# Resting Sarcomere Length-Tension Relation in Living Frog Heart

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ABSTRACT   The sarcomere pattern and tension of isolated resting frog atrial trabeculae were continuously monitored. In the absence of any resting tension the sarcomere lengths varied with the diameter of the trabeculae. In over 75 % of the trabeculae the value exceeded 2.05 μm, the estimated in vivo length of the thin filaments, and it was never less than 1.89 μm. When the trabeculae were stretched the increase in length of the central undamaged portion could be completely accounted for by an increase in sarcomere length. The width of the A band was constant only at sarcomere lengths between 2.3 and 2.6 μm; it decreased at smaller and increased at larger sarcomere lengths. A group of spontaneously active cells stretched the sarcomeres in cells in series to longer lengths than could be produced by passive tension applied to the ends of the trabeculae, but they did not influence the sarcomeres of adjacent cells. It is proposed that the connective tissue is a major factor in determining sarcomere length and that there are interactions between thick and thin filaments in resting muscles.

Although the relation between the resting volume of a heart and the tension that it develops during systole was described over 50 years ago by Frank (1895) and by Starling (1918), the basis is still unknown. The most obvious length-dependent contractile function to which this relation may be attributed evolves from the sliding hypothesis of contraction of skeletal muscle and depends on the amount of overlap of thick and thin filaments. Force generators are believed to be uniformly distributed along the length of the thick filament except for its central 0.10–0.15 μm, but to be active they must be overlapped by thin filaments. The number of force generators available during a given contraction is, therefore, length dependent and, as has been shown by Gordon et al. (1966), can be accurately estimated by the sarcomere length. If the basis of the length-tension curve in cardiac muscle is the number of force generators that can interact with the thin filaments, the lengths of sarcomeres in cardiac tissue at different positions on its length-tension curve must have quite specific values.

There have been several attempts to evaluate this possibility by determining

sarcomere length in sections of heart muscle histologically fixed at different positions on the length-tension curve (Sonnenblick et al., 1964; Grimm et al., 1970), but the utility of these studies has been limited by the changes in the tissue produced by the fixation procedure itself. The only study of living heart used a muscle strip with an abnormally large proportion of connective tissue (Gay and Johnson, 1967), and consequently its general applicability to all heart tissue is questionable. In the following work the sarcomere pattern of living trabeculae from frog atria has been studied with the aid of Nomarski optics to obviate the problems of fixation.

## METHODS

Atria from medium-sized frogs (*Rana pipiens* and *Rana temporaria*) were isolated, spread open in a chamber and pinned down on a small amount of polymerized Sylgard 184 (Dow Corning Corp., Midland, Mich.). The luminal surface of an atrium consisted of many trabeculae varying in diameter from 10 to 200 $\mu$m and in length from about 1 to 3 mm. It was generally possible to isolate two and occasionally three trabeculae from a single atrium, and this permitted the comparison of the properties of trabeculae with the same dimensions from different parts of the atrium as well as of trabeculae with different dimensions from the same part of the atrium. Great care was taken during the dissection to avoid any stretching of the tissue. An isolated trabecula was suspended in a chamber on a gliding stage of a microscope in one of three ways: (*a*) both ends held by fine stainless steel forceps; (*b*) one end held with a fine forcep and the other end tied with filaments of raw silk to an Endevco semiconductor force transducer (Endevco Div. Becton, Dickinson & Co., Pasadena, Calif.); (*c*) one end tied to a mechanical ground with raw silk and the other end tied to the transducer with raw silk. Muscles were suspended so that their largest diameter was as close as possible to horizontal. The output of the transducer was continuously recorded on a Brush Mark 280 oscillograph (Gould Inc., Instrument Systems Div., Cleveland, Ohio).

The tissue was then positioned over a portion of the chamber in which a no. 3 cover slip formed the bottom in order to minimize the distance of the tissue from the condenser. This permitted the use of a condenser and an objective with high numerical apertures. The suspended tissue and transducer were lowered by means of a common micromanipulator to within less than a millimeter of the cover slip and the tissue was observed with Zeiss differential interference (Nomarski) optics (Carl Zeiss Inc., New York, N. Y.). The use of the gliding stage allowed the movement of the entire muscle bundle without alteration of either the length or tension of the tissue. The resting length of the trabeculae was varied by moving one end of the trabeculae with a micromanipulator. Four different objectives: the Zeiss water immersion × 40 numerical aperture 0.75, the × 40 numerical aperture 0.65, and × 16 numerical aperture 0.35; and the Nikon × 20 numerical aperture 0.40 (Nikon, Inc., Div. of EPOI, Garden City, N. Y.), were used with either the standard (numerical aperture 1.4) or modified (numerical aperture 0.6) Zeiss Nomarski aplanatic achromatic condenser. The modification was devised as a result of a suggestion by Professor A. F. Huxley for overcoming the limitations of the short working distance. It consisted of

