three phenomena reported by Endo: (a) a limitation of the apparent increase of sensitivity to Ca\(^{2+}\) by increasing sarcomere length to a very low range of [free Ca\(^{2+}\)], (b) a decrease of the slope of the force-pCa curve by increasing sarcomere length, and (c) a rate of tension development of individual contractions upon Ca\(^{2+}\) activation higher at long than at short sarcomere lengths. In agreement with our results, however, Moisescu and Thieleczek (1978) have obtained in the same preparation as used by Endo a parallel shift of ~0.1 pCa unit of the relative tension-pCa curve when the mean sarcomere length was increased from 2.25 to 2.60 \(\mu\)m.

In the skinned cells from the rat ventricle, the resting tension increased considerably more steeply as the sarcomere length increased than in the cells from the frog ventricle (Fig. 6). In addition, the level of tension developed during partial Ca\(^{2+}\) activation of skinned cells from the rat ventricle decreased steeply when the sarcomere length was increased from 2.20 to 2.70 \(\mu\)m.
Figure 6. Effect of varying the mean sarcomere length on the resting tension measured at pCa 9.0 (△), the active tension obtained for a partial Ca\(^{2+}\) activation with pCa 6.0 (□) and for a full Ca\(^{2+}\) activation with pCa 5.0 (□) in 8-13-μm-wide skinned cells from the rat ventricle. The pMg was 3.5, pMgATP was 2.5, and Γ/2 was 0.16 M. The tension developed by a given cell at a given sarcomere length and pCa was expressed as a percentage of the tension developed by the same cell at pCa 5.0 and at a sarcomere length between 2.12 and 2.23 μm (bold solid bar). For active tension, tension was plotted as a function of the sarcomere length reached during the plateau of contraction.

The curve of tension induced by full Ca\(^{2+}\) activation presented no plateau in the rat ventricular cells and decreased much more rapidly than in the frog tissue when the sarcomere length was increased. The extrapolation of the curve to zero tension would correspond to a sarcomere length of only 2.90-3.00 μm.

These observations suggest the following interpretation of the discrepancies between the data obtained from the two tissues. Because of the compliance of the transducer, the average sarcomere length decreased by 0.03-0.12 μm during contraction. If it had been measured, the resting tension at this sarcomere length (reached during contraction) would have been found smaller than the resting tension actually measured before and after contraction. The difference would have been small in the frog preparations but considerable in skinned cells from the rat ventricle because of the steepness of the length-resting tension
relation. Because at least part of the resting tension was borne by elastic elements parallel to the contractile elements, the shortening unloaded the parallel elastic elements and thereby reduced the amplitude of the plateau of tension. Thus, the true force generated by the contractile elements at a given sarcomere length was much larger than actually recorded, and this discrepancy increased as the length-passive tension relation became steeper at greater sarcomere lengths.

The results obtained in the small bundles of myofibrils from the dog ventricle (Fig. 7) were intermediary between those obtained in the two other animal species. The resting tension of the dog preparation increased at a slow rate

![Graph]{https://example.com/graph.png}

**FIGURE 7.** Effect of varying the mean sarcomere length on the resting tension measured at pCa 9.0 (△), the active tension obtained for a partial Ca\(^{2+}\) activation with pCa 6.0 (△), for a partial activation by pMgATP 5.5 with pCa 9.2 (○) and for a full activation with pCa 5.0 (□) in 2-7-μm-wide bundles of myofibrils from the dog ventricle. For both resting tension and active tension induced by partial Ca\(^{2+}\) activation, the triangles pointing upward correspond to > 5-μm-wide bundles of myofibrils, whereas the triangles pointing downward correspond to < 5-μm-wide bundles. No distinction between these two sizes of preparations is made for the other symbols. In all cases, tension developed by a given cell at a given sarcomere length and pCa was expressed as a percentage of the tension developed by the same cell of pCa 5.0 and at a sarcomere length between 2.13 and 2.27 μm (bold solid bar). The composition of the solutions used to obtain pMgATP 5.5 is given in Fig. 3. In the other solutions, the total EGTA was 10.0 mM, pMg was 3.5, pMgATP was 2.5, and Γ/2 was 0.16 M.
when the sarcomere length was increased up to \( \sim 2.80 \ \mu m \). In this range the data were similar to those obtained in the frog cells, including the occurrence of a positive slope of the length-tension diagram for partial \( \text{Ca}^{2+} \) activation. At sarcomere lengths \( \geq 2.80 \ \mu m \), the resting tension increased steeply as the sarcomere length increased, and the measured active tension for both full and partial activation decreased more rapidly than in skinned cells from the frog ventricle. This is consistent with the hypothesis of the unloading of a parallel elastic element during the imperfectly isometric contraction. Finally, the rigor tension induced by decreasing \([\text{MgATP}]\) to \( p\text{MgATP} \) 5.5 decreased when the sarcomere length was increased above 2.20 \( \mu m \) (Fig. 7, ○). The deviation of the few points corresponding to sarcomere length \( \geq 2.85 \ \mu m \) from the straight line can also be explained by the unloading of a parallel elastic element during the contraction.

