Sarcomere Length-Tension Relations in Living Rat Papillary Muscle

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ABSTRACT

Small papillary muscles about 2 mm long and 0.2 mm thick were dissected from the right ventricles of 16-19-day-old rats. Resting (between twitches) and active (at twitch peaks) striation patterns were photographed in living muscles using a light microscope. External muscle length was varied from L_max, the length at which peak twitch tension was maximum, to 0.75L_max, the length at which peak twitch tension was about 10% of maximum. Resting and active tension versus muscle length curves were similar to those obtained from other papillary muscle preparations. Resting average sarcomere length at L_max was about 2.23μm; it decreased with decreasing muscle length in the range between L_max and 0.75L_max. Near 0.75L_max, resting average sarcomere length was about 1.5-1.6μm. Considerable internal shortening occurred during contractions, and the active average sarcomere lengths measured at the twitch peaks were less than the resting values. At L_max, the active average sarcomere length was 1.98μm. At 0.75L_max, there was only about a 3-6% decrease in average sarcomere length at the twitch peaks. However, at external muscle lengths between L_max and 0.75L_max more internal shortening was present than was at L_max, since average sarcomere length decreases of about 15% were observed. The finding that peak active tension decreases as sarcomere length decreases below about 2.0μm suggests that some of the factors limiting force generation at short lengths in skeletal muscle may also limit it in mammalian cardiac muscle.

In mammalian heart muscle, length, for isolated papillary muscle preparations, or chamber volume, for whole heart preparations, significantly influences the strength of contraction (1). As in the ascending limb of the skeletal muscle length-tension relation, increases in initial lengths or volumes are associated with progressively stronger contractions. At the same time, resting or diastolic tension also progressively increases so that, in contrast to vertebrate skeletal muscle, heart muscle becomes increasingly less compliant as the optimum length or volume is approached.

Gordon et al. (2) have shown that the plateau and the descending limb of the active sarcomere length-tension diagram for frog skeletal muscle can be simply accounted for by the sliding filament, cross-bridge model (2-4). The ascending limb of the active sarcomere length-tension relation, which is of great interest in the study of heart striated muscle, is more difficult to explain. However, as Gordon et al. (2) have pointed out, the variation of active tension in this region of the diagram can be attributed to an interference with the normal mechanism for tension generation by the double overlap of the thin filaments and to internal loads produced by double overlap of the thin filaments and compression of the thick filaments against the Z disks. Recently, Taylor and Rüdel (5) have shown that, as the sarcomere length decreases below the point at which thick filaments first begin to contact the Z disks in isolated frog skeletal muscle fibers, some of the drop in active tension is due to a failure of activation in the central part of a muscle fiber.

The work described in the present paper was undertaken in an attempt to determine how peak twitch tension and active sarcomere length are related in living mammalian heart muscle. The complexity of heart muscle compared with single frog skeletal muscle fibers makes it difficult to obtain results that can be simply interpreted. Even so, our findings indicate that an active sarcomere length-tension relation can be determined from living mammalian papillary muscles; moreover, the relation bears some resemblance to that obtained from frog skeletal muscle.

Methods

Male 16-19-day-old CD Wistar rats (Charles River Breeding Laboratories) weighing 35-43 g were anesthe-
tized with sodium pentobarbital (approximately 0.05 mg/g body weight, ip). The heart was removed from each rat and transferred to a dish; the right ventricle was then opened, and the ventricle wall was pinned back, exposing the papillary muscles. We found that the optimum muscles for these experiments were 2 mm or more long and approximately 0.2 mm in cross-sectional diameter. When a suitable muscle was located, a short piece of nylon monofilament 10-0 suture (Ethicon 2814) was tied around the tendinous end very near the junction of the tendon with the muscle, and another piece was passed through the ventricular wall at the base of the papillary muscle, using a BV-3 needle attached to the suture. During this procedure, the actively contracting heart was submerged in Krebs-Ringer’s solution bubbled with a 95% O2-5% CO2 mixture. The Krebs-Ringer’s solution contained (in mm): NaCl 117, NaHCO3 27, KCl 4.2, CaCl2 1.2, H2O 1.9, MgSO4 7 H2O 1.2, NaHPO4 1.2, and glucose 5.6. The pH was 7.4 after bubbling.