replacing the front lens of the Zeiss Nomarski condenser with one of longer focal length (the auxiliary lens from the Zeiss aplanatic achromatic condenser that is used to increase the working distance and decrease the numerical aperture to 0.63) and increasing the distance of the condenser optics from the prism to superimpose the fringe patterns of the upper and lower prisms of the Nomarski system. The amount of separation was determined empirically in the following way. The lens and the prism of the condenser were mounted separately and the lens positioned to focus the iris of the Kohler illumination in the plane of the specimen. The aperture of the condenser was opened maximally and the exit pupil of the objective was examined with a telescope replacing the eyepiece. The position of the prism was set to produce the sharpest image of the fringe pattern in the exit pupil of the objective. The bias compensator of the upper prism was adjusted to center the zero order fringe, and if it did not fill most of the exit pupil of the objective, the height of the condenser was adjusted slightly to broaden the zero order fringe. When the optimal positions of the prism and lens had been determined, their separation was carefully measured, and a brass collar, machined to the identical width, was added to the condenser between the lens and the prism to produce a permanent separation. The modified condenser system had a working distance of over 12 mm and a numerical aperture of about 0.6 with little sacrifice of contrast within the image. Images of similar quality were produced by the air objective with a numerical aperture of 0.65 and the water immersion objective (numerical aperture 0.75) with this condenser.

The chamber was perfused with Ringer's solution (117 mM NaCl, 5 mM KCl, 1.0 mM $CaCl_2$, 2 mM $PO_4$ buffer at pH 7.2) at room temperature. After the muscle had been positioned it was photographed on Polaroid film (Polaroid Corp., Cambridge, Mass.) and allowed to recover from the manipulation for 1 h. It was then photographed again, and if any significant change in the striation pattern of the middle third of the trabecula's length had occurred the preparation was rejected. Photographs during experimental runs were taken on Kodak Tri-X 35-mm film (Eastman Kodak Co., Rochester, N. Y.) with a Nikon Microflex AFM (Ehrenreich Photo-Optical Industries, Garden City, N. Y.) and a Zeiss Ukatron UN60 Strobe that produced an adequate flash of 1-ms duration, and they were processed with a fine-grain developer. Sarcomere lengths were determined by measuring the total lengths of groups of approximately 20 sarcomeres and dividing by the number of sarcomeres. The widths of A bands were measured both directly from enlarged prints of the 35-mm negatives and by densitometer tracings of the enlargements.

RESULTS

*Sarcomere Structure*

The Nomarski system of differential interference optics has certain advantages over other optical systems for studying the sarcomere pattern of a thin bundle of living muscle. The high degree of contrast in images that are formed at relatively large numerical apertures favors both resolution and optical sectioning, and contrary to phase contrast optics no reversal of contrast occurs with focusing (Huxley and Niedergerke, 1958). Several optical sections

