Effects of Calcium on the Sarcomere Length-Tension Relation in Rat Cardiac Muscle

Implications for the Frank-Starling Mechanism

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SUMMARY The objective of these experiments was to better understand the factors responsible for the decline in tension at short sarcomere lengths in cardiac muscle. We measured the effects of variation of extracellular calcium concentration on the sarcomere length-tension relation in thin trabeculae and papillary muscles excised from right ventricles of rats. Sarcomere lengths were measured by optical diffraction. Experiments were carried out using two protocols. In the primary protocol, the muscle was allowed to contract isometrically. The sarcomeres in the central region, shortened and stretched the end regions adjacent to the clips. The sarcomere length was measured at the time of peak tension and plotted against the active tension. In a secondary protocol, the sarcomere length and tension were measured during contractions in which sarcomere length in the central region was held approximately constant. Both protocols were carried out at extracellular Ca\textsuperscript{2+} concentrations ranging from 0.3 to 5.0 mM. The height of the length-tension curves was progressively depressed as extracellular Ca\textsuperscript{2+} was reduced from 2.5 to 0.3 mM; the variation of the shape of the curve was modest. On the other hand, when Ca\textsuperscript{2+} was increased to 5 mM, there was less upward shift of the sarcomere length-tension relation, indicating a tendency toward saturation. The results obtained using the sarcomere isometric protocol were similar to those obtained with the muscle isometric protocol. Extracellular Ca\textsuperscript{2+} appears to act principally as a modulator of the height of the length-tension curve, though it has a modest effect on the shape as well. On the basis of these results, we deduce that several of the factors previously cited as possible explanations of the decline in tension at short sarcomere length are not likely candidates.

THERE is great interest in the length-tension relationship in cardiac muscle, particularly in understanding what limits force production at short sarcomere lengths. This is of both basic and applied interest in that it is the cellular basis of the Frank-Starling relationship for intrinsic control of the heart.

One of the ways that has been used to distinguish between various possible explanations of what could limit force production at short sarcomere lengths has been the effect of Ca\textsuperscript{2+} on the length-tension curve (Jewell, 1977). The effect of a reduction in Ca\textsuperscript{2+} on the length-tension relation of isolated cardiac muscle has been studied by several investigators, but the results have not been entirely consistent. In one study, the length-tension relation was shifted downward by the same increment at all lengths (Bodem et al., 1976) while, in others, the downward shift was nonuniform (Allen et al., 1974; Jewell, 1977; Huntsman and Stewart, 1977).

In these experiments, the relation between tension and muscle length, not sarcomere length, was studied. We now know that any quantitative inference of sarcomere length changes based upon muscle length changes may not be correct since isolated cardiac muscle preparations may contain highly compliant end regions caused by clamping (Krueger and Pollack, 1975; Julian and Sollins, 1975). This not only allows considerable sarcomere shortening during isometric contraction but causes the relation between muscle length changes and sarcomere length changes to be nonlinear (Krueger and Pollack, 1975; Julian and Sollins, 1975; Pollack and Krueger, 1976; Julian et al., 1976; Huntsman et al., 1977). Thus, conclusions reached in previous studies warrant reconsideration.

In the present experiments, we measured the effects of variation of extracellular Ca\textsuperscript{2+} on the length-tension relation at the sarcomere level. By so doing, we hoped to avoid the possible pitfalls inherent in the previous studies and to be able to draw inferences about the nature of the mechanism underlying the falloff of tension at short sarcomere lengths, specifically whether variations in Ca\textsuperscript{2+} activation, restoring forces, or shortening-induced "deactivation" are responsible. The conclusion is that none is solely responsible and that some other factor or combination of factors must be considered.

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Methods

Figure 1 shows the apparatus used in these experiments. It consisted of a mechanical servo capable of controlling the length of the specimen, a tension transducer, and an optical apparatus based on light diffraction used to measure sarcomere length on-line, as used previously in this laboratory (Pollack et al., 1977; ter Keurs et al., 1978).