In conclusion, the observation of a positive slope of the length-tension diagram required exactly the same three conditions that were necessary for the occurrence of myofilament-generated tension oscillations: (a) activation by \( \text{Ca}^{2+} \), (b) partial activation, and (c) sarcomere length shorter than \( \sim 3.00 \ \mu m \). This similarity between the two phenomena led to the proposal of a model explaining the tension oscillations on the basis of an increase of the apparent sensitivity of the myofilaments to \( \text{Ca}^{2+} \) by increasing sarcomere length.

**Mechanism of the Tension Oscillations and Factors Influencing Their Characteristics**

It is proposed that the tension oscillations are caused by sarcomeres that are less activated (or weaker, as will be proposed in the Discussion) and are consequently rapidly stretched (yield phenomenon) to a greater length during the contraction of the other sarcomeres. This stretch increases the tension developed by the initially less activated sarcomeres because of the sarcomere length-tension relation observed during partial activation. The increased activation of the contractile element and the parallel elastic tension cause these sarcomeres to contract and shorten until they again inactivate and a new cycle begins.

According to this rationale, a mathematical model was developed which describes the tension oscillations (Appendix). This model is based on Hill's equation (1938) applied to the reshortening of the small number of sarcomeres that have yielded. The model shows that the rate of tension redevelopment is proportional to \( V_{max} \) (the maximum velocity of reshortening of the sarcomeres that have yielded) and to the stiffness and inversely proportional to \( P_o \). The model permits the derivation of a value for \( V_{max} \) per sarcomere that may reflect indirectly the rate of reattachment of the cross-bridges. This rate of cross-bridge attachment has been inferred previously in models describing different types of tension oscillations observed in glycerinated preparations of various striated muscles (Steiger, 1971; White and Thorson, 1973). Unlike previous studies, however, the present study includes structural observations which demonstrate that the sarcomeric oscillations are not homogeneous throughout the preparation. Consequently, these data and proposed model (Appendix) indicate that a modification in the frequency of the oscillations induced by physical, ionic, or metabolic interventions do not permit any inference relative to the effect of
these interventions on $V_{\text{max}}$ per sarcomere and the cross-bridge kinetics unless the number of oscillating sarcomeres is observed under microscope. Accordingly, the effects of varying the concentrations of ions and metabolites will be described only for the small bundles of cardiac myofibrils from the dog ventricle because that was the only preparation for which quantitative studies using microscopic information were done. Qualitative (Fig. 8) and quantitative (Table I) data will be discussed simultaneously to point out the inferences that cannot be made from some of the observed modifications in the frequency of the oscillations.

Calculations of $V_{\text{max}}$ from the model were done only in small bundles of cardiac myofibrils from the dog ventricle in which the yield of the sarcomeres was limited to one segment of the length of the preparation with all the myofibrils yielding at this level. When two different segments were yielding, as in the experiment of Fig. 8 A, the time-course of the phase of tension redevelopment was irregular and did not follow the prediction of the model. It was also necessary that the location of the yielding segment did not change during the interventions studied.

Increasing [free Ca$^{2+}$] produced an increase in both $P_o$ (Fabiato and Fabiato, 1975 a) and the frequency of the tension oscillations (Fig. 8 A and B). The increase in frequency of the oscillations was mainly caused by an increase in the rate of tension redevelopment. Calculations with the mathematical model showed that increasing [free Ca$^{2+}$] increased significantly $V_{\text{max}}$ (preparation nos. 1–3 in Table I), which is consistent with the data of Julian (1971) and Wise et al. (1971) in glycerinated skeletal muscle fibers but inconsistent with the data of Podolsky and Teichholz (1970) in skinned skeletal muscle fibers. Thames et al. (1975) have explained the discrepancy between the data by Podolsky and Teichholz and those by Julian on the basis of the lower ionic strength used by Julian. The present data in skinned cardiac cells show that both $P_o$ and $V_{\text{max}}$ were lower at the same pCa in the presence of a $\Gamma/2$ of 0.24 M as compared to 0.16 M, but increasing [free Ca$^{2+}$] still increased $V_{\text{max}}$ in the presence of 0.24 M $\Gamma/2$ (preparation nos. 4–6 in Table I). These data show larger percentages of increase of $V_{\text{max}}$ than produced by increasing extracellular [Ca$^{2+}$] in intact cardiac muscle (Brutsaert et al., 1973). However, even large variations of the extracellular [Ca$^{2+}$] are unlikely to change the myoplasmic [free Ca$^{2+}$] reached during contraction by more than ~0.1 pCa unit if a Ca$^{2+}$-induced release of Ca$^{2+}$ from the sarcoplasmic reticulum is operating (see Fig. 9 in Fabiato and Fabiato, 1975 a).