through an opened frog atrium are shown in Fig. 1 and they indicate the capacity of the optical system for defining the sarcomere structure of individual myocardial cells. With a numerical aperture of about 0.6 the depth of focus equaled one to two cell diameters. The thickness of the connective tissue surrounding the trabeculae was estimated by optically sectioning the tissue at the highest possible numerical aperture to produce the shallowest depth of focus. Regardless of the diameters of the myocardial bundles the connective tissue layer was never greater than one optical section, which was approximately 3 µm.
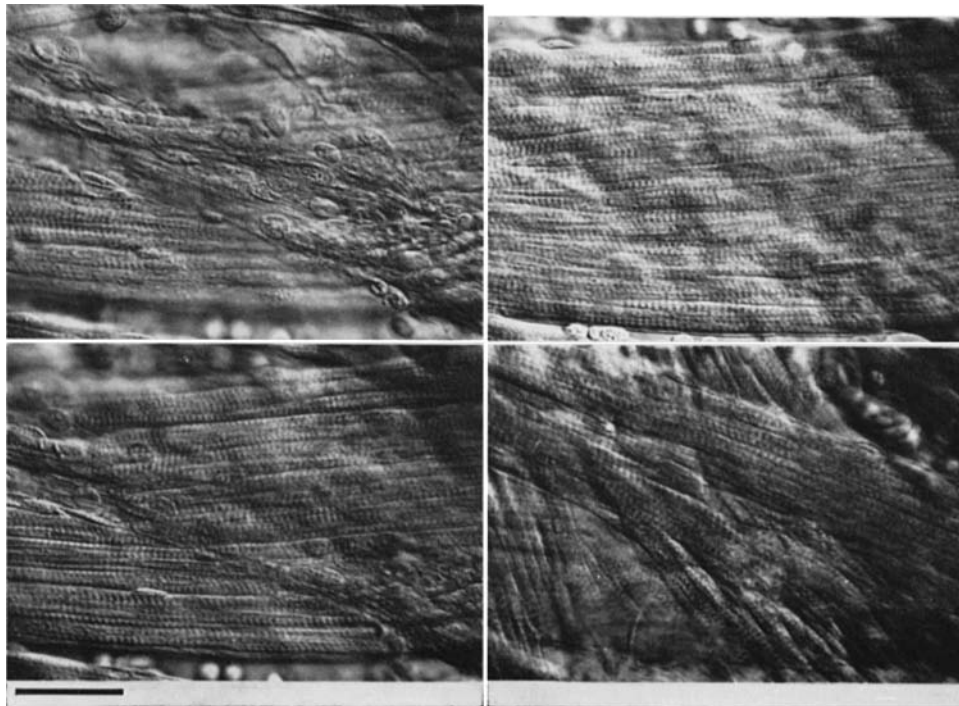


FIGURE 1.   Four photomicrographs showing optical sections (not consecutive) through an opened frog atrium using Nomarski optics. Calibration bar equals 35 µm.

The ends of the trabeculae were damaged during the dissection and suspension of the tissue, and therefore only the characteristics of the central third of the length of the trabeculae have been analyzed in order to eliminate the influence of damaged tissue. The dimensions of the sarcomeres in the undamaged region were relatively uniform when resting tension was zero or small (less than 50 g/cm²). In the absence of resting tension the largest difference in the lengths of 20 sarcomeres from the center and each edge of four different optical sections of any trabecula was 0.03 µm per sarcomere. The

variability increased when the resting tension of the trabeculae was increased. With a stretch of about 15% sarcomere length was about 2.3–2.4 $\mu$m and the spread in lengths within the central third of the trabeculae increased to 0.05 $\mu$m per sarcomere. When the sarcomeres had been stretched to about 2.8 $\mu$m the spread increased to 0.11 $\mu$m per sarcomere. No pattern of distribution of larger and smaller sarcomeres across the thickness of the tissues was observed.

The length of sarcomeres was not the same in all resting trabeculae at zero tension, but instead it appeared to be related to the diameter of the tissue (Fig. 2). Because of the fortuitous absence of trabeculae between 110 and 150 $\mu$m
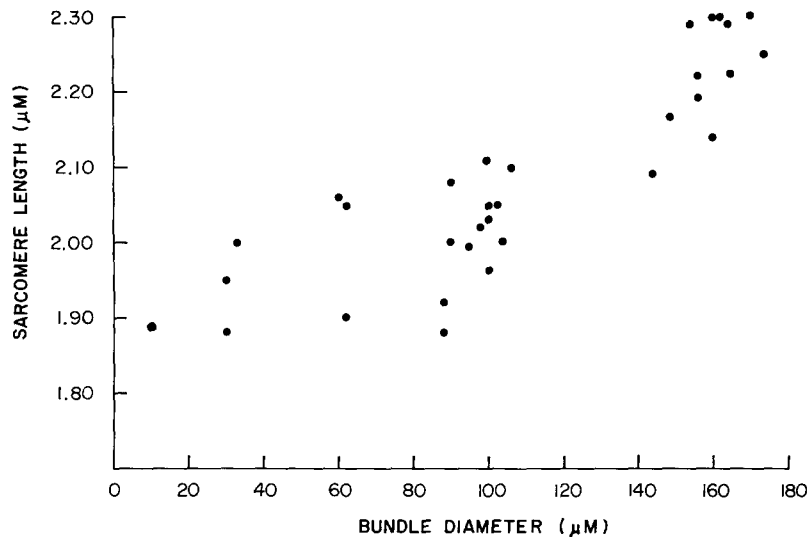