Optical Methods

The optical methodology is described more fully elsewhere (Iwazumi and Pollack, 1979). Briefly, the beam from a 5 mW He-Ne laser (Spectra Physics 120) was compressed to approximately 200 μm and directed toward a region along the specimen (Fig. 1). Since the muscle consists of regularly alternating bands of different refractive indices, the light emerging from the muscle is broken into diffracted orders whose angular spacings are uniquely related to sarcomere length in the specimen. The diffracted light was collected by a water immersion objective lens (Zeiss, 40x, N.A. 0.75) and focused onto a plane about 50 cm from the specimen by means of a phase centering telescope.

A beam splitter was used to direct the light emerging from the phase centering telescope on to two different sensors. The first was a vidicon tube. This permitted observation of the diffraction pattern on a video monitor. We found this to be a useful method of observing gross features of the diffraction pattern; for example, any skewing of the diffracted orders resulting from striations not normal to the longitudinal axis could be detected easily and the specimen remounted. Also bubbles originating from the stimulating electrodes, lodging on the muscle surface, and distorting the diffraction pattern could be detected and removed.

The second sensor was a photodiode array (Reticon RL 128) with 128 elements in series, 25 × 430 μm in size with an interelement spacing of 50 μm. A cylindrical lens (focal length, 25 mm) was used to condense the diffracted orders onto the meridian so as to increase their intensity and reduce the intensity fluctuations due to laser speckle. The photodiode array was positioned to capture only one of the two first orders of the compressed diffraction pattern and a portion of the zeroth order “skirt,” permitting a range of measurement corresponding to sarcomere lengths between the 1 and 4 μm.

Each scan of the photodiode array generated a profile of intensity vs. distance, d, along the array (Fig. 1). The area under the profile of the first order peak was calculated on-line by integrating the intensity distribution with respect to d, starting from the zeroth order side. The value of d at which the integral reached 50% of the total area was computed, and the respective sarcomere length was calculated. This was done every 200 μsec, giving an effectively continuous record of sarcomere length vs. time. The resolution of this technique was limited by noise which was about 5 nm peak-to-peak. Calibration was achieved by replacing the specimen with a series of gratings spanning the range 1.33-3.33 μm. Accuracy was within 1% over this range.

The specimen was visible at all times through the oculars of a compound microscope (Leitz Ortholux), the vertical tube of which contained the phase centering telescope. In this way, translation of the specimen across the axis of the laser beam during contraction could be monitored.

Mechanical Measurements

The specimen was mounted between two miniature (250-mg) stainless steel clips, one connected to a tension transducer and the other connected to the servo-motor. The tension transducer used in some experiments was a Statham UC-2, which had a sensitivity of 1 V/g and a resonant frequency of 150
Hz. In the other experiments, we used an improved force transducer (Kuhlite BG-50) which, with clip attached, had a resonant frequency of approximately 400 Hz. Its sensitivity was 10 V/g, and its compliance was negligible relative to the compliance of the damaged ends of the specimen in series with it.

The servo-motor system was of conventional design, consisting of a copper-wound, cylindrically shaped coil suspended in the magnetic field of a permanent magnet. The coil could translate along its cylindrical axis and was able to achieve a step change of position of 1 mm in about 3 msec.

Solutions

Physiologic salt solutions with the following millimolar concentrations of added species were used throughout the experiments: Mg$^{2+}$, 1.0; SO$_4^{2-}$, 1.0; HPO$_4^{2-}$, 1.0; K$^+$, 5.0; HCO$_3^-$, 24.0; C$_2$H$_5$O$_2^-$, 20.5; Cl$^-$, 102.4; Na$, 145.5; and CaCl$_2$, 0.3, 0.6, 1.25, 2.5, or 5.0. Insulin (Iletin; 150 units/ml) was added (0.1 ml/liter), along with dextrose (1.8 g/liter). The solution was gassed continuously with a gas consisting of 95% O$_2$ and 5% CO$_2$ and had a pH of approximately 7.4. This solution was used in the experiments and during dissection procedures. Experiments took place at a solution temperature of 28°C, at which temperature the muscles behaved satisfactorily.

