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MYOCARDIAL SARCOMERE DYNAMICS DURING ISOMETRIC CONTRACTION

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SUMMARY

1. Sarcomere lengths were measured during rest and throughout the time course of isometric contractions in thin, isolated rat papillary muscles using light diffraction techniques.

2. Shortening of the sarcomere length occurred upon contraction at all muscle lengths, averaging 7% at optimal length and more at shorter lengths. Relative to the narrow range of sarcomere lengths spanning the length–tension curve, this degree of shortening was considerable.

3. Local changes of sarcomere length were quantitatively paralleled by local changes of tissue segment length, the latter demarcated by microspheres lodged within the muscle tissue. At all but the shortest muscle lengths, sarcomere shortening was fully accounted for by equivalent lengthening of the non-striated regions near the clamped ends of the preparation.

4. It seems likely that these regions constitute the source of the large series elasticity characteristic of isolated papillary muscle preparations such as this.

INTRODUCTION

Current concepts of cardiac mechanics rest almost entirely upon results obtained from isolated papillary muscle preparations. Yet little is known about the internal dynamics of such preparations. Many hypotheses have been advanced and are being debated (Brady, 1973; Meiss & Sonnenblick, 1974), but there are few data available at the microscopic level to confirm or rule out any of these hypotheses.

Because the mechanics at the microscopic level are crucial to the interpretation of the macroscopic measurements, we sought to investigate sarcomere length changes that might occur during contraction in a mammalian papillary muscle preparation. The results are straightforward, and provide some clues as to the source of the many uncertainties regarding cardiac muscle mechanics.

METHODS

Thin papillary muscles from the right ventricles of rats were mounted in a chamber seated on a microscope stage. Muscle length was adjusted by a micrometer and muscle force was measured. Sarcomere length (measured using laser diffraction techniques) and the length of small segments of tissue (defined by the positions of microspheres lodged within the capillary beds of the muscle) were sampled at rest and during isometric contraction using a video microscopy system. All optical data were recorded for subsequent analysis.

Since many of the features of this system have been detailed elsewhere (Pollack & Huntsman, 1974), only those modifications pertinent to this study will be described.

Preparation of muscles. The hearts of Sprague–Dawley white rats were rapidly exposed after the animals had been anaesthetized with ether. Oxygenated physiological salt solution at room temperature was immediately infused into the coronary arteries via the aorta to remove blood from the myocardial tissues. The blood-cleared heart was then placed in a dissection chamber submersed in recirculating, oxygenated physiological salt solution also at room temperature. The right ventricle was carefully opened. Papillary muscles suitable for diffraction studies (long, thin, and uniform) were present in only one-eighth of the hearts inspected.

The tendon and fleshy end of the papillary muscle were secured between the flat, opposing surfaces of two stainless steel clips (Pl. 1). The preparations were mounted horizontally, submerged in a recirculating, oxygenated physiological salt solution maintained at 30° C. Parallel platinum wire electrodes were used to impose 12 stimuli/min, 5 msec duration, at a voltage 20% above threshold. The pH, P_{CO_2}, and P_{O_2} were sampled near the muscle in the first eleven experiments, and consistent values of 7·4, 45 mmHg, and 550 mmHg (respectively) were routinely achieved. These gas tensions were maintained by vigorously bubbling 95% O_2–5% CO_2 gas through the physiological salt solution in a reservoir. All solutions used in this experiment consisted of the following components, in mM: Na^+, 140; K^+, 5; Ca^{2+}, 2·25; Mg^{2+}, 1·0; Cl^-, 103; HCO_3^-, 24; SO_4^{2-}, 1·0; HPO_4^{2-}, 1·0; acetate, 20·5; glucose, 10; and insulin, 2·5 units/l.

The length of the muscles was adjusted by a micrometer, and the resting and developed forces were measured with a Statham UC2 strain gauge bridge transducer and recorded on a strip chart recorder. The motion of the clip ends was examined during contraction as part of the experimental procedure, and the largest excursion was 10 μm which amounted to 0·3% muscle length. This movement was considered to be insignificant, so that the total muscle length was regarded as constant.

Marking internal tissue segments. In six of the thirteen muscles studied, discrete tissue segments were defined by the positions of carbonized microspheres (15 ± 5 (S.D. of observation) μm diameter, Nuclear Products Division, 3 M Co.) which had been lodged within the capillary beds of the muscles. In these preparations, a 0·1% suspension of microspheres (dry vol. microspheres/vol. saline) was infused via the severed aortic stump. Microspheres were initially suspended with a 0·02% solution polyoxyethylene-80 sorbitan monovaleate (Tween-80 detergent) in saline to prevent aggregation; washed twice with freshly oxygenated saline; and, after a third resuspension only those microspheres remaining in suspension were used. Microscopic inspection of the tissue showed microspheres 10–20 μm in diameter lodged within the fine vessels of the muscle (Pl. 2). That these particles were securely lodged within the vessel was confirmed by direct microscopic observation: they did not migrate from their initial resting positions after a repeated number of contractions and the relation of the microspheres to the surrounding tissue did not change during contraction.

Optical methods

Measurement of muscle dimensions. Muscle length, width, and thickness were measured at that muscle length at which actively developed force was maximal (i.e. L_{max}). The average half-thickness of each muscle was determined at its mid length by selectively focusing on the near surface and the two edges of the muscle and noting the excursions on a stage focusing micrometer. The cross-sectional area of the muscle was calculated by considering muscle width and thickness as major and minor axes of an ellipse. Average thickness of the thirteen muscles was 171 μm (range 45–300); the average width, 267 μm (range 125–462); the average length, 2·50 mm (range 1·38–4·63); and the cross-sectional area, 0·04 mm^2 (range 0·004–0·09).