FIGURE 2.   Plot of the average sarcomere length in a series of atrial trabeculae in the absence of resting tension as a function of the largest diameter of the central third of the length of the trabeculae. Each point represents one trabecula.

in diameter, it is not possible to distinguish between a continuous relation between the length of a sarcomere and the diameter of a trabecula and a discontinuous relation with essentially two populations: trabeculae less than 120 $\mu$m in diameter have an average sarcomere length of 1.99 ± 0.06 um (SD) and those thicker than 120 $\mu$m have a mean sarcomere length of 2.24 ± 0.07 $\mu$m (SD). The smallest sarcomere length was 1.89 um, but values as high as 2.30 $\mu$m were seen in the thicker specimens. There was no correlation between the location of the trabecula within the atrium and the resting sarcomere length at zero tension. On several occasions after the sarcomere length at zero tension had been measured with the tissue fixed at both ends, the attachment at one end was released, but the sarcomere length in the unre-

strained tissue remained the same. In two ventricular strips, in which the contrast produced by the striations was much less because of the thickness of the tissue, the lengths of sarcomeres in the absence of any resting tension were 2.25 and 2.27 μm.

After the resting sarcomere length had been determined in the absence of resting tension, the bundle was stretched by increasing the tension on the ends of the trabeculae (Fig. 3). The increase in the length of the middle segment of the trabecula, as determined by the change in distance between either nuclei in the connective tissue sheath surrounding the muscle or carbon particles
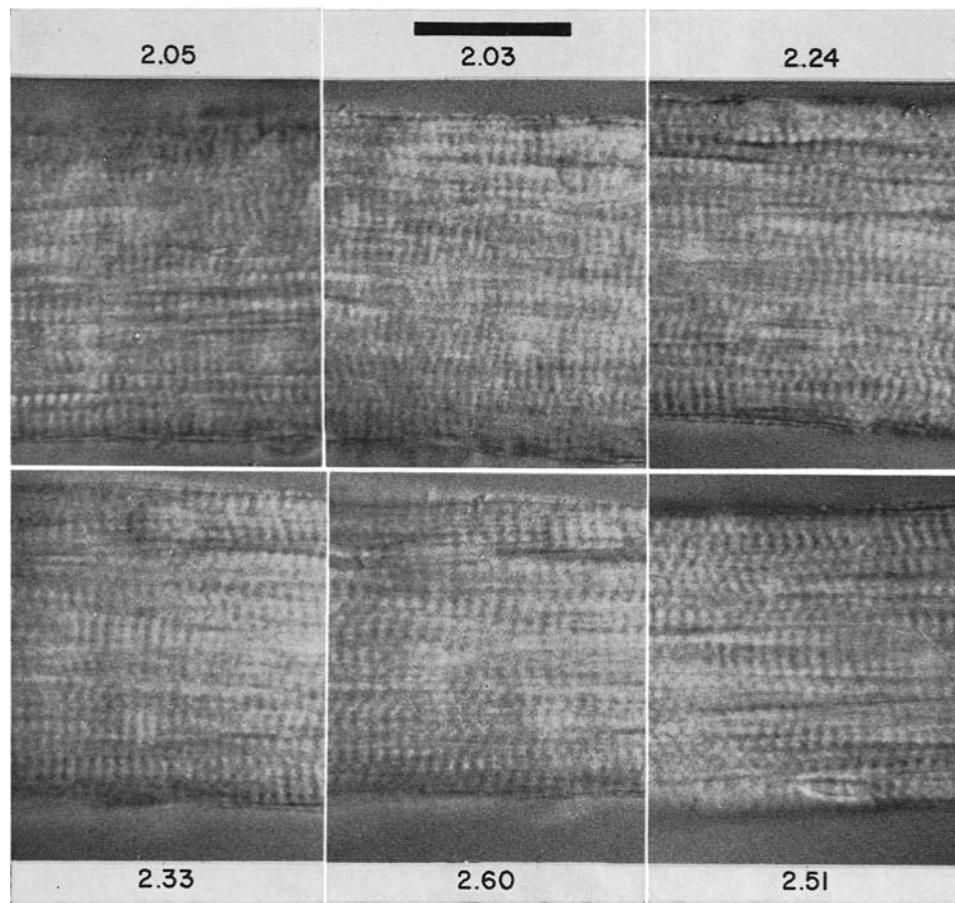


FIGURE 3.