Muscle Chamber

The muscle chamber was made of Lucite, except for the bottom where a glass slide was mounted to admit laser light. Solution was passed through the chamber at a replacement rate of about 5 times/min and removed with the aid of an aspiration pump which was mechanically isolated from the chamber to prevent vibrational coupling to the transducer. Stimulus electrodes were of platinum wire, one on either side of the specimen. Stimulation was monopolar and took place at a rate of 12/min with a pulse duration of 5 msec and intensity of about 20% above threshold. At this rate, the muscles maintained their performance satisfactorily.

Preparation and Selection of Specimens

Adult albino rats were used in these experiments. Rats were selected because, in this species, it is not difficult to find trabeculae or papillary muscles that are thin enough to give a good diffraction pattern. Since there are some apparent differences between cardiac muscle of rat and other mammalian species in action potential duration and the heart rate-force relation, it may be questioned how generally applicable results using the rat will be. However, Henry (1975) has shown that rat cardiac muscle can show a positive staircase phenomenon at physiological stimulation rates given the proper metabolic substrates. Thus, the actual differences may not be great.

The rats were anesthetized with ether. The hearts were rapidly excised and placed in a dissection chamber. The coronary circulation was perfused with the physiologic salt solution in a Langendorff-type setup; this cleared the blood from the preparation, which ultimately resulted in sharper diffraction patterns. The right ventricle was exposed to view, and an appropriate specimen was carefully removed. Specimens consisted of thin (less than 250 μm) papillary muscles or, in the majority of cases, trabeculae. The latter were usually ribbon-shaped, 2–3 mm long, 50–200 μm wide, and 50–150 μm thick.

The specimen was clamped in the apparatus, stimulation was started, and the muscle was allowed to equilibrate in a solution containing 2.5 mM Ca$^{2+}$, until it demonstrated the following: stable contractile tension, relatively uniform activation as confirmed by visual inspection, low resting tension (usually less than 15% of total tension at L$_{max}$), all or none response to stimulation, and a relatively noise-free diffraction pattern with a sharp first order. Remounting was sometimes necessary to minimize nonuniform contraction and the consequent large amounts of lateral and longitudinal translation during contraction. Only those muscles which satisfied the above criteria were considered for use in the experiments. All muscles used produced between 9 and 15 g/mm$^2$ at L$_{max}$.

The specimen then was subjected to tests of uniformity. As a rule, both cross-sectional area and sarcomere length were nonuniform toward the ends of the specimen. However, a central segment of 30–50% of the fiber length could be found in perhaps one-half the specimens counted in which the criteria for uniformity, as described below, were met. The others were discarded.

First, uniformity of cross-section was measured by stereo microscopy. Width was measured directly. Thickness was measured with the aid of a 45° mirror mounted parallel to and alongside the specimen. If the variation of cross-sectional area along the central segment exceeded 10%, the specimen was discarded.

We then measured sarcomere length uniformity with the muscle stretched to L$_{max}$. Local sarcomere length was measured at positions along the central segment when the muscle was at rest and at the time of peak tension. If the peak-to-peak variation of sarcomere length along the muscle either at rest or at peak contraction exceeded 0.1 μm, the muscle was discarded. Usually the variation at rest was less than 0.05 μm while, at the time of peak tension, it was slightly larger. In some experiments, we measured the uniformity across the muscle, i.e., from edge to edge. In all cases, the peak-to-peak variation was less than 0.05 μm.

Experimental Procedure

The muscle was allowed to equilibrate at some intermediate length, which denoted the control length. Then the muscle was adjusted to a new
length and allowed to contract. Peak tension was measured on the third beat after the length change at which time a new, steady state in peak tension had been reached. The muscle was then returned to the control length, allowed to contract 10 times, and adjusted to a new experimental length. The sequence of experimental lengths was chosen randomly for each run.