Direct measurement of sarcomere length. In six additional muscles sarcomere length was measured by focusing directly upon the sarcomeres during contraction. This method was used only to evaluate the diffraction methods. The muscles were illuminated by a strobe light (Strobex No. 71B, Chadwick Helmuth) synchronized with the vertical sweep of a television camera (Cohu No. 4300), giving a light pulse every 16·7 msec. These pulses were gated to occur only during the time course of the contraction. The illumination signals were electromagnetically picked up and superimposed on the original force traces to serve as time references. Flash duration of the strobe (*ca.* 50 μsec) was sufficient to freeze the motion of the image. Sarcomere length was measured directly from videotaped images of the muscles obtained with a 40 × 0·75 N.A. water immersion lens (Carl Zeiss). Sarcomere length was obtained by including 15–30 consecutive striations and averaging the values. Length was measured by a movable cursor generated by a video-analyser (Colorado Video Instruments No. 321).

Light diffraction methods. A continuous wave helium-neon laser (Spectra-Physics no. 120, 3 mW) or a pulsed argon-ion laser (TRW no. 71A, 2 mW average power, 40 μsec pulse length) was used to obtain the diffraction information from the papillary muscles. Light diffracted by the muscle was collected by a Zeiss 40 × 0·75 N.A. water immersion lens. The focused diffraction pattern at the rear focal plane of the lens was projected on to the vidicon tube of a television camera using an eyepiece telescope (Auxiliary Magnifier no. 513123, Ernst Leitz Co.). The telescope was focused by minimizing the diameter of the laser spot without the muscle in place.

An example of the focused diffraction pattern is shown in Pl. 2*B*. Bands of diffracted light run parallel to the striations in the muscle and are symmetrically spaced on either side of the bright band of non-diffracted light. The distance between the bands is inversely related to sarcomere length. Because an objective lens was used to collect the diffracted light, the observed diffraction pattern represents the two dimensional Fourier transform of the striation pattern contained in the muscle (Goodman, 1968). The horizontal variable in this focused pattern, D, corresponds to $\lambda f/d$, where f equals the effective focal length of the lens system, λ equals the wavelength of the incident illumination, and $1/d$ is the spatial frequency of the striated pattern with a spacing, d. The spatial frequency of peak intensity corresponds to that striation spacing which occurs most often, i.e. the statistical mode (Gradmann & Hort, 1964). The width of the focused diffraction orders is a function of the dispersion (i.e. distribution) of sarcomere lengths in the muscle. As such, there is a theoretical possibility of inferring the sarcomere length distribution directly from the shape of the first order diffracted light. However, certain features of cardiac muscle structure discourage this approach. The chief difficulty centres about the low angle scattering by abundant non-sarcomeric structures (i.e. nuclei, mitochondria, connective tissue). Scattering from these elements alters substantially the collimation of the zeroth order; then layering gives rise to significant broadening of the higher orders. The resultant broadening is unrelated to sarcomere length dispersion.

The uncertainty associated with evaluation of sarcomere length distribution does not carry over to the evaluation of sarcomere length (i.e. the most prevalent, or modal, sarcomere length) from the positions of the first order peak spectral intensity. The effects of striation misregistration, layering, finite aperture of the lens system, and non-sarcomeric scattering can be shown not to compromise the accuracy of sarcomere length measurement (D. A. Christensen, personal communication).

The striation spacings were determined by displaying the light intensity distribution as a function of the video signal voltage, as shown in Pl. 2*C* and *D*. (In these oscilloscopic displays, all of the raster lines forming the television image of Pl. 2*B* are superimposed, giving rise to the fuzziness.) The positions of peak intensity were determined with a movable cursor generated by a video-analyser. The cursor's position was read potentiometrically with a digital voltmeter. The striation spacings corresponding to these positions were determined by suitable calibration as discussed next.

Calibration of optical measurements. When sarcomere lengths were measured by directly focusing upon the specimen, the image of an eyepiece graticule was superimposed and recorded with that of the sarcomeres. This graticule was pre-calibrated with a stage micrometer.

With the diffraction methods, the modal striation spacing in the specimen was determined by referencing the lateral separations of the two first order peak intensities to similar patterns obtained from calibrated diffraction gratings. A series of phase gratings of 2400, 7500, 13,400 and 15,000 lines/in. were used to calibrate positions throughout the full range of spatial frequencies expected.

Comments about the diffraction methods

Sample size. The microscope objective lens collected and focused light diffracted from a field approximately 100 μm in diameter. (In our system, the field size is determined by the magnification of the objective lens and limited by the field stop in the focusing telescope.) For a representative muscle we estimate that the sampled volume contained the equivalent of 100 cells with a length 40 striations. Although the cross-sectional dimension of the ultimate unit forming the diffraction pattern is uncertain, it is undoubtedly subcellular, possibly myofibrillar. In any case, the striation spacing measured by diffraction represents sarcomere length measured over a very large number of these units.

Precision of measurements. Sarcomere length changes can be measured very precisely by light diffraction techniques, as shown by Cleworth & Edman (1972) who detected spectral motions resulting from perturbations 5 nm/sarcomere in single skeletal muscle fibres. However, a large amount of light scattering from non-contractile material, striation misregistration, and non-uniformity of striation spacing broaden and weaken the spectra obtained from papillary muscles. The precision is consequently less than that achieved in single skeletal muscle fibres.

Precision was assessed empirically from the S.D. of repeated measurements made by different observers. Six cases were considered: spectra from a representative muscle at rest and at peak shortening were analysed for three lengths spanning the experimental range (i.e. L/L_{max} = 0·84, 0·93, 1·00, where L_{max} equals length at which peak active tension is maximal). Sarcomere length ranged from 1·8 to 2·2 μm. Fifteen measurements were made by each of five observers at each situation. The largest S.D. of any observer's measurements in any situation was 0·05 μm. The average S.D. for all observers in all situations was 0·025 μm. These data provide some estimate of the level of precision of our methods.