The papillary muscle was removed from the heart by cutting out a small section of ventricular wall around the base of the muscle and severing the tendon between the suture and the valve. It was then quickly transferred to the experimental chamber. The tendinous end was directly knotted to a wire connected to a force transducer, and the other end was directly knotted to a wire connected to a micrometer movement. The wires were stainless steel, 0.2 mm in diameter. The experimental apparatus was essentially the same as that described previously (6) except that the servomotor was replaced by a micrometer movement that made it possible to change the overall length of the muscle with a resolution of 5μ. The muscles were stimulated with platinum plate electrodes to which one cycle of a sine wave, 50 or 100 Hz, was applied at a rate of about 5/min throughout the course of an experiment. The intensity of the stimuli was adjusted to be slightly above threshold. The temperature of the bubbled Krebs-Ringer’s bath was maintained at 20°C.

The striations were viewed and photographed using a 40x water-immersion objective and a 5x eyepiece. A 200-w d-c mercury vapor arc lamp was used with a wide-band green interference filter. The condenser aperture was stopped down to enhance the visibility of the striations. This procedure produced a depth of field of approximately 20-25μ. Striation patterns were recorded at random positions along the length and at various depths in the muscles. In no case, however, were positions near either end of the muscles used. To provide lower magnification views of the entire muscle, a 6x objective could be substituted, and the eyepiece and the camera were moved nearer to the objective lens. Striations were photographed using a 35-mm camera having a focal plane shutter. There was no lens in the camera. In each frame, about 0.2 mm of the width and about 0.3 mm of the length of a muscle could be recorded. Magnification was determined by photographing a Zeiss stage micrometer on which the small divisions were spaced 10μ apart. The calibrating photographs were obtained under exactly the same conditions as were the striation pattern photographs. The shutter was electrically triggered so that the exposure could be made either between stimuli or at any predetermined time during the twitch. Resting (between twitches) and active (at twitch peaks) striation photographs were obtained at seven muscle lengths in each of five muscles to make a total of 70 frames of film analyzed. The resting photographs were obtained at about the middle of the interval between twitches at which time no sign of activity was discernible optically or in the force trace. An exposure time of 1/125 seconds was used. To record striations, Kodak high-contrast copy film, 5069, was used.

Sarcomere lengths were measured by projecting the film with an enlarger having a 50-mm focal length, f/2.8 lens. In general, at least three or four groups of striations, each containing at least ten lines were analyzed in each frame. In each group, an average sarcomere length was determined by measuring the total length of the group and dividing it by the number of lines minus one, i.e., by the number of sarcomeres. The mean ± SE for the several values of sarcomere length determined from each frame was calculated and plotted (see Figs. 5 and 6). The papillary muscles in a large number of rat hearts were examined, and we were able to record some resting and active striation patterns of usable quality in 18 muscles. All of these muscles gave similar results, but only 5 will be described in detail in the present paper, since in these muscles resting and active striation patterns of reasonable quality were recorded over the complete range of muscle lengths studied.

Results

A typical papillary muscle is shown in Figure 1; it is long, thin, and fairly uniform in cross-sectional diameter. It is evident, however, that a discernible tapering of the papillary muscle occurs toward the tendinous end. We often observed through the microscope that there was some “give” during contraction at the muscle-tendon junction where the muscle diameter was smallest, but the greatest amount of give during contraction always took place at the heart wall end of the muscle. Here, no striations could be seen for several tenths of a millimeter from the suture knot. Clearly, this region was not contracting; rather, it was being extended by the central part of the shortening muscle. We tried other methods of attaching the heart wall end of the papillary muscle, e.g., by using a tiny clothespinlike clamp, but we could not substantially reduce the large amount of give during contraction.

The five papillary muscles used in this series of experiments had the following pertinent characteristics (means ± SE). The cross-sectional diameter, measured optically, in the central region of the muscles was 0.22 ± 0.03 mm, the calculated cross-section area in the central region, assuming a circular cross section, was 0.04 ± 0.01 mm2, the peak developed twitch tension at Lmax was 7.5 ± 0.58 g/mm2, the resting tension at Lmax was 1.3 ± 0.21 g/mm2, the ratio of resting tension to peak developed twitch tension at Lmax was 0.17, and the muscle length at Lmax was 2.3 ± 0.18 mm. Lmax
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Figure 1
Series of low-magnification photographs of a resting papillary muscle. The bar in A indicates 0.5 mm. The ends of the muscle were tied with 10-0 monofilament nylon suture to stainless steel wires 0.2 mm in diameter, one of which is visible at the left side. The muscle lengths relative to the length at $L_{\text{max}}$, the length at which peak twitch tension is maximum, are 1.03 (A), 1.01 (B), 0.96 (C), 0.83 (D), 0.77 (E), and 0.77 (F). E shows the muscle immediately after its length was reduced to 0.77 $L_{\text{max}}$. F was recorded at the same length but several minutes later. Stimuli recurred at a rate of 5/min throughout the experiment. Note that the muscle is bowed in E but straight in F.