After a run at a calcium concentration of 2.5 mM, the calcium level was changed, and the specimen was allowed to stabilize for approximately one-half hour before data were collected. The sequence of calcium levels was randomized. At the end of the experiment, the specimen was returned to the bath containing 2.5 mM calcium and performance checked. If the tension at L_{\text{max}} differed by more than 10% from that measured during the first run at the same calcium concentration, the data were discarded. The data reported in this study were collected in eight successful experiments.

Results

Figure 2A shows traces of the first order intensity profile obtained at different times throughout contraction in a representative specimen. Two degrees of stretch of the resting trabecula are shown. The first orders always moved rightward during isometric contraction, indicating sarcomere shortening. Shortening was modest at highly stretched lengths (right), more substantial at intermediate lengths (left), and again modest at the shortest lengths (not shown). The first order intensity usually diminished progressively during contraction, particularly at the shorter initial length. In most specimens, the order remained sufficiently distinct to permit unambiguous determination of median sarcomere length; in instances when this was not so, the traces of sarcomere length vs. time became noisy, and data were discarded.

Figure 2B shows three traces of median sarcomere length computed on-line from the intensity profiles such as those shown in Figure 2A. The traces of Figure 2B were obtained from three regions along the specimen, separated by 125 \( \mu \text{m} \). Sarcomere length at rest was uniform along the fiber, usually to within 0.05 \( \mu \text{m} \). By the time of peak tension, there was a slight increase of nonuniformity. Sarcomere shortening often persisted beyond the time of peak tension. Continued shortening in the face of diminishing tension could be accounted for by premature "give" of the regions flanking the central segment, i.e., in the marginally contractile regions between the viable region and the damaged, nonstriated regions adjacent to the clamps. Some variation in the onset of relaxation also could be found within the central region (Fig. 2B).

Figure 3 shows the influence of variation of extracellular calcium on the waveforms of tension and sarcomere shortening. Peak tension diminished when calcium was reduced from the control level of 2.5 mM. Increases to 5 mM produced only slight increases in tension. The rate of rise of tension diminished with reduced calcium, but the time to peak tension did not vary by more than \( \pm 10\% \) at any given initial sarcomere length. The time to peak tension did increase with increases in initial sarcomere length (e.g., by 25% as the initial sarcomere length increased from 1.9 to 2.3 \( \mu \text{m} \)). The amount of shortening that occurs by the time of peak tension varied with initial sarcomere length, reaching a maximum (0.37 \( \mu \text{m} \)) for an initial sarcomere length intermediate between the extremes used in this study (1.85 and 2.35 \( \mu \text{m} \)). The extent of this maximum did not vary with external Ca\(^{2+}\) concentration, but its position within the range of initial sarcomere length did, decreasing as the external Ca\(^{2+}\) was increased (e.g., the position of maximum shortening changed from 2.20 to 2.05 \( \mu \text{m} \) as Ca\(^{2+}\) changed from 0.3 to 5 mM). This will be seen later to spread the data on the effects of external Ca\(^{2+}\) when it is plotted as a function of sarcomere length at the time of peak tension rather than initial sarcomere length (see Figures 4 and 5 compared to Figure 6).

Length-tension plots were constructed as follows. For each contraction, we measured passive tension, peak tension, and sarcomere length at the time of peak tension. As discussed by Jewell (1977), computation of the tension of significance, the active, developed tension produced by the contracting sarcomeres in the central, healthy part of the muscle, depends upon knowledge of the location of the elements supporting the passive tension. If the passive tension is borne by a structure that runs continuously from one end of the muscle to the other and bears tension dependent on the total muscle length, the tension of significance would be the "active" tension developed above the passive tension [DT in Jewell’s nomenclature (1977)]. If the passive tension is borne by a structure that is parallel to and fixed to the individual cells or sarcomeres in the muscle or is part of the contractile filaments and bears tension dependent on the sarcomere length, then the tension of significance would be the total tension minus the passive tension measured at the sarcomere length that occurs at the peak tension [DT* in Jewell’s nomenclature]. In the latter case, much of the resting tension residing in the parallel elastic elements would have been discharged during the substantial shortening occurring prior to the time of peak tension. Since resting tension became a significant fraction of total tension only above 2.3 \( \mu \text{m} \), the passive tension at peak shortening is insignificant at the sarcomere lengths included in this study; thus, active tension would be in this model simply equal to total tension. Because the data are not available to decide definitively between these two models, the data have been plotted under both assumptions above.