Accuracy. It is difficult to determine how faithfully the positions of the peak spectral intensities delineate the true modal sarcomere length. An estimate of the

extent of inaccuracies due to all sources of error can be made by comparing sarcomere lengths measured by focusing upon the muscle with those measured in the same field by diffraction. Mean sarcomere lengths obtained by direct measurement in three muscles at rest were 2·16, 2·24, and 2·30 μm. Light diffraction measurements of the same populations indicated sarcomere lengths of 2·18, 2·22 and 2·26 μm, respectively. These values agree within the level of precision established in the previous section.

Spectral changes during contraction and between stimuli. A consistent pattern of changes characterized the diffraction spectra during contraction, as illustrated in Pl. 2*C* and *D*. With shortening, the distribution of the diffracted light broadened and its peak intensity decreased substantially. Increased sarcomere length dispersion, misregistration, and decreased I band length are possible explanations of these changes. Our present techniques do not permit us to evaluate dispersion quantitatively: even qualitative changes are difficult to assess since changes of zero order intensity and breadth (Pl. 2*C* and *D*) may affect the distribution of the diffracted light.

First order intensities with multiple peaks were occasionally observed during contraction in thinner specimens, indicating multiple peaks in the sarcomere length distribution. This was more characteristic of muscles *after* the time of peak force, so it was not relevant in this study.

Immediately following contraction the diffraction pattern was motionless. Toward the end of the interval between contractions, especially when the stimulus frequency was reduced, we saw a tendency for motion of the speckle spots comprising the diffraction pattern. Occurrence of speckle movement coincided with the appearance of small foci of contractile activity similar to those seen in frog atrial muscle (Winegrad, 1974). The loci of spontaneous activity propagated longitudinally. Throughout this event the position of the first order peak spectral intensity did not appear to change, and the resting force remained constant.

Profuse speckle movement and sarcomere motions enveloping the whole specimen characterized most muscles immediately after mounting in the apparatus. This initial, random contractile activity receded with subsequent stimulation and subsided with the onset of stable mechanical properties. Regions of sarcomere motion sometimes persisted near the ends of the preparations.

Experimental procedure

After 1 hr of mechanical stabilization during which time the random sarcomere and speckle motions between contractions disappeared, the length–tension characteristic of each muscle was obtained. Tensions from multiple contractions at a given muscle length were averaged, but the first few beats after each lengthening increment were not included. The muscle's length–tension characteristics obtained by stepwise shortening from L_{max} and by stepwise stretch from rest length were then averaged. The muscle's dimensions were determined at L_{max}. Four to five muscle lengths were selected to obtain data spanning the muscle's length–tension diagram.

Diffraction and segment length changes were sampled at contiguous positions along the muscle at each of the pre-selected lengths. The motion of the clips holding the ends of the muscle was observed. Force was monitored throughout the acquisition of data, and the length–tension characteristics of the muscle were re-checked after observation as each muscle length was completed. The first and last determinations of length–tension gave rise to curves which were often, but not always, superimposable in absolute force: the position of L_{max} was never seen to change significantly. Nine muscles were studied in order of increasing length, four in order of decreasing length.

All of the data were stored on one-inch videotape and analysed at a later date.

RESULTS

Sarcomere length behaviour during contraction

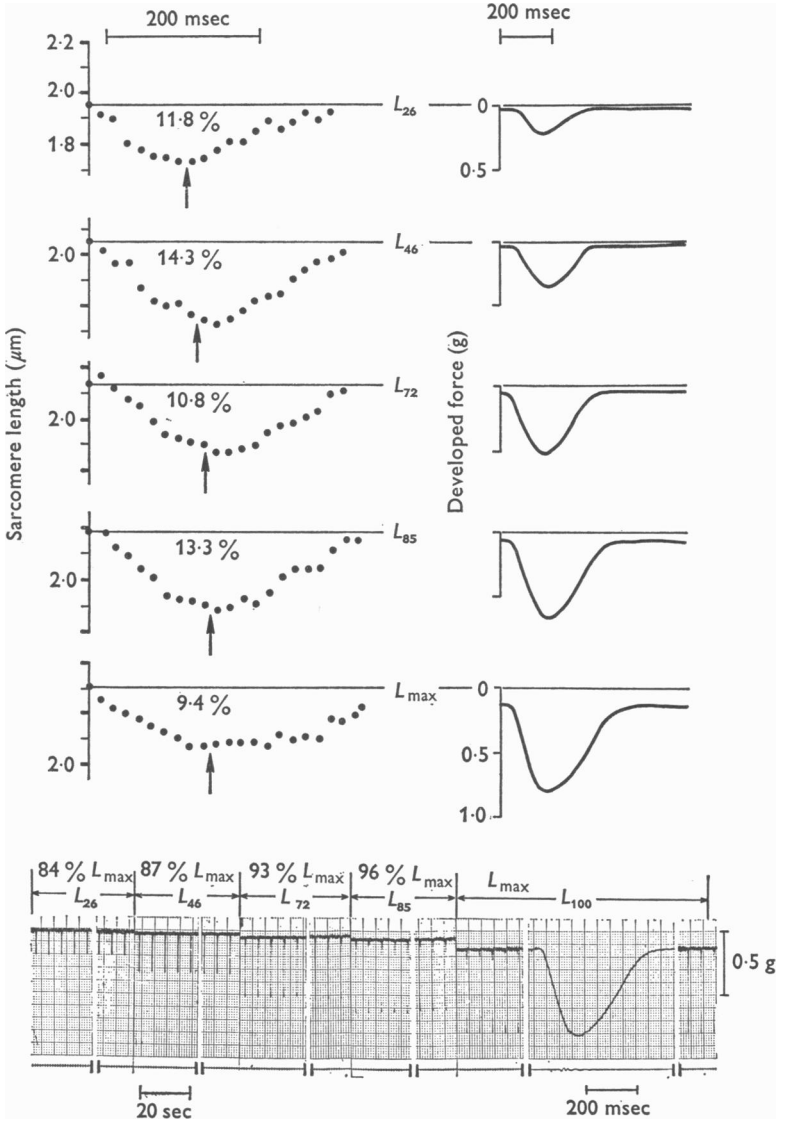
Microscopic inspection revealed regions near the ends of the muscles which appeared either non-striated or partially striated. (These regions corresponded to tendinous material or those parts of the muscle damaged by the clips.) Sarcomere length was sampled from fields spanning the length of the fully striated part of the muscle, usually 60 % preparation's length. Unless otherwise stated, results consisted of combining the observations at all positions to obtain the 'average' sarcomere length within the fully striated portion of the muscle.