Figure 2
Series of fixed-end twitches obtained while external muscle length was varied over a complete cycle of release followed by reextension to the original length. Length changes were made in the interval between twitches. The numbers above the brackets at the top of the figure refer to the size of the length change, i.e., the first four length changes on the left were decreases of 25μ. These changes were followed by seven decreases of 75μ. The arrow points to the very small twitch at the shortest length. Then, seven increases of 75μ followed by four of 25μ were applied. The muscle lengths relative to $L_{\text{max}}$, the length at which peak twitch force is maximum, are: 1.03, 1.02, 1.01, 1.00, 0.99, 0.96, 0.93, 0.90, 0.86, 0.83, 0.80, and 0.77. The time interval marked by T is 30 seconds, and the amount of force denoted by F is 83 mg or 3.3 g/mm². The trace labeled zero is a baseline obtained by shortening the muscle so that the twitch was completely undetectable.

The results shown in Figure 2 were taken from a papillary muscle whose length was changed over a complete cycle from longest to shortest and back to longest again. The length changes were made between each successive stimulus. At longer lengths, resting tension began to rise steeply in the vicinity of $L_{\text{max}}$. Beyond $L_{\text{max}}$, peak twitch height decreased only slightly, but resting tension increased very rapidly. Microscopic examination of the papillary muscles while they were being...

forces resist sudden changes in length in this region of the length-tension diagram, although it is not possible to be certain of their magnitude. This phenomenon of gradual straightening of the muscle outline was closely correlated with the time course of the gradual, small increase in peak twitch height to a final steady value which followed sudden length decreases at short muscle lengths when resting tension was near zero. In single skeletal muscle fibers (7), decreasing the length well below that at which optimum tension is generated produces marked bowing. On subsequent stimuli, the fiber outline becomes straight during the contraction, but it becomes bowed again in the relaxed state. In contrast, according to Hill’s work (8), it seems that whole skeletal muscles do not reextend themselves after repeated stimulation at short lengths.

The series of low-power photographs shown in Figure 1 was taken between twitches at various muscle lengths ranging from greatly stretched at the top to very short at the bottom. In going from top to bottom, the length was decreased between successive twitch contractions (stimuli occurred at a rate of 5/min). The general outline of the muscle remained nearly straight until the shortest length (Fig. 1E) was reached. As shown in E, the muscle was initially quite bowed at this length. However, after several minutes during which stimuli recurred at a rate of 5/min, the outline of the muscle became nearly straight again (Fig. 1F) even though the overall length was unchanged. Apparently, internal...

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stretched to lengths beyond L\(_{max}\) revealed that the heart wall ends of the muscles were the parts that were being mainly lengthened. It was impossible, therefore, in this series of experiments to produce substantial decreases in peak twitch height by stretching the muscles beyond L\(_{max}\). Also, heavily stretching these muscles tended to produce prolonged periods during which contractions recurred spontaneously.

The relation between resting tension and relative muscle length obtained in this series of experiments is shown in Figure 3 (open circles). Small increases in length beyond L\(_{max}\) would have increased the resting tension to values very near to that obtained for the maximum peak developed twitch tension. Such extensions were not attempted in this series of experiments because of the aforementioned deleterious effects produced in the muscles. The relation between peak developed twitch tension and relative muscle length is also shown in Figure 3 (solid circles); these points were obtained by decreasing muscle length between each successive stimulus. A decrease in length of only about 10-12% below L\(_{max}\) reduced the peak developed twitch force by about 50%. The peak developed twitch tension generated when the muscles were stretched beyond L\(_{max}\) decreased slightly, but we were unable, for reasons already mentioned, to produce a significant descending limb in the length-tension relation. The results shown in Figure 3, relating resting and peak developed tension to muscle length, can be compared with those obtained from rat papillary muscles by Grimm et al. (9). Their rat muscles were considerably longer and thicker than the ones we used in this work, but in the main there is agreement between our curves and those of Grimm et al. (9) with the exception that the resting tension at L\(_{max}\) expressed relative to the peak developed tension in our muscles was less than half that present in their muscles.