An increase of [free Mg$^{2+}$] with no modification of [MgATP] or [free Ca$^{2+}$] decreased both $P_o$ (Fabiato and Fabiato, 1975 b) and the rate of tension redevelopment (Fig. 8 C). The model showed that an increase of [free Mg$^{2+}$] decreased both $P_o$ and $V_{\text{max}}$ (preparation nos. 7–9 in Table I). Thus, Mg$^{2+}$ in the millimolar range appears to compete with Ca$^{2+}$ in the micromolar range in its regulatory roles on both the amount of tension developed and the velocity of shortening. Even in the presence of a pMg of 2.5, an increase of [free Ca$^{2+}$] increased $V_{\text{max}}$ (preparation no. 10 in Table I).

An increase of [MgATP] with no modification of [free Mg$^{2+}$] or [free Ca$^{2+}$] slightly decreased $P_o$ (Fabiato and Fabiato, 1975 b) but dramatically increased
FIGURE 8. Effects of varying pCa (A and B), pMg (C), pMgATP (D), [ADP]/[ATP] ratio (E), or mean sarcomere length (F) on the level of tension ($P_0$) and the tension oscillations in six different bundles of myofibrils from the dog ventricle. All preparations had a width of ~5 μm. All solutions had a $I/2$ of 0.16 M. Unless indicated otherwise, pMg was 3.5, pMgATP was 2.5, and no ADP was added. In (E) the pMg was maintained constant by appropriate variations of the total magnesium concentration, and creatine phosphate and phosphokinase were deleted from the ADP-containing solution. The mean sarcomere length was continuously monitored during contraction and was between 2.25 and 2.35 μm in panels A–E.
| Preparation | Width | Sl. | M | pCa | pMg | $P_o$ | $V_{max}$ | $\mu$m/s per sarcomere |
|-------------|-------|----|---|-----|-----|-------|-----------|-----------------------|
| No. 1       | 5     | 2.31 | 0.16 | 6.3 | 3.5 | 0.112 | 5.75      | 18.60                |
|             |       |     |    |     |     |       |           |                       |
| No. 2       | 3     | 2.24 | 0.16 | 6.2 | 3.5 | 0.048 | 9.22      | 21.11                |
|             |       |     |    |     |     |       |           |                       |
| No. 3       | 5     | 2.71 | 0.16 | 6.3 | 3.5 | 0.152 | 4.28      | 15.02                |
|             |       |     |    |     |     |       |           |                       |
| No. 4       | 3     | 2.78 | 0.24 | 6.2 | 3.5 | 0.035 | 3.74      | 6.27                 |
|             |       |     |    |     |     |       |           |                       |
| No. 5       | 4     | 2.27 | 0.24 | 6.2 | 3.5 | 0.051 | 2.84      | 7.11                 |
|             |       |     |    |     |     |       |           |                       |
| No. 6       | 5     | 2.32 | 0.24 | 6.2 | 3.5 | 0.082 | 2.24      | 6.12                 |
|             |       |     |    |     |     |       |           |                       |
| No. 7       | 5     | 2.27 | 0.16 | 6.2 | 3.5 | 0.161 | 8.24      | 4.20                 |
|             |       |     |    |     |     |       |           |                       |
| No. 8       | 4     | 2.41 | 0.16 | 6.3 | 3.5 | 0.107 | 6.11      | 3.07                 |
|             |       |     |    |     |     |       |           |                       |
| No. 9       | 4     | 2.33 | 0.16 | 6.2 | 3.5 | 0.141 | 9.12      | 4.21                 |
|             |       |     |    |     |     |       |           |                       |
| No. 10      | 5     | 2.34 | 0.16 | 6.3 | 2.5 | 0.102 | 3.18      | 7.04                 |
|             |       |     |    |     |     |       |           |                       |
| No. 11      | 4     | 2.19 | 0.16 | 6.0 | 3.5 | 0.171 | 19.14     | 19.04                |
|             |       |     |    |     |     |       |           |                       |
| No. 12      | 5     | 2.17 | 0.16 | 6.0 | 3.5 | 0.201 | 20.01     | 18.17                |
|             |       |     |    |     |     |       |           |                       |
| No. 13      | 3     | 2.20 | 0.16 | 6.0 | 3.5 | 0.099 | 21.12     | 21.78                |
|             |       |     |    |     |     |       |           |                       |
| No. 14      | 5     | 2.31 | 0.16 | 6.1 | 3.5 | 0.115 | 14.92     | 8.80                 |
|             |       |     |    |     |     |       |           |                       |
| No. 15      | 5     | 2.21 | 0.16 | 6.0 | 3.5 | 0.248 | 20.12     | 15.10                |

Only the experimental condition that has been changed is indicated on the second line for each preparation. The number of sarcomeres in the length of the preparations varied between 16 and 27. In all cases only a single one-sarcomere-long segment yielded at the same level in all the myofibrils of the preparation. The data of the following preparations are illustrated in the following figures: No. 1 in Fig. 8 B, No. 7 in Fig. 8 C, No. 14 in Fig. 8 F.
the frequency of the tension oscillations (Fig. 8 D). In the absence of structural observations this result might suggest that increasing [MgATP] in the millimolar range increases the rate of attachment of the cross-bridges. In fact, it was observed directly under microscope and on ciné-film that increasing [MgATP] from pMgATP 2.5 to pMgATP 1.7 dramatically increased the number of sarcomeres oscillating. This suggested that $V_{\text{max}}$ per sarcomere was not necessarily increased. Unfortunately, when presumably ~50% of the sarcomeres were yielding, localizing and counting them on ciné-film became impossible, and printed individual frames of the film failed to detect measurable differences in the length of individual sarcomeres. Accordingly, these experiments did not provide quantitative data on a possible effect of increasing [MgATP] in the millimolar range on the cross-bridge kinetics but indicated that if such an effect exists, it is much smaller than suggested by the modification of the frequency of the tension oscillations (Fig. 8 D).