All muscles consistently exhibited sarcomere shortening during a twitch. Text-fig. 1 present typical results. The sequence of spatially averaged sarcomere length changes and the developed force are shown for five muscle lengths. In all muscles the sarcomere length changed with essentially the same time course as force development. The minimal sarcomere length during contraction occurred near the time of peak force. Comparable results were seen in the six additional muscles in which sarcomere length was measured directly from the striated image.

The amount of shortening varied among specimens, from 5 to 16 % resting sarcomere length. This degree of shortening means that there was a large velocity of shortening during isometric contraction of the muscle. As a representative example (from Text-fig. 1), a 12 % shortening within 120 msec (time to peak force) is equivalent to an average shortening velocity of 1 muscle-length/sec. Peak velocity of shortening was larger than this average, since the velocity was zero at the moment of peak force development.

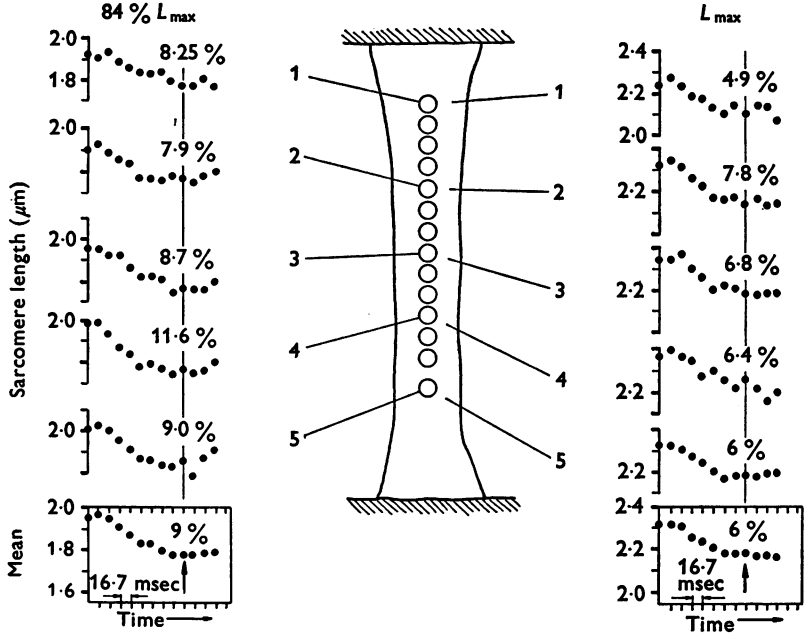
Although the spatially averaged sarcomere length presents a smooth sequence of changes during contraction, minor differences in localized shortening activity could be found. Text-fig. 2 demonstrates some of the common regional variations within a muscle held at optimal length (L_{max}) and 84 % L_{max}. The relative positions at which these observations were made are shown in the muscle diagram which is drawn to scale at 84 % optimal length. The circles on the muscle denote the relative size of the area sampled in the diffraction measurements.

Shortening occurred soon after stimulation at most positions. Minor variations in the onset of shortening might be attributable to variations in latency within the muscle and the temporal resolution of the measurements (i.e. 16·7 msec), so that bulk shortening within the fully striated regions of the muscle is approximately synchronous and uniform until the moment of peak force development (indicated by arrows in the Text-figure). Variations in the degree of shortening were observed as well. Near



Text-fig. 1. Sarcomere shortening and contractile tension at five degrees of stretch. The sequence of sarcomere length change, averaged over all sampled positions along the muscle, is shown at the left. The contractile force traced from the original records is shown at the right for the respective muscle lengths. L_{26}, L_{46}, etc. denote % maximal active force developed at the selected muscle length, where active force is the difference between developed and resting forces. The nos. 11·8, 14·3, etc., denote the percent sarcomere shortening calculated at the moment of peak force development (arrow). The original force records at the start and end of each observation period are shown in the bottom panel. The forces during the period of switchover to the next length, and subsequent few contractions are not shown.

the boundaries of the fully striated regions shortening was often reduced. Shortened sarcomeres sometimes persisted beyond the time of peak force development, resulting in the greatest disparity of regional sarcomere shortening after this moment (not shown). Between the onset of shortening and the time of peak force development, sarcomere lengthening was not seen over the fully striated region of the muscle.



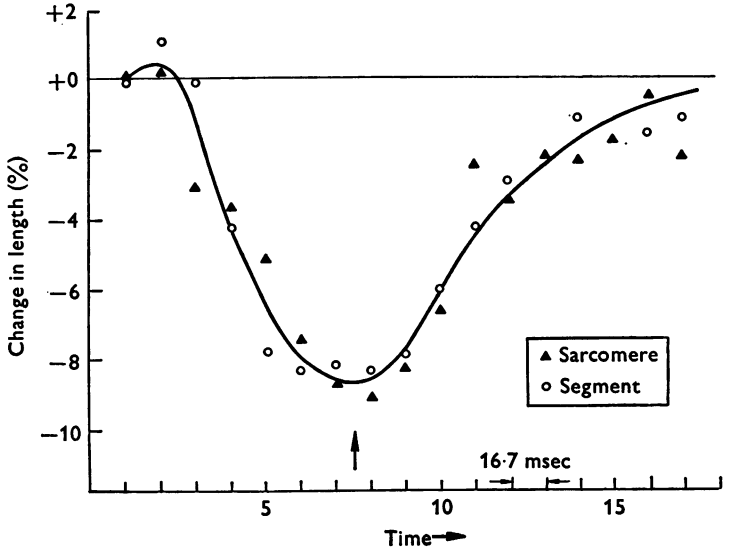
Text-fig. 2. Localized sarcomere length behaviour. The sequence of sarcomere length changes during the first part of contraction is shown for different positions sampled throughout the fully striated region of the muscle. Two muscle lengths are shown, optimal and 84% optimal length. The relative position of each sample point is shown in the diagram of the muscle drawn to scale for 84 % L_{max}. The diameter of circles on the muscle is equivalent to area of sampled field. While regional differences in % shortening exist (value given for each position), over-all shortening is synchronized. The shortening sequence appeared similar as the sample field was moved across the width of the muscle. The sequence of sarcomere length changes representing the mean of all fourteen positions is shown at the bottom.