The collection of resting striation pattern photographs did not present a particularly difficult problem once a papillary muscle preparation of sufficiently small diameter was utilized. However, the collection of striation pattern photographs from active muscles did present problems. It became apparent that good photographs were least difficult to obtain at the peak of the twitch responses. Twitches had a fairly broad peak at the temperature used in this study, which is the reason for lowering the temperature. By taking photographs throughout the time course of a twitch, we found that the peak of the twitch was associated with the period of least overall motion of the muscles. It is also obvious that it would be most useful to try to correlate active sarcomere length with the maximum force produced so that our results could be more nearly compared with those of Gordon et al. (2).

Relative resting and active tension plotted against relative muscle length. Muscle lengths were expressed relative to L\(_{max}\), the length at which peak developed twitch tension was maximum, and tensions were expressed relative to the peak developed twitch tension at L\(_{max}\). All points were obtained by analyzing the decreasing length phase of records similar to that shown in Figure 2. The open circles represent resting tension. The solid circles show the peak developed tension that would be present in the contractile component of a Maxwell model, i.e., simply the difference between peak total and resting tension. The crosses indicate the total tension, i.e., resting plus peak developed tension, when this value is significantly different from the peak developed tension. The active contractile component tension in a Voigt model would lie somewhere between the two extremes given by the solid circles and the crosses. Note that, for each muscle length, the resting, peak developed, and total tension points are vertically in line. The curves were drawn by eye but with the constraint that they pass through the average values of resting and developed tension at a relative muscle length of 1.0.
outline remained buckled even if they were stimulated. At these lengths, very little, if any, active tension was recorded. Generally, at the shorter lengths used in this study, about 0.75 $L_{\text{max}}$, muscle outlines soon became straight, during rest, following decreases in length. In such cases, it could be seen microscopically that the initial waviness in the striation pattern gradually disappeared as the general muscle outline became straight. Striation pattern photographs were not obtained at the same position along or at the same cross-sectional level in each muscle, so that there is reason to believe that the results are a valid indication of the average behavior of most of the contracting sarcomeres.

The relation between average resting sarcomere length and relative muscle length is shown in Figure 5. The average value for sarcomere length at $L_{\text{max}}$ was 2.23μ. Increasing the muscle length beyond $L_{\text{max}}$ was not associated with an obvious increase in average sarcomere length. At the shortest muscle lengths, there was a tendency for the average sarcomere lengths to bottom out at about

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**FIGURE 4**

Striation patterns in a resting (A) and an active (B) papillary muscle. In B, the record was obtained at the time of peak twitch force by electrically triggering the camera shutter. The horizontal bar indicates 0.1 mm. The muscle length relative to $L_{\text{max}}$ was 1.00. Note that the striation pattern seen in the resting muscle in A remains visible while the muscle is contracting in B. Average sarcomere lengths are 2.3μ in A and 2.1μ in B.

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1.5–1.6μ, which is near the thick filament length of 1.5–1.6μ (10). Between L_max and 0.75L_max, there was an approximately linear relation between average resting sarcomere length and muscle length. This finding was expected, since the general outline of the muscles remained nearly straight in this region. In resting skeletal muscle, fibers become slack and bowed once sarcomere length reaches about 2.0–2.1μ, the length at which the thin filaments meet in the center of the A band. Apparently, in these heart muscles, regular periodic stimulation and the presence of resting tension can lead to further decreases in resting sarcomere length. The results presented in Figure 5 are also relevant to the well-known findings of Gay and Johnson (11), who studied sarcomere length and muscle length in living thin trabeculae taken from the right ventricles of rabbits. Gay and Johnson (11) concluded that there is no accurately predictable relation between overall strand length and resting sarcomere length. This conclusion does not apply to the rat papillary muscles that we studied. The reasons for the difference are not readily apparent, but, as pointed out by Page (10), the discrepancy may be related to the fact that Gay and Johnson (11) did not stimulate their strands regularly.