We first considered data obtained with control levels of Ca\(^{2+}\). Data from all experiments (\( n = 8 \)) were pooled and then assembled into bins of different sarcomere length ranges; the size of each bin...
was 0.05 μm. The mean total tension obtained at 2-μm sarcomere length was taken as 100% for that fiber, and all other tensions for that fiber were expressed relative to that.

We were interested in obtaining the isometric tension but, as mentioned above, we often noted that the sarcomeres in the central region were still shortening somewhat at the time of peak tension in the muscle isometric contractions (Figure 2B). This small velocity would not be expected to diminish the tension substantially below isometric since the force-velocity curve, at least for single skeletal muscle fibers, shows that muscles produce virtually isometric tension so long as the shortening velocity is low (Edman and Hwang, 1979). That this tension is not substantially different from isometric is demonstrated below by the similarity between the muscle isometric and sarcomere isometric data (Fig. 8).

The length-tension relations are shown in Figures 4 and 5. In Figure 4, the tension of significance is

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**Figure 2** A: Scans of first order intensity profile at different times throughout contraction, proceeding downward at 80-msec intervals. Left: Initial sarcomere length, 2.28 μm; sarcomere length at time of peak force, 2.03 μm. Right: Initial sarcomere length, 2.36 μm; sarcomere length at time of peak force, 2.24 μm. Traces from several successive contractions are superimposed on each panel, demonstrating repeatability. B: Sarcomere length changes during contraction (top traces) recorded from regions along the specimen 125 μm apart. Sarcomere length was computed online, electronically, from output scans (as in Figure 2A) of the photodiode array. Tension and stimulus pulse shown below. Note the uniformity of sarcomere length changes during contraction. The onset of relaxation, however, varied somewhat from region to region.
FIGURE 3  Sarcomere length (upper traces) and tension development (lower traces) in contractions at three levels of extracellular Ca$^{2+}$.

considered to be the total tension. In Figure 5, the tension of significance is considered to be the "active" tension produced above the passive tension. The most striking feature of both was the progressive downward shift of the curve as the extracellular Ca$^{2+}$ was reduced. This occurred over the range 2.5-0.3 mM. When Ca$^{2+}$ was elevated by a factor of 2 to 5 mM, there was some elevation of the curve over that obtained at 2.5 mM but less than the depression for an equivalent factor of 2 decrease in Ca$^{2+}$, indicating a tendency toward saturation. There may be a progressive change in the shape of the length-tension relation as Ca$^{2+}$ was diminished as plotted in Figure 4 but not Figure 5. As seen in Figure 4, at high Ca$^{2+}$, the ascending limb was linear at short sarcomere lengths but grew flatter at longer lengths; between 2.0 and 2.2 μm, the tensions differed by less than 15%. At progressively lower Ca$^{2+}$ concentrations, the shape became progressively more linear. The possibility of a concave upward relation at 0.3 mM as Endo (1973) has observed is not excluded.

Figure 6 shows the relation between peak "active" tension (i.e., total tension minus resting tension) and initial resting sarcomere length. This plot might be expected to be similar to the one obtained by previous workers where active tension was plotted against muscle length. The shapes of the curves are similar to those of Figures 4 and 5, except for two features. First, there is more scatter in Figure 6, possibly because of the variability in the extent of the damaged end regions from specimen to specimen; and second, the curves are more compressed along the sarcomere length axis.