The partial replacement of ATP by ADP without modifying [free Ca$^{2+}$] or [free Mg$^{2+}$] caused a dramatic decrease in the rate of decrease of tension followed by a disappearance of the oscillations and by an increase of $P_o$ (Fig. 8 E). Here again, quantitative derivations from the model were not possible, because with ADP the amplitude of the sarcomeric oscillations was so small that it was not possible to decide whether ADP changed the number of sarcomeres oscillating.

The amplitude of the tension oscillations increased when the mean sarcomere length during contraction was increased to an optimum of ~2.30-2.40 μm. Increasing sarcomere length above this optimum increased $P_o$ but either did not modify the amplitude of the tension oscillations or else slightly decreased it (Fig. 8 F). Calculations from the mathematical model showed that an increase of the mean sarcomere length did not modify $V_{\text{max}}$ in the low range of sarcomere length (preparation nos. 11-13 in Table I) or decreased it in a high range of sarcomere length (preparation nos. 14 and 15 in Table I). Thus, the effects of increasing sarcomere length on $V_{\text{max}}$ (i.e., probably on the rate of cross-bridge formation) were different from those of increasing [free Ca$^{2+}$], although both interventions had similar effects on $P_o$. Finally, the model explains that increasing the mean sarcomere length to >3.00-3.10 μm abolishes the tension oscillations. Then, the sarcomeres that had yielded would be further deactivated by their lengthening so that no tension redevelopment would occur.

A decrease of temperature with an appropriate correction of the pH and constant [free Ca$^{2+}$], [free Mg$^{2+}$], [MgATP], and sarcomere length decreased the frequency of the oscillations with a $Q_{10}$ of 3.1 (data not shown).

The model suggests that the higher stiffness of the rat ventricular cells could explain the higher frequency of the oscillations observed in this preparation as compared to the frog or dog cells without necessarily implying a difference in the cross-bridge kinetics between the animal species.

**DISCUSSION**

Many types of tension oscillations have been described in partially activated cardiac (Steiger, 1971), skeletal and insect flight muscles (see White and Thorson, 1973, for review). The tension oscillations described in the present
study are different in their characteristics because, unlike the previously described oscillations, they are spontaneous and asymmetrical. In addition, previous descriptions of tension oscillations do not include simultaneous microscopic observations. Probably the most relevant findings are those of Bonner and Carlson (1975), who demonstrated by laser light-scattering studies that structures much larger than the cross-bridges moved during contraction, but these studies were done in skeletal muscle. Accordingly, the discussion will be limited to that of the mechanism and physiological relevance of the tension oscillations described in the present study. We have proposed that these tension oscillations are caused by the yield of some sarcomeres that subsequently redevelop tension because their passive lengthening increases their activation. But neither the cause of the yield nor the mechanism whereby lengthening increases activation have been clarified yet. This discussion, therefore, will focus on these two problems.

Cause and Physiological Relevance of the Yield Phenomenon

Because the occurrence of the tension oscillations corresponds to a region of the force-pCa curve for which force varies very rapidly when the pCa is varied, it is plausible that any type of small difference may cause some sarcomeres to be less activated. Only in 3 cases among 44 observations could the lesser activation be explained by a shorter length of some sarcomeres before activation. Although the yield phenomenon was not preferentially located in a narrower area of the preparation, the homogeneity of the thickness along the preparation could not be accurately ascertained under optical microscopy. Similarly, it could not be excluded that, for instance, the connectin (Maruyama et al., 1976, 1977 a, b) was unequally distributed along the preparation. Thus, it seems very difficult to eliminate the possibility that all preparations presented some slightly weaker sarcomeres. Alternatively, it could be proposed that the number of cross-bridges formed per sarcomere could vary in a random manner during partial activation with Ca\textsuperscript{2+}. After some sarcomeres had yielded because they happened to have fewer cross-bridges formed when the other sarcomeres developed $P_o$, they could be modified by the stretch so that the yield would occur repetitively at the level of these sarcomeres during a given contraction, as was generally observed. This hypothesis of a random location of the initial yield was supported by the observation that the yielding location changed when two applications of an activating solution were separated by a return to relaxing solution.