Anatomical source of sarcomere length changes

We sought to determine why sarcomeres should shorten in an apparently isometric contraction. Two possibilities seemed worth considering. First, the damaged ends might constitute a source of series compliance, permitting shortening throughout the remainder of the muscle. Alternatively, local shearing, slippage between adjacent cells, or slackened intercellular

connexions might permit sarcomeres to shorten independently of the surrounding tissue, leading to sarcomere shortening in excess of tissue shortening.

The latter possibilities were tested by comparing sarcomere and tissue length changes throughout the contraction. Tissue segments were defined by the distance between microspheres lodged within the capillaries of the muscle. Only the thinner muscles were considered. Small segment lengths

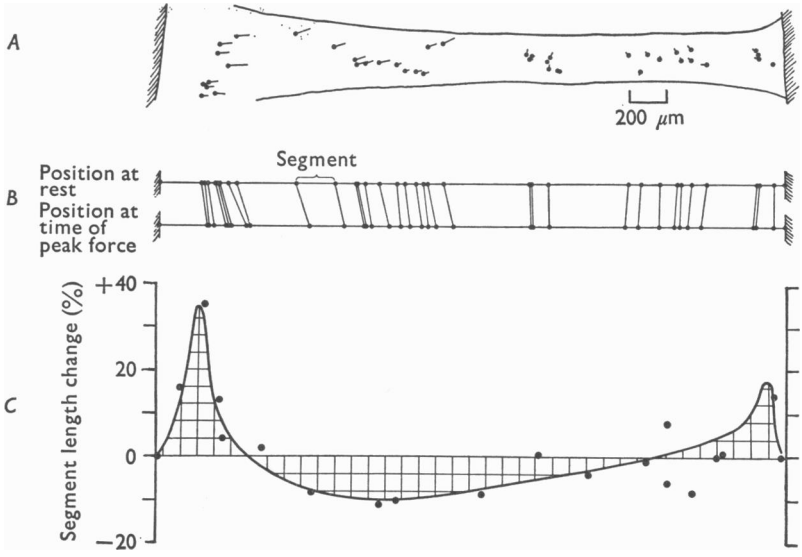


Text-fig. 3. Contractile sequence of sarcomere and tissue segment length changes. The amounts of shortening measured in the segment of tissue and by diffraction were expressed as $\frac{\text{change of length}}{\text{initial length}} \times 100\,\%$. (Segments were defined by the longitudinal distance between any two microspheres embedded within the tissue.) The tissue segment shown was small; its dimension approximating the field diameter sampled by diffraction and it was located mid length in the muscle. Arrow denotes moment of peak contractile force. Muscle held at 93 % optimal length.

were chosen, the separation of the microspheres approximating the diameter of the area sampled by the diffraction measurements. A representative result is illustrated in Text-fig. 3, for a muscle held at 93 % L_{max}. The degree and sequence of tissue length changes paralleled the changes indicated by the diffraction measurements. This parallelism did not hold up near the bottom of the length–tension relation (i.e. 79–85 % L_{max}), where sarcomere shortening significantly exceeded tissue shortening, sometimes by as much as 30 %; however, over the remainder of the physiological length region, and particularly above 90 % L_{max}, tissue

shortening and sarcomere shortening were in excellent agreement. In this length range it appears that any microscopic inhomogeneity intrinsic to cardiac tissue cannot be the cause of the sarcomere shortening we observed.

To investigate the other possibility, that high end-compliance might give rise to sarcomere shortening, we compared lengths of adjacent segments throughout the length of the muscle at rest and at the time of peak force. (Since we established that segment length changes reflect sarcomere length changes, we were able to substitute the former for the latter, thereby retaining the same measurement technique throughout the length of



Text-fig. 4. Internal tissue movements at L_{max}. *A*, pattern of microsphere translation within muscle. The relative positions of the microspheres at rest (●) and at peak contractile force (end of straight line) are shown. *B*, distribution of net movements in tissue during shortening. The longitudinal translation of selected microspheres is illustrated by projecting upon reference lines the positions at rest and at the time of peak force development. Trajectories connect a microsphere's two positions. The over-all pattern of these net motions indicates qualitatively that tissue shortening occurs in the middle of the muscle at the expense of the stretched ends. *C*, extent and distribution of tissue segment length changes. A tissue segment is defined as the longitudinal component of distance between any two microspheres. Segment length changes (●) are shown along the length of the muscle: these length changes are expressed as $\frac{\text{change in length}}{\text{initial length}} \times 100\,\%$ and are assumed to be zero at the clip edge. Note that the area included below the abscissa is approximately equal to the sum of the areas above it, indicating that tissue shortening is accounted for by lengthening at the ends of the muscle.

the muscle.) Text-fig. 4*A* maps the net translation of microspheres during contraction in a muscle held at L_{max}. Relative positions of microspheres at rest and at the moment of peak contractile force are shown. Considerable tissue movement is evident during the contraction. Clearly, the ends of the muscle can be held fixed, but the tissue is not isometric.