The relation between active average sarcomere length and relative muscle length is shown in Figure 6. The active average sarcomere length at L_max was 1.98μ, which represents a decrease of about 11% from the average value for the resting sarcomere length at L_max obtained from Figure 5. This decrease indicates a very large amount of internal shortening. Probably, the decrease can be mostly accounted for by give in the heart wall end of the papillary muscles as previously mentioned. Krueger et al. (12) have recently presented some studies of passive and active sarcomere lengths in rat trabeculae in which sarcomeres shortened by 11% in a fixed-end contraction at L_max. At lengths greater than L_max, active average sarcomere length remained in the range of 1.9 to 2.15μ so that the descending limb of the length-tension relation described by Gordon et al. (2) was never attained. Below L_max, active average sarcomere lengths decreased along a curvilinear path and appeared to reach a limit at an average sarcomere length of 1.4–1.5μ, which is not very much less than the average values shown in Figure 5 for resting sarcomeres near the bottom of the length-tension relation. Therefore, at the shortest muscle lengths, sarcomeres shorten by only a small amount during contraction. In contrast, in the midrange of muscle lengths, average sarcomere length decreased by about as much as 15%, which is even greater than the figure given earlier for internal shortening taking place at L_max.

In Figure 3 relative peak developed twitch tension was plotted against relative muscle length, and in Figure 6 the average active sarcomere

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lengths obtained at the peak of the twitches were plotted against relative muscle length. Each pair of measurements of relative peak developed twitch tension and simultaneous average active sarcomere length had a common relative muscle length. Therefore, we constructed a graph of relative peak developed twitch tension plotted against average active sarcomere length (Fig. 7, solid circles).

Furthermore, using the data shown in Figures 3 and 5, we plotted relative resting tension against average resting sarcomere length (Fig. 7, open circles).

Discussion

The unusually small mammalian papillary muscle preparation used in this work was obtained from very young rats. The temperature and the frequency of contraction maintained were well below the physiological normals for this animal. However, the macroscopic behavior as reflected in the resting and peak developed muscle length-tension relations is similar to that obtained from larger muscles from either the same or different species. This fact is our only justification for applying the results obtained regarding the relations between tension and average sarcomere length to other papillary muscle preparations.

In the graph of relative tension versus average sarcomere length shown in Figure 7, the solid line shows the results of Gordon et al. (2) relating tetanic, steady tension to sarcomere length for frog skeletal muscle fibers. To compare our results with theirs, the lengths of the thick and thin filaments, together ideally with the widths of the Z line and the bare zone in the middle of the thick filament, should be known. It appears that cardiac thick filaments have about the same length, 1.5-1.6μ, as those of frog skeletal muscle (10). Thin filament lengths are not exactly known for rat heart muscle, but it seems likely that their length is about 1μ (10). Therefore, the lengths of the thick and thin filaments from frog skeletal and rat heart muscles are approximately the same so that the form of the active tension versus active average sarcomere length relation for the two muscles could also be similar. That there is some resemblance can be seen in Figure 7 by comparing the peak developed tension data (solid circles) with the curve of