The major argument against the hypothesis that the yield phenomenon could be an artifact caused by the technique used for preparing skinned cardiac cells was the observation of this phenomenon in more intact cardiac tissues. The demonstration relied mostly on microscopic observations that permitted the distinction of the sarcomere motion accompanying the myofibrilament-generated tension oscillations from that occurring during the sarcoplasmic reticulum-generated tension oscillations. In skinned cardiac cells, the yield phenomenon, corresponding to the myofibrilament-generated tension oscillations, was localized in one or a few sarcomeres and never propagated throughout the preparation. In contrast, the phasic contractions caused by Ca\textsuperscript{2+}-induced release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum corresponded to an apparently homogeneous con-
traction (within the limits of the resolution of the optical system) of all the sarcomeres of the preparation in single skinned cardiac cells. In larger preparations made of several cardiac cells with disrupted but not skinned sarcolemmas, the contractions elicited by Ca$^{2+}$-induced release of Ca$^{2+}$ from the sarcoplasmic reticulum propagated as a wave at a velocity of 50–100 μm/s along the preparation (Fabiato and Fabiato, 1975b). In addition, the myofilament-generated tension oscillations were observed, with the same general characteristics, in the three skinned preparations studied, including the single skinned cells from the frog ventricle in which the sarcoplasmic reticulum-generated tension oscillations were absent (Fabiato and Fabiato, 1975a).

Directly under microscope and on ciné-film we have observed sarcomere oscillations with rapid yield and slow reshortening in preparations of frog ventricular muscle made of ~20 cells that were chemically skinned with 10 mM total EGTA and partially activated by pCa 6.0. However, tension recording from the end of these preparations showed sinusoidal oscillations of <5% of $P_0$. Both the decrease in amplitude of the tension oscillations and the loss of their asymmetrical character were attributed to the asynchrony of these tension oscillations in different areas of the preparation. This interpretation was supported by the observation of large tension changes occurring at irregular time intervals that were attributed to the occasional synchronization of the myofilament-generated tension oscillations. The same type of chaotic sarcomere motion was observed in 50-μm-wide trabeculae of frog ventricle that were not chemically skinned but were either depolarized by high [KCl] solutions or perfused with 20 mM caffeine in the presence of 10 mM [Ca$^{2+}$].

These multifocal sudden sarcomeric lengthenings followed by much slower shortenings were clearly different from the slow waves of contraction that were superimposed on them in depolarized mammalian ventricular trabeculae and were attributed to a propagated Ca$^{2+}$-induced release of Ca$^{2+}$ from the sarcoplasmic reticulum. Finally, the rapid (10 Hz) stimulation of the mammalian cardiac trabeculae treated with caffeine increased the level of base-line tension into a perfectly smooth plateau of contraction. Yet, microscopic observations during this apparent cardiac tetanus showed the same chaotic multifocal sarcomeric oscillations that demonstrated the unhomogeneity of this partial contractile activation.

These observations indicate that the myofilament-generated tension oscillations are not a peculiarity of the skinned cardiac cells but represent a property of partially activated cardiac myofilaments apparent under microscope in all the preparations that we have studied.

Apparent Increase of the Sensitivity of the Myofilaments to Ca$^{2+}$ Caused by an Increase of Sarcomere Length during a Partial Ca$^{2+}$ Activation

The present data show that the length-tension diagram of rigor tension follows the prediction of the cross-bridge theory (Gordon et al., 1966). This renders it unlikely that the results obtained during Ca$^{2+}$ activation are caused by some physical damage of the skinned cardiac cells. The latter results are not necessarily arguments against the cross-bridge theory (Noble and Pollack, 1977) but may suggest that some additional length-dependent regulatory mechanisms
are unmasked during Ca\textsuperscript{2+}-induced contraction, possibly because the cross-bridges are less stable than in rigor.

Already difficult to explain on the basis of the sole cross-bridge theory is the abrupt change of slope of the diagram of maximum tension as a function of sarcomere length when the sarcomere length is increased >3.00-3.10 \( \mu \text{m} \). This finding is consistent with data recently reported by Iwazumi et al. (1977) for the plateau of tension in tetanized intact skeletal muscle. Julian et al. (1978 b) have explained the data by Iwazumi et al. (1977) on the basis of an unhomogeneity of the sarcomere length during contraction. Using a much higher resolution than Julian et al. (1978 b), we failed to demonstrate (but could not completely eliminate) an unhomogeneity of sarcomere length capable of explaining that the descending limb of the sarcomere length-tension diagram was less steep between 2.35 and 3.00 \( \mu \text{m} \) than predicted by the cross-bridge theory. It seems that a phenomenon yet to be identified occurs at ~3.00-3.10-\( \mu \text{m} \) sarcomere length because this is also the sarcomere length for which tension starts to decrease during partial Ca\textsuperscript{2+} activation.

The even more puzzling observation that the tension induced by partial Ca\textsuperscript{2+} activation increased when the sarcomere length was increased well above that corresponding to the maximum overlap of the thin and thick filaments is, in fact, consistent with an increasing number of findings reported by other investigators in a variety of preparations. Similar observations have been made not only by Endo (1972, 1973) and Moisescu and Thieleczek (1978) in skinned skeletal muscle fibers, but also by Winegrad et al. (1976) and Endo (1976) in chemically skinned cardiac muscles, by Rack and Westbury (1969) and Close (1972) for the twitch of the intact skeletal muscle, and by Nassar et al. (1974) and Blinks and Allen (1978) for the twitch of the intact cardiac muscle.