The distribution of net tissue movements is qualitatively shown by projecting the microsphere's relative positions on to a reference grid in Text-fig. 4*B*. (Only the longitudinal component of microsphere motion is considered.) Trajectories link a given particle's position in resting muscle (upper line) to that at the time of peak force development (lower line). The local degree of tissue segment length change is shown in Text-fig. 4*C*. Tissue segment length was defined as the projection upon the reference line of the distance between any two respective microspheres (i.e. the longitudinal component of microsphere separation). Analogous to strain, localized changes in segment length have been expressed as the changes in length divided by initial length. It is apparent from Text-fig. 4*C* that shortening occurs within the central region of the muscle at the expense of the ends. Very high localized strains occur near the ends of the muscle, where the tissue appears non-striated or only partially striated.

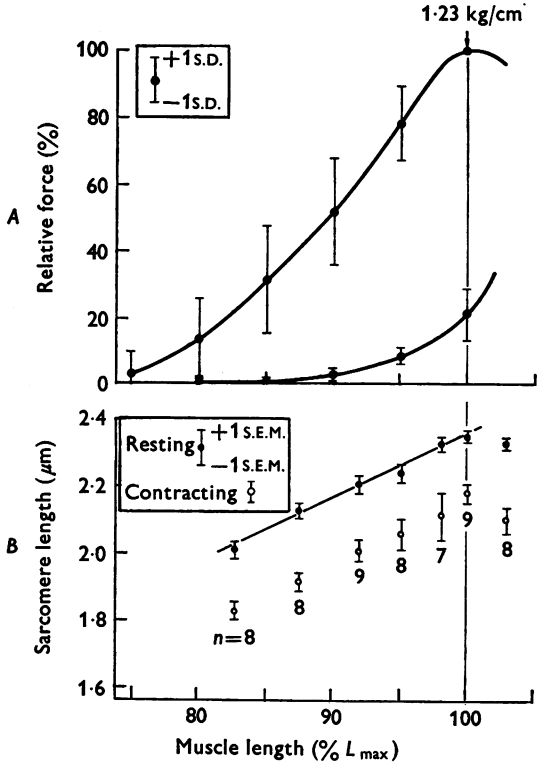
That tissue shortening is fully accounted for by stretch at the ends is demonstrated by the approximation of shortening (area enclosed below reference line in Text-fig. 4*C*) to stretch (areas above this line). The average of the ratio of stretched area to shortening area for four muscles encompassing thirteen muscle lengths ranging from 79 to 100 % L_{max} was 0·91. The difference between the experimental value (0·91) and that expected (unity) was within the limits of experimental error.

As a check of these measurements, it should be noted that in all muscles not only the extent, but also the distribution of sarcomere shortening was reflected by tissue shortening. (For example, this can be seen by comparing Text-figs. 2 and 4, since both diagrams illustrate the same muscle at L_{max}.) At the boundaries of the fully striated tissue the shortening measured by diffraction often exceeded the local tissue shortening. This is not unexpected, since in these regions the segments may include both striated and non-striated tissue. Integration of both shortening and stretch within this inhomogeneous region would account for the discrepancy.

Effect of varying muscle length

Sarcomere length and degree of shortening were considered with respect to the initial muscle length. A composite force–length curve was constructed from data pooled from all muscles as shown in Text-fig. 5*A*. In all cases muscle length is relative to the length–tension diagram, expressed as a fraction of the optimal length for force development (L_{max}). At L_{max}, peak active contractile tension averaged $1{\cdot}23 \pm 0{\cdot}53$ (S.D. of observation, $n = 13$) kg/cm^2 and the ratio of peak active force to resting force was 5:1.

Sarcomere lengths (mean values of measurements throughout the fully striated region of the muscle) in resting muscle and at the moment of peak contractile force are shown in Text-fig. 5*B*. Data points were divided into bins of approximately equal sample size, and the mean for each compartment is shown. The % increments in sarcomere length in resting muscle tended to be less than corresponding increments in muscle length, especially when the muscle was stretched beyond optimal length. We suspect that the striated tissue is stiffer than the ends at this length, giving rise to these differences. At sarcomere lengths less than 2·3 μm the sarcomere



Text-fig. 5. Effect of variation of muscle length on force and sarcomere length for all muscles. Muscle length is expressed as a fraction of that length, L_{max}, at which maximum active force is produced. *A*, length–tension relations. The peak active tensions averaged 1·23 ± 0·53 S.D. kg/cm² at optimal length. The ratio of active to resting forces at optimal length averaged 5:1. *B*, average sarcomere length in resting muscle and at time of peak contractile force. A moderately consistent amount of shortening occurred at all degrees of stretch. *n* indicates the number of data points in each group. Note the relatively narrow range of sarcomere length encompassing the length–tension diagram.

length–force curves coincided with those recently reported in frog atrial muscle (Winegrad, 1974).

Confirming an earlier observation (Pollack & Huntsman, 1974), our measured sarcomere lengths were always larger than those obtained in histologically prepared rat papillary muscles after fixation at analogous muscle lengths by Grimm, Katele, Kubota & Whitehorn (1970). The mean sarcomere length in resting muscles held at 100 % L_{max} was $2{\cdot}35 \pm 0{\cdot}02$ (S.E. of mean, $n = 10$) μm, while the mean sarcomere length at a muscle length of 83 ± 2 % L_{max} (where 25 % of force at L_{max} was generated) was $2{\cdot}01 \pm 0{\cdot}03$ (S.E. of mean, $n = 8$) μm. Both values agree well with recent measurements in similar preparations (Pollack & Huntsman, 1974) and in the frog atrial trabeculae (Winegrad, 1974). Whether measured by diffraction or by direct observation of striations (J. W. Krueger & G. H. Pollack, unpublished results from six muscles), sarcomere lengths in resting muscle were not less than 1·8 μm. Resting muscles appeared to be slack at lengths less than 80 % L_{max}. It can be readily appreciated that (at least in muscles in which shortening occurs) the major portion of the resting length–tension relation encompasses a relatively narrow range of sarcomere lengths.