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**Figure 7**

Relative tension plotted against average sarcomere length. Tensions are expressed relative to the peak developed twitch tension at Lmax. Peak developed twitch tension points (solid circles) were obtained by replotting the active sarcomere length data given in Figure 6 and the peak developed twitch tension data given in Figure 3 with relative external muscle length as a parameter, which is appropriate for a Maxwell model. The crosses indicate the maximum possible contractile component tension, peak developed tension plus all of the resting tension, in a Voigt model. The actual value would lie somewhere between the solid circles and the crosses depending on the extent to which parallel elastic component tension is transferred to the contractile component during contraction. The crosses have been omitted where the values are not significantly different from those for the solid circles. The pair of arrows indicates, for a set of points obtained at one muscle length, that active sarcomere length is decreased from the resting value. Note that the crosses are vertically in line with their respective solid circles but that the open circles are not. A correction to the peak developed and total tension values plotted in this figure was necessary because the active striation pattern photographs were recorded during contractions that occurred several minutes after the changes in muscle length were made. As discussed in the text, there was a small, gradual increase in peak twitch tension to a final steady value after the muscle length was reduced from values well below Lmax. The result of this correction is to raise slightly the points below active tension values of 0.85. The open circles represent resting tension and were obtained by replotting the resting sarcomere length data given in Figure 5 and the resting tension data given in Figure 3 with relative external muscle length as a parameter. The broken curve was fit to the resting points by eye with the constraint that it pass through the relative resting tension of 0.17 at the resting average sarcomere length of 2.23μ. The solid curve is taken from the results of Gordon et al. (2), and it shows the relation between active tension and sarcomere length for tetanically stimulated frog skeletal muscle. No distinction was made, in the work of Gordon et al. (2), between resting and active sarcomere lengths, since the methods used made it unlikely that significant internal shortening occurred during contraction.
Gordon et al. (2). The results indicate that an ascending limb relating active tension and sarcomere length exists and is associated with a range of sarcomere lengths below about 2.0μ. This finding suggests that some of the factors limiting force generation in skeletal muscle at short lengths may also limit it in mammalian cardiac muscle. These factors may be related to both structural limitations, e.g., amount of double overlap and compression of thick filaments against Z disks, and time, shortening- and length-dependent limitations on the degree of activation. The broken curve shown in Figure 7 relating resting tension to resting sarcomere length is, of course, much different from that obtained from isolated frog skeletal muscle fibers in which significant resting tension does not appear below a sarcomere length of about 3.0μ (13). The fact that in rat heart muscle the resting tension increases steeply in the vicinity of Lmax may explain the absence of a plateau region in the length-tension diagram similar to that observed in frog skeletal muscle (2). The high resting tension prevents rat papillary muscles from being lengthened to such an extent that the internal shortening of about 11% occurring at the peak of a twitch would place the active sarcomere lengths in the range of 2.0 to 2.2μ.

Up to this point, the active tension developed by the contractile component has been taken to be the difference between the peak total tension developed during contraction and the resting tension, i.e., the peak developed tension. This definition is correct if it is assumed that a Maxwell model is the most suitable analogue for cardiac muscle (Jewell and Blinks [14]). In this analogue, the parallel elastic component is in parallel with the series combination of the series elastic component and the contractile component so that during a fixed total length contraction the contractile component active tension is easily obtained since the parallel elastic component length and resting tension are held constant. However, there are some reasons for believing that a Maxwell model may not be applicable to our preparation. It is clear that the central parts of the muscles shortened considerably while the ends lengthened during contraction. In addition, as already mentioned, more internal shortening occurred during contractions at intermediate muscle lengths. Preliminary attempts to simulate these findings have suggested that a Voigt model may be more appropriate for our preparation. In a Voigt model, the series elastic component is in series with a parallel combination of the parallel elastic component and the contractile component. During a contraction, the series elastic component is further extended while parallel elastic component extension is reduced. The active tension generated by the contractile component in a contraction can have any value between the peak developed and the total tension, depending on the tension-extension characteristics of the series and the parallel elastic components and the amount that parallel elastic component extension is reduced.

From the data that we have presented in the present paper, it is not possible to determine exactly which value to use for contractile component active tension in the Voigt model. The greatest difference from the peak developed tension data occurred in the region of the length-tension diagram where resting tension began to increase steeply. The steeply rising resting tension implies that both the parallel and the series elastic components are operating in very stiff parts of their tension-extension relations. Since the amount of internal shortening occurring in this region was large, it is possible that the true contractile component active tension was nearly equal to the total tension, because parallel elastic component shortening reduced its tension to near zero. For these reasons, each peak developed tension point in Figures 3 and 7 has associated with it a point (crosses) which indicates the total tension present, i.e., the peak developed tension plus all of the resting tension present at the beginning of the contraction. The main effects produced by plotting the active tension in this way are, in Figure 3, to eliminate the peak in the active length-tension curve, and, in Figure 7, to call into question whether a plateau exists in the active sarcomere length-tension diagram.

The plot of active total tension against sarcomere length in Figure 7 bears some resemblance to the plot of peak twitch tension against sarcomere length presented by Close (15) for large cross-sectional area frog skeletal muscles at 20°C in that a definite tension plateau is absent over the range of sarcomere lengths from about 2.0 to 2.2μ. Close (15) thought that this result was characteristic of muscles that were only partially activated, since the results he obtained from tetanized, large cross-sectional area muscles were very similar to those obtained by Gordon et al. (2). If total active tension more nearly indicates the contractile component contribution, then our results suggest that this papillary muscle preparation was incompletely activated in a twitch. This finding is not surprising, because it has been shown by Kavaler et al. (16)
that heart muscle is probably not fully activated by a single stimulus in a bathing medium of the kind used in this work.