Available biochemical data do not support (but do not completely eliminate) the hypothesis that increasing sarcomere length increases Ca\textsuperscript{2+} binding to troponin. To the contrary, Fuchs (1977 a, b) has shown that stretching glycercinated skeletal muscle fibers beyond the sarcomere length of maximum overlap, if anything, reduces both the number and the affinity of the Ca\textsuperscript{2+} binding sites. However, Fuchs's studies were carried out in fibers in rigor, as were previous studies referred to in Fuchs's articles (1977 a, b). Accordingly, Fuchs's conclusion may not be safely extrapolated to partial Ca\textsuperscript{2+} activation. The present data bring another difficulty for the hypothesis of a length-induced increase of sensitivity to Ca\textsuperscript{2+} because they show that, unlike an increase of [free Ca\textsuperscript{2+}], an increase in sarcomere length does not increase \( V_{\text{max}} \) but only \( P_o \). It could be argued, however, that Ca\textsuperscript{2+} may regulate contraction not only in binding to troponin but also in binding to the myosin light chains (see Julian, 1971, for references) and that only the second type of regulation would control the rate of cross-bridge formation and would not be influenced by a change in length. Such a hypothesis seems ruled out by the observation that, even in the presence of a [free Mg\textsuperscript{2+}] as high as pMg 2.5, an increase of [free Ca\textsuperscript{2+}] increased \( V_{\text{max}} \), because binding of Ca\textsuperscript{2+} to myosin does not seem to be significant, at least in skeletal muscle, when the [free Mg\textsuperscript{2+}] is higher than pMg 4.0 (Bremel and Weber, 1975; Potter, 1975). Another possibility is that the effect of Ca\textsuperscript{2+} on the rate of cross-bridge formation could be accounted for by its binding to an activator protein.
interacting with a kinase to phosphorylate the myosin light chains (Yagi et al., 1978). It could be proposed that this Ca\(^{2+}\)-induced phosphorylation could be length-independent. Not enough biochemical data supports such a hypothesis to permit us to devise experiments for testing it, especially inasmuch as the biochemical data are available only for skeletal muscle, which differs from cardiac muscle with respect to the myosin light chains.

Alternatively, the increase of tension observed at sarcomere lengths longer than those corresponding to the maximum overlap between thin and thick filaments could be explained by a length-dependence of one of the steps of the contractile process that is subsequent to the binding of Ca\(^{2+}\). Ishiwata and Oosawa (1974) have proposed that increasing sarcomere length could increase tension by decreasing the spacing between thin and thick filaments and thereby facilitating the formation of cross-bridges. However, increasing length decreases much less the interfilament spacing in skinned muscle fibers than predicted by a constant volume behavior (Matsubara and Elliot, 1972; April and Wong, 1976). Moreover, the shrinkage of skinned cardiac cells by the addition of 100 mg/ml of polyvinylpyrrolidone to the solution did not increase the sensitivity of the myofilaments to Ca\(^{2+}\), but produced a decrease of the maximum tension induced by a saturating [free Ca\(^{2+}\)], which is another unexplained finding (Fig. 9).

Therefore, the present discussion proposes no conclusion for the mechanism causing tension to increase when sarcomere length is increased above that corresponding to the maximum overlap of the thin and thick filaments. Our purpose in presenting this finding together with the myofilament-generated tension oscillations is to stress our impression that both phenomena are complex, but physiologically relevant, properties of partial Ca\(^{2+}\) activation which complicate the study of the cross-bridge properties but do not negate their existence.

APPENDIX

Mathematical Model for the Myofilament-Generated Tension Oscillations

The model describing the tension oscillations uses Hill's equation (1938) to derive \(V_{\text{max}}\) of the sarcomeres that redevelop tension after having yielded. The oscillations are localized, and only a small fraction of the total length is involved. This oscillating area is referred to by the subscript "osc." Consequently, the velocity outside this region is low, so that the contractile element of the continuously activated part (referred to by the subscript "act") can be represented simply as a force generator (shown as a battery in Fig. 10) in parallel with an elastic component.

To simplify the writing of the equations, it first will be assumed that the oscillations of \(P\) (instantaneous tension) are near \(P_o\) (maximum tension reached at a given pCa, pMg, pMgATP, and \(\Gamma/2\)). Appropriate corrections for oscillations with large amplitude will be indicated later. In this high range of \(P\), Hill's data (1938) show that the velocity, \(V\), is almost directly proportional to \(P_o - P\). Thus, Hill's equation, \((P + a)V = b(P_o - P)\), where \(a\) and \(b\) are constants defined by Hill (1938), can be linearized to a good approximation.