The sarcomere length at peak contractile force (average of all muscles) is also shown in Text-fig. 5*B*. Active shortening occurred at every muscle length, varying from 5 to 16 % initial sarcomere length for individual muscles. The amount of averaged sarcomere shortening was moderately consistent at all muscle lengths, being 7 % at L_{max} with some slight increase at shorter lengths. The mean values of fully 'contracted' sarcomere lengths at L_{max} and 83 % L_{max} were $2{\cdot}18 \pm 0{\cdot}03$ (S.E. of mean, $n = 9$) and $1{\cdot}83 \pm 0{\cdot}3$ (S.E. of mean, $n = 8$) μm, respectively. Thus, peak active force decreased by 75 % over the same range of sarcomere length in which force decreased by only 7 % in truly isometric tetanic contractions of frog skeletal muscle (Gordon, Huxley & Julian 1966), an observation also made by Close (1972) in isometric twitches of skeletal muscle.

DISCUSSION

Two primary observations are presented. First of all, considerable sarcomere shortening occurs during contraction in an isometric mammalian papillary muscle preparation. Secondly, a source of simultaneous and equivalent stretch could not be found within the normal cardiac tissue; rather, the stretch occurred exclusively at the ends of the preparation.

Sarcomere shortening during isometric contraction

The existence of some degree of internal shortening upon isometric contraction in an isolated papillary muscle preparation is not surprising since similar phenomena have been reported in whole skeletal muscles (Sandow, 1936) and single skeletal muscle fibres (Cleworth & Edman, 1972; Kawai & Kuntz, 1973). Sarcomere shortening greater than the level of 2 % seen in single skeletal muscle fibres (Kawai & Kuntz, 1973) might have been predicted in papillary muscle preparations from differences in the series elastic properties of the two types of muscle: the maximum series elastic element strain measured from quick release experiments in cardiac muscle (Meiss & Sonnenblick, 1974) is three to six times that measured in skeletal muscle (Jewell & Wilkie, 1958).

The amount of shortening in fact ranged from 5 to 16 % resting sarcomere length in different preparations. Several features of this shortening are noteworthy. First, the amount of internal shortening could be as much as half the range of sarcomere lengths spanning the entire length–tension curve of these muscles (Text-fig. 5). By this criterion the contractions were far from isometric. Shortening is thought to diminish the level and the duration of mechanical contractile activity so that more force might have been produced had no shortening occurred. Shortening also introduces some uncertainty into the estimate of the force developed by the sarcomeres. If a force (e.g. resting tension) is sustained by elements in parallel to the sarcomeres, it decreases as sarcomere shortening occurs, i.e. an analogous 'parallel element' is unloaded. Thus, at lengths near L_{max}, the force borne by the sarcomere during contraction may be larger than the active muscle force shown in Text-fig. 4. Clearly, the manner in which the sarcomeres and the surrounding tissues shorten will influence the calculation of sarcomere force.

Second, the velocity of shortening was on the order of one to two sarcomere lengths/sec, a level seemingly high since it approximates previous estimates of the velocity of unloaded shortening, V_{max}, of cardiac muscle (Sonnenblick, 1962; Parmley, Chuck & Sonnenblick, 1972). V_{max} may be higher in the rat than in other species (Henderson, Brutsaert, Parmley & Sonnenblick, 1969), so it is difficult to assess relative shortening velocity by this criterion alone.

A third feature of shortening is noteworthy. The velocity of shortening remains relatively unchanged throughout most of the period of force development. A constant velocity of shortening might be the coincidental result of complex interactions among several molecular mechanisms and merits further study under controlled conditions. Nevertheless it is notable that Nassar, Manring & Johnson (1974) also report this result in frog

atrial trabecular strips, where important physical differences might be expected to rule out any coincidental similarity to our results.

Source of sarcomere shortening

Our results provide direct evidence that sarcomere shortening occurred simply because the contracting tissues could not be held isometrically by holding constant the length of the muscle. It was evident that the stretch at the ends occurred in regions where the striations were disrupted, presumably as a consequence of clip attachment. The stretch at the ends of the preparation fully accounted for the sarcomere shortening at all but the shortest lengths.

The possibility that inhomogeneities provide an explanation for sarcomere shortening was not supported by our data. With minor allowances for slackened tissues below rest length, the sarcomere shortening measured by diffraction at any position along the muscle was paralleled by the local tissue shortening. Moreover, sarcomere shortening during the period of force development was synchronized along the length and width of the muscle. Clearly, inhomogeneities at the cellular or regional level cannot play a significant role in accounting for the sarcomere shortening we observed.

Generality of the results

Many heart muscle preparations are isometrically held by spring-loaded clips, the same method of attachment used in this study. Yet, it is a matter of speculation whether the extent of shortening that we measured in isometric contractions is characteristic of other preparations. Indirect evidence indicates that this may, in fact, be the case. The maximum series elastic strain reported by others is on the same scale as the lengthening we observed at the ends of the muscle. Therefore, it is likely that the degree of internal shortening may be quantitatively similar.

Although this analogy between series elasticity and damaged ends is tempting and may provide some qualitative insight into internal dynamics, the application of simple analogues for quantitative description of mechanics must be approached with caution. For example, if one attempts to equate the contracting sarcomeres with the contractile element (*CE*), and the ends with the series elastic component (*SEC*), thereby synthesizing a simple two or three element analogue, it should then be possible to reverse the process and deduce the contractile element mechanics from the characteristics of the series elastic component (measured by quick release) and the boundary conditions on the muscle. In recent attempts at this (Parmley *et al.* 1972), the time course of contractile element velocity did not resemble the time course of sarcomere velocity measured here. Even

if a quantitatively adequate model could be derived, it would be of little value in describing ventricular muscle in general unless that portion of the model corresponding to the attachment artifact could be isolated.

Finally, it is worth while to reflect on the significance of our findings relative to previous measurements of papillary muscle mechanics. We have demonstrated that the time course of sarcomere velocity may bear little relation to the time course of muscle velocity or computed contractile element velocity. Consequently, previous attempts to deduce contractile properties of cardiac sarcomeres by relating muscle force, length and velocity to contractile element velocity may be misleading.