In five experiments, a supplementary protocol was adopted. We used open-loop control to main-
Initial sarcomere length

Figure 6. Active force vs. initial sarcomere length in muscle isometric contractions at five levels of extracellular Ca²⁺. The data are the same as used in Figures 4 and 5. Individual SEM’s omitted for clarity. Average value of SEM for all points is ±6.8%.

Figure 8. Active force vs. sarcomere length at the time of peak force in muscle isometric (MI) and sarcomere isometric (SI) contractions at two levels of Ca²⁺. For the muscle isometric points, total force above resting force is plotted. Muscle isometric points are the average of five different muscles and are the same data as on Figure 4. Sarcomere isometric points are the average of three different muscles.

Figure 7. Constrictions of a specimen under two conditions: muscle isometric (top) and sarcomere isometric (bottom). In the lower panel, muscle length is altered so as to keep the sarcomere length from changing during the early part of the contraction. Note the higher rate of rise of tension and the elevated peak tension in the lower panel. Traces from several successive contractions are superimposed.

tain the sarcomere length constant during contraction. The method used was similar to that described by Pollack and Krueger (1976). It involves using an approximately exponential stretch that can be varied in amplitude, time constant, and time of application. With some practice, it was possible to adjust the variables while observing the effect on measured sarcomere length so that the measured sarcomere length stayed approximately constant to within 0.05 μm up to and beyond the peak of active tension. The disadvantage of this open-loop method is the time taken to adjust the condition for each initial sarcomere length; the advantage is that the muscle stretch waveform is a smooth rather than jagged one. With this method of controlling sarcomere length, length-tension relations could be constructed for “sarcomere isometric” contractions. Figure 7 shows an example of such a contraction (lower) and the corresponding “muscle isometric” contraction (upper). In the sarcomere isometric contraction, the tension rises more rapidly and reaches a higher level than in the muscle isometric contraction. Unfortunately, contraction at a constant sarcomere length cannot be achieved for sarcomere lengths below the minimum resting sarcomere length (1.85–1.90 μm).

Figure 8 compares the length-tension relations obtained with muscle isometric and sarcomere isometric contractions at two Ca²⁺ levels. The force plotted is the total force for the muscle isometric conditions (where there is substantial sarcomere shortening; this is identical to the curves in Figure 4) and the active force above the resting tension for the sarcomere isometric conditions. The assumption for both is that the appropriate resting tension
to be used is that for the sarcomere length at peak tension. The difference between the two sets of data is small. There is considerable scatter in the data, and there are no sarcomere isometric points below 1.9 μm, the minimum resting sarcomere length. For these reasons, equality of the two sets of data cannot be claimed, but the similarity is clear.

**Discussion**

The relation between tension and sarcomere length at the peak of contraction in an extracellular Ca\(^{2+}\) concentration of 2.5 mM found here agrees with that observed by Pollack and Krueger (1974) for rat papillary muscle and by ter Keurs (personal communication) for rat trabeculae. The shape of the curve is also similar to that found by Julian and Sollins (1975) for rat cardiac muscle, except that the sarcomere lengths at rest and at zero active force are less (about 0.2 μm) in their study than in ours. The difference might be due to the fact that they used younger rats (cf. Hopkins et al., 1973, and Schiebler and Wolff, 1966).

Extracellular Ca\(^{2+}\) affects mainly the level rather than the shape of the length-tension curve. The fact that the effect of extracellular Ca\(^{2+}\) saturates not far above the normal level for rat muscle has been known for some time (Forester and Mainwood, 1974). However, the effects of decreasing Ca\(^{2+}\) are not just to shift the curve down by a constant factor at all sarcomere lengths. This is obvious in Figure 4, but it is also true for Figure 5. If the data from Figure 5 are scaled so that the maximum tension is the same for all Ca\(^{2+}\) concentrations, the tension falls off more steeply at short sarcomere lengths for low Ca\(^{2+}\) than for higher Ca\(^{2+}\). This was implied earlier by Allen et al. (1974), Huntsman and Stewart (1977), and Jewell (1977).