With \(P\) near \(P_o\), the velocity of shortening can be written as \(V = b(P_o - P)/(P_o + a)\). At \(P = 0\), the maximum velocity of shortening is \(V_{\text{max}} = bP_o/a\). Thus, at \(P\) near \(P_o\), \(V = V_{\text{max}}(P_o - P)/(1 + P_o/a)\). Defining \(\mu\) as
Figure 9. Effects of the addition of 100 mg/ml polyvinylpyrrolidone (40,000 mol. wt.) to the solution on the relation between pCa and the tension developed by 3-13-\mu m-wide skinned cardiac cells from the dog ventricle. The pMg was 3.5, pMgATP was 2.5, and \Gamma/2 was 0.16 M. Each point represents the mean and each vertical bar represents 1 SD. The filled circles represent the tension developed in the absence of polyvinylpyrrolidone. The addition of polyvinylpyrrolidone decreased the width of the skinned cells by 21 ± 3% SD. The filled triangles and the dotted curve correspond to the tension developed when polyvinylpyrrolidone had been added to the solutions. Both for filled circles and filled triangles, the tension developed by a given cell in the presence or absence of polyvinylpyrrolidone was expressed as a percentage of the maximum tension developed by the same cell at pCa 5.0 in the absence of polyvinylpyrrolidone. The open triangles correspond to the normalized values of the tension developed in the presence of polyvinylpyrrolidone expressed as a percentage of the tension developed at pCa 5.0 in the presence of polyvinylpyrrolidone (relative tension). Statistical analysis showed no significant difference between these normalized points and the filled circles.

Figure 10. Model for myofilament-generated tension oscillations. The subscript "osc" refers to the oscillating area and the subscript "act" to the continuously activated area. $K$ = stiffness; $\mu$ is the dashpot constant; and $P_o$ is the maximum tension developed at the pCa, pMg, pMgATP, and $\Gamma/2$ considered. $A$ is the point used for equating forces.
\[ \mu = \frac{P_a (1 + P_a/\sigma)}{V_{\text{max}}}, \quad (1) \]

This can be reduced to \( P_a - P = \mu V \). The reciprocal of \( \mu \) is the slope of the tension-velocity curve at \( P = P_a \).

The stiffness of the springs corresponding to the oscillating area (\( K_{\text{osc}} \)) and the continuously activated part of the muscle (\( K_{\text{act}} \)) are inversely proportional to the corresponding rest lengths (\( l_{\text{rest}} \)):

\[ (K_{\text{osc}})(l_{\text{osc}}) = (K_{\text{act}})(l_{\text{act}}), \quad (2) \]

where \( l_{\text{act}} \) and \( l_{\text{osc}} \) are the lengths of the continuously activated and oscillating areas, respectively.

Tension rise is slow and tension fall is rapid. This is schematized (Fig. 10) by having a dashpot in parallel with a force generator during tension redevelopment while having neither during inactivation. The dashpot constant, \( \mu \), is defined by Eq. 1. For simplicity, the isometric tension developed by the oscillating segment (\( P_{\text{osc}} \)) is taken as a step function of the length, being either \( P_a \) or zero (although not returning to zero until \( l_{\text{osc}} \) is less than the activation length).

The tension must be at all times the same in the oscillating and activated areas. Thus, equating forces at point A in Fig. 10 gives:

\[ K_{\text{osc}}(l_{\text{osc}} - l_{\text{osc}}^{\text{rest}}) + \mu l_{\text{osc}} + P_{\text{osc}} = K_{\text{act}}(l_{\text{act}} - l_{\text{act}}^{\text{rest}}) + P_a, \quad (3) \]

where \( l_{\text{osc}} \) is the time derivative of \( l_{\text{osc}} \). When the oscillating area becomes activated by being stretched, \( P_{\text{osc}} \) becomes equal to \( P_a \) and Eq. 3 can be reduced to a simple differential equation in \( l_{\text{osc}} \):

\[ \mu \dot{l}_{\text{osc}} + (K_{\text{act}} + K_{\text{osc}})l_{\text{osc}} = K_{\text{act}}L, \quad (4) \]

where \( L = l_{\text{osc}} + l_{\text{act}} \). Solving this equation for \( l_{\text{osc}} \) gives:

\[ l_{\text{osc}} = \frac{K_{\text{act}}L}{K_{\text{act}} + K_{\text{osc}}} + Ce^{-\tau t}, \quad (5) \]

where \( \tau = \frac{1}{K_{\text{act}} + K_{\text{osc}}} \) and \( C \) is the constant of integration. At \( t = 0 \), just before the oscillating area becomes activated, \( \mu = 0 \) and \( P_{\text{osc}} = 0 \). The constant of integration can be found by substituting these conditions into Eq. 3 and comparing the result with Eq. 5. Doing this, Eq. 5 becomes:

\[ l_{\text{osc}} = \frac{1}{K_{\text{act}} + K_{\text{osc}}}[K_{\text{act}}L + P_a e^{-\tau t}]. \quad (6) \]

From Eq. 6 an expression for the time-course of the tension redevelopment can be obtained. The tension contributed by the oscillating segment is:

\[ \Delta P = P_a - (K_{\text{osc}})\Delta l_{\text{osc}}, \quad (7) \]

where \( \Delta l_{\text{osc}} \) is the change in the length of the oscillating segment with respect to its length at \( t = 0 \).