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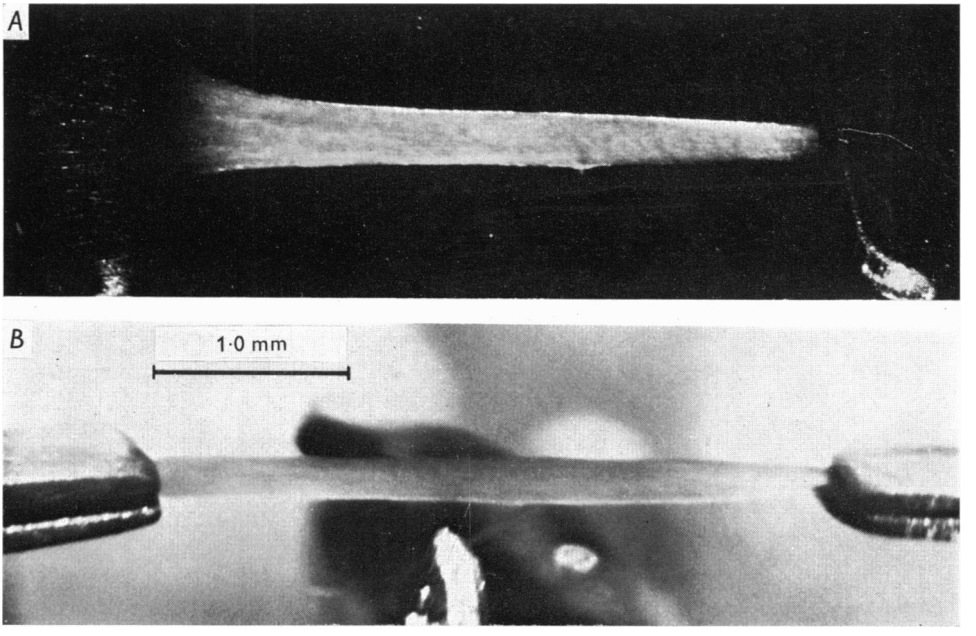
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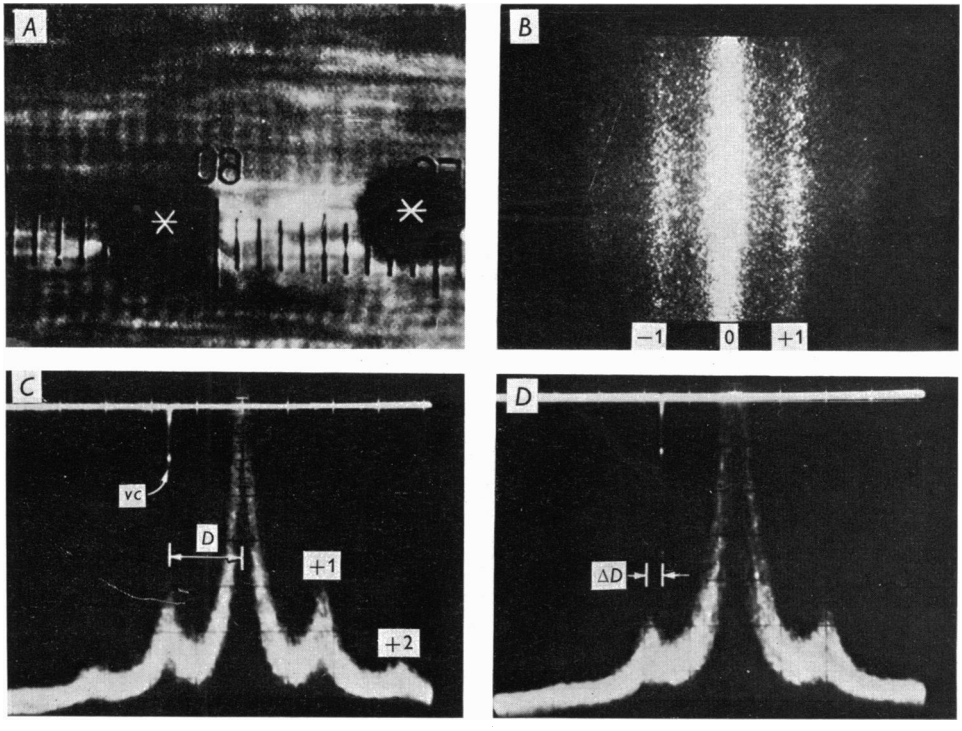
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EXPLANATION OF PLATES

PLATE 1

Experimental muscle preparation. *A*, top and, *B*, side views of a small rat papillary muscle mounted in the experimental chamber. The muscle's tendinous end is grasped by the right-hand clip. Muscle length is near that optimal for maximum force development.

PLATE 2

Details of experimental methods. *A*, carbonized microspheres lodged within capillary beds of rat papillary muscle. Calibration, 2·7 μm/division; asterisk denotes microsphere. *B*, focused diffraction pattern from rat papillary muscle. A bright band of non-diffracted light (zero order, '0') is straddled by the two first order bands (± 1) comprising the fundamental spectral frequency contained in the striated pattern of the muscle. Sarcomere length is inversely proportional to the separation of the first order bands. Muscle thickness is approx. 230 μm. *C* and *D*, one dimensional distribution of spectral intensities at rest (*C*) and at peak shortening (*D*). The video signals comprising Pl. 1 *B* are electronically compressed in the *y* direction and displayed oscillographically. Thus the wave forms in *C* and *D* represent the horizontal distribution of light intensity across the compressed image. A movable vertical cursor (*vc*) indicates the position of the peak first order intensity at rest. During contraction the first order spectra shift apart by $2\Delta D$ indicating sarcomere shortening. With active shortening, a reduction in peak first order spectral intensity and an increase in the zero order light intensity are evident. The breadth of the zero order also increases.