The presumption that a decrease in extracellular Ca\(^{2+}\) leads to a decline in the Ca\(^{2+}\) activating the contractile proteins has been confirmed by Allen and Blinks (1978) in frog cardiac muscle. This decline probably accounts for the general depression of the length-tension curve for Ca\(^{2+}\) below 2.5 mM.

**Muscle Isometric and Sarcomere Isometric**

The ability to keep the sarcomere length in the center section approximately constant during a contraction allowed us to compare contractions with muscle length constant to those with the sarcomere length of the central segment constant. This, evidently, could be done only for sarcomere lengths above the minimum resting sarcomere length. Although the results showed some scatter, there was no tendency for one set of data to be consistently above or below the other.

The several major differences between the muscle isometric and sarcomere isometric cases deserve comment. When contractions are considered in which the sarcomere length at the time of peak active tension is the same under the two conditions, both the initial sarcomere length and the amount of sarcomere shortening during contraction are considerably greater in the muscle isometric case than in the sarcomere isometric case. For example, consider the data points where the sarcomere length at peak tension is 2.0 μm. In the muscle isometric condition, the initial sarcomere length would have been about 2.24 μm and the shortening about 0.24 μm, compared to 2.0:μm and less than 0.05-μm shortening for the sarcomere isometric case. These differences notwithstanding, the similarity of the two length-tension relations implies either that there is not a substantial amount of shortening-induced deactivation (Edman, 1975) or that, if there is, it may be offset by some other compensating factor such as perhaps an effect of initial muscle length.

The differences in initial sarcomere length for the two cases lead to a second conclusion, that the peak force is not determined by initial muscle length. For example, in the muscle isometric case, a contraction with sarcomere length of 1.7 μm would have started at an initial sarcomere length of 2.0 μm but generates less than 40% as much tension as if it had either remained at 2.0 μm throughout contraction or had shortened from an initial sarcomere length of 2.24 μm. Evidently, it is the sarcomere length at the peak of contraction that is relevant, not the initial sarcomere length.

**Why Does Tension Decline at Short Sarcomere Lengths?**

The manner in which the length-tension relation is affected by variations in extracellular Ca\(^{2+}\) provides some clues about the factors underlying the relation itself. The factors most likely to limit force at short sarcomere lengths have been considered recently by Jewell (1977). They include: (1) reduced twitch duration; (2) increased "deactivation" due to shortening; (3) increased restoring forces; and (4) decreased activation of the contractile system. Other possible factors cited by Jewell include: (5) decreased Ca\(^{2+}\) sensitivity; (6) increased interfila-ment spacing; and (7) diminished myofilament interaction.

The first factor was ruled out by arguments presented by Jewell (1977).

The second factor, shortening-induced deactivation (Edman, 1975), was discussed above and considered unlikely on the basis of similarity of the muscle isometric and sarcomere isometric data.

Restoring forces, the third factor, could exist as a result of external constraints which limit fiber swelling during shortening or from internal constraints which impair fiber shortening (Winegrad, 1975). Either one would cause a progressive falloff of force at short sarcomere length. As argued by Jewell (1977), if restoring forces are responsible for the falloff of tension at short sarcomere lengths, the sum of the absolute value of restoring force and
measured force should be independent of sarcomere length. From Figures 4 and 5, it is apparent that there is no unique restoring force-sarcomere length function that satisfies this requirement at all values of Ca$^{2+}$. Thus, the restoring forces, unless they were Ca$^{2+}$-dependent, would not explain the falloff of force at short sarcomere length.