Substituting for \( \Delta l_{\text{osc}} \) in Eq. 7, the tension redevelopment is described by the equation:

\[ \Delta P = [P_a K_{\text{act}}/(K_{\text{act}} + K_{\text{osc}})] [1 - e^{-\tau t}], \quad (8) \]

which is in the form of \( A[1 - e^{-\tau t}] \).

**Derivation of \( V_{\text{max}} \) per Sarcomere from the Model**

An estimate of \( \tau \) can be found by analyzing the curve of tension redevelopment obtained experimentally. An example of such an estimate is shown in Fig. 11 for a fiber that was 47 \( \mu \)m long, presented 19 sarcomeres, and had a mean sarcomere length of 2.48 \( \mu \)m during activation by a solution at pCa 6.1, pMg 3.5, pMgATP 2.5, and \( \Gamma/2 \) 0.16 M.
a single one-sarcomere-long segment of the length of the preparation yielded during contraction, with all the myofibrils yielding at this same level.

The tension redevelopment curve was extrapolated to $t = \infty$ to obtain $P_0$ (Fig. 11). The base line for measuring tension corresponded to the lowest value that $P$ reached during the tension oscillation. Tension was measured at points equally spaced in time and was divided by $-A$, $A$ being the difference between $P_0$ and the base line. This quotient was added to 1. The natural logarithm (ln) of this result gave values for $1/\tau$. A linear regression analysis was done on these points (Fig. 11), giving a value of $\tau = 2.18$.

![Graph showing tension redevelopment and tension-velocity curve](image)

**Figure 11.** Method used for the computation of $1/\tau$ from the data in a 5-μm-wide bundle of myofibrils from the dog ventricle. At the time indicated by the arrow, the pCa was decreased from 9.0 to 6.1. The pMg was 3.5, pMgATP was 2.5, and $F/2$ was 0.16 M. See text for discussion.

The overall stiffness, $K$, of this fiber was $2.11 \times 10^{-3}$ mg/μm. Because $K = (K_{act})(K_{osc})/(K_{act} + K_{osc})$, $K_{act} = 19/18 K$, and $K_{osc} = 19/1 K$. Thus, $K_{osc} + K_{act} = 42.2 \times 10^{-3}$ mg/μm. From the definition of $\tau$, $\mu$ is then found to be $92.04 \times 10^{-3}$ mg/μm. $P_0$ was 0.125 mg (Fig. 11). Substituting the value of $\mu$ into Eq. 1 and assuming that $P_0/a = 4$ as in skeletal muscle (Hill, 1938) gives $V_{max} = 0.79$ μm/s per sarcomere.

Until now it has been assumed that $P$ was nearly equal to $P_0$. In fact, the amplitude of tension oscillations was up to 50% of $P_0$. A typical tension-velocity curve generated by
Hill's equation (1938) shows that for \( P \) varying between 50% \( P_o \) and \( P_o \), the slope is nearly constant. Then, the average slope \( (1/\mu') \) is related to the slope at \( P = P_o \) by \( 1/\mu' = [1/\mu'][5/(4P_{min}/P_o + 1)] \), where \( P_{min} \) is the lowest level of tension reached during the oscillations. In the particular case of the data shown in Fig. 11, \( P_{min} = 0.60 P_o \). Then \( 1/\mu' = 1.47(1/\mu) \). Substituting \( \mu' \) into the derivation of \( V_{max} \) gives \( V_{max} = 1.47(6.79 \mu m/s) \), i.e., \( V_{max} = 9.98 \mu m/s \) per sarcomere, a value which happens to be in the same range as that found for \( V_{max} \) per sarcomere in intact cardiac muscle (Pollack and Krueger, 1976).

In contrast, from direct measurements of the velocity of shortening in a preparation of partially skinned cardiac cells from the rat ventricle, De Clerck et al. (1977) reported data that would suggest a much lower \( V_{max} \). This can be explained by the slow and unhomogeneous activation that De Clerck et al. produced by Ca\(^{2+}\) iontophoresis and by a competition between the sarcoplasmic reticulum and the myofilaments for Ca\(^{2+}\). Even if the 0.125 mM total EGTA that these investigators used may be sufficient to decrease considerably the amplitude of the phasic contractions generated by the Ca\(^{2+}\)-induced release of Ca\(^{2+}\) from the sarcoplasmic reticulum in skinned cardiac cells from the rat ventricle, this small [total EGTA] is certainly insufficient to prevent the sarcoplasmic reticulum from interfering with the rate of tension development (see Text-Fig. 3 G and H in Fabiato and Fabiato, 1975a). Thus, it seems to us that De Clerck et al. (1977) did not measure the direct effect of increasing [free Ca\(^{2+}\)] on the velocity of shortening of the myofilaments.

We thank our friend Dr. Morton Orentlicher for his help with the mathematical model.
This study was supported by Established Investigatorship 73-186 and grants-in-aid 75-783 and 78-1137 from the American Heart Association and by a research grant 1 R01 HL 19138-02 and -03 from the National Heart, Lung, and Blood Institute.

Received for publication 31 January 1978.

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