There are further difficulties encountered in Figure 7 in attempting to compare the plots of either active peak developed tension or total tension against sarcomere length with similar plots obtained from frog skeletal muscles. It is obvious that in our work considerable internal shortening occurred during the rising phase of the twitches. This fact means that our results were not obtained under truly isometric, or fixed sarcomere length, conditions. It certainly would not be justifiable to assume that peak tension in a twitch would be unaffected by internal shortening. Even if internal shortening could have been prevented, the sarcomere length-tension relation obtained using peak twitch tensions would very likely be different from that obtained using stimulating conditions more nearly equivalent to the tetanic state in skeletal muscle because of the time limitation on activation in a twitch. The situation becomes even more complicated if the effects of shortening during contraction on activation are considered. Deactivation can be caused by suddenly changing the length of a muscle as shown by Brady (17) and Edman and Nilsson (20). Abrupt length changes did not occur in this work, so this effect was probably not significant. However, Jewell and Wilkie (19), in skeletal muscle, and Edman and Nilsson (20), in cardiac muscle, have shown that if shortening takes place during a contraction the capacity to produce tension is reduced. This phenomenon may be thought of as a form of deactivation caused by shortening, and its effect could have substantially influenced our results.

In addition, it is now commonly believed that muscle or sarcomere length can influence excitation-contraction coupling and the level of activation. This effect has been shown by Taylor and Rüdel (5) for frog skeletal muscle fibers contracting at lengths well below the plateau region. In cardiac muscle, Blinks (21), Nilsson (22), and Allen et al. (23) have all presented evidence indicating that muscle length may be an important factor in determining duration of mechanical activity, decay of active state, and degree of activation. We have obtained results similar to those reported by Blinks (21), which show that twitch duration in the papillary muscles used in the present study becomes progressively shorter as muscle length is decreased below $L_{\text{max}}$. Therefore, at least three additional factors must be taken into account in interpreting the results shown in Figure 7. These factors can be conveniently separated into time-dependent, shortening-dependent, and length-dependent influences on the level of activation. These factors in addition to the simple structural factors such as the degree of double overlap also affect the form of the active sarcomere length-tension relation. The form indicated in Figure 7 may only approximate the true curve obtained under conditions in which internal shortening is prevented and activation is complete.

The results presented in the present paper are not easily related to those obtained from fixed specimens of heart muscle examined with either light or electron microscopes. Page (10) has reviewed the results obtained using fixed material, and her paper should be consulted for details and references. Page (10) points out that there are a number of problems involving shrinkage and other sources of error that make it difficult to obtain accurate measurements of either sarcomere or filament lengths from fixed material. She finds, if suitable corrections are made for probable amounts of shrinkage, that the values reported in the literature for resting sarcomere length at $L_{\text{max}}$ range from 2.2 to 2.45µ compared with our value of about 2.2µ. There are also discrepancies concerning the values for the resting sarcomere lengths at short muscle lengths for which peak twitch tension is small. Closer agreement would be expected between our results and those of Pollack and Huntsman (24) because both studies used the light microscope to obtain sarcomere length measurements in living rat right ventricular papillary muscle and trabecular strand preparations. The values reported for the average sarcomere length at $L_{\text{max}}$ are similar: our value is about 2.2µ, and Pollack and Huntsman obtained a value of 2.3µ. There is also agreement concerning the relative muscle length at which peak twitch tension has declined to about 25% of that produced at $L_{\text{max}}$, but there is disagreement over the value of resting sarcomere length for this muscle length. Our value is about 1.75µ, but Pollack and Huntsman (24) have reported a value of about 2.0µ. The reasons for this discrepancy are not yet clear, but in our experiments resting sarcomere lengths at $L_{\text{max}}$ were rarely greater than 2.3µ. Since the external outlines of our muscles remained straight in the region between $L_{\text{max}}$ and the relative length at which peak twitch tension declined by about 75%, it is likely, as shown in Figure 5, that resting sarcomere lengths decreased in at least the same proportion as did muscle length. In addition, we did not detect any waviness in the striation pattern that would

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indicate the presence of myofibrillar curling or buckling in this region.

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Sarcomere length-tension relations in living rat papillary muscle.
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Circ Res. 1975;37:299-308
doi: 10.1161/01.RES.37.3.299

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