Regarding factor four, our data allow us to draw inferences about whether length is affecting myofilament activation. This could occur through the action potential (Kaufman et al., 1971) or through Ca$^{2+}$ release (Fabiato and Fabiato, 1975a and b). Since in the rat both are early events (Allen and Kurihara, 1979; Coraboeuf, 1960), their effects would likely depend more on initial sarcomere length than on the sarcomere length at the time of peak active force. Thus, if variations in either the action potential or in Ca$^{2+}$ release accounted entirely for the length-tension relationship, sarcomere isometric and muscle isometric contractions reaching the same sarcomere length at peak contraction should give very different tensions because, in the latter case, the initial sarcomere length is considerably greater than in the former. However, there appears to be little difference. Consequently, diminished activation is unlikely to be entirely responsible for the falloff of tension. Our finding that even "saturating" levels of extracellular Ca$^{2+}$ fail to eliminate the tension decline at short sarcomere lengths is consistent with this conclusion.

Length-dependent activation is even more strongly negated by the results of experiments using aequorin to measure Ca$^{2+}$ release. In frog cardiac muscle, Allen and Blinks (1978) observed that increases in muscle length caused decreases in the height of the Ca$^{2+}$ transient and, paradoxically, increases in force. However, their sarcomere lengths were probably longer than those in our study. In rat ventricular muscle, Allen and Kurihara (1979) showed that the peak of the light emission from aequorin-injected cells did not vary significantly with muscle length, implying that, although peak tension changed dramatically, the Ca$^{2+}$ release was not affected by length.

The decline in force at short sarcomere length could also arise out of a length-dependent Ca$^{2+}$ sensitivity of the filaments, factor five. Fabiato and Fabiato (1978) have shown that this can influence the shape of the length-tension curve at longer sarcomere lengths, but there are no published data at the shorter sarcomere lengths of interest here. Once again, as with factor four, the effect should depend on events occurring relatively early in contraction [the Ca$^{2+}$ transient is already back to the resting level at a time when force is near the peak value (Allen and Kurihara, 1979)] and therefore seems inconsistent with our results.

The sixth factor is the increase of interfilament spacing that occurs as the fiber shortens (Matsubara and Elliott, 1972; Matsubara and Millman, 1974). Two observations argue against this. First, changes of interfilament spacing, whether brought about by variations in osmolarity (Edman and Andersson, 1968) or by addition of polyvinylpyrrolidone (PVP) to the bathing medium (Fabiato and Fabiato, 1976), have little effect on the shape of the length-tension curve at sarcomere lengths between 1.6 and 2.2 \(\mu\)m. Second, the effect of increasing interfilament spacing is not to decrease force but to increase it (Edman and Andersson, 1968; Fabiato and Fabiato, 1976).

Finally, the myofilaments themselves might be incapable of generating as much force at short sarcomere length as at intermediate sarcomere length. The data of Fabiato and Fabiato (1975b) and Schoenberg and Podolsky (1972) indicate a relatively flat length-tension curve in skinned cardiac and skeletal muscle at short sarcomere length, which would argue against this possibility. However, Moss (1979) found a rather substantial falloff of Ca$^{2+}$-activated tension similar to the decline seen on the ascending limb for tetanic tension in intact fibers (Gordon et al., 1966). Though Moss’ experiments were on skeletal, not cardiac muscle, fundamental differences among preparations would be unexpected. The differences need to be resolved before a definitive conclusion can be reached about the intrinsic force-generating capability of the myofilaments at short sarcomere length.

To recapitulate, the analysis presented above implies that variations of activation, of "deactivation," of Ca$^{2+}$ sensitivity, of restoring forces, or of interfilament spacing are not unique determinants of the decline of force at short sarcomere length. This sharply reduces the number of potential candidates. It appears that the falloff of tension is due either to: (1) some complex combination of the above factors; (2) an intrinsic property of the myofilaments; or (3) some factor that is yet to be identified.

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Note Added in Proof
Since acceptance of this manuscript for publication, a report of a similar study has been published; H.E.D.J. ter Keurs, W.H. Rijnberger, R. van Heuningan, and M.J. Nagelom (1980) Tension development and sarcomere length in rat cardiac trabeculae: Evidence of length-dependent activation, Circ Res 46: 703-714. The results of the two papers are in agreement, although the interpretation of the results is somewhat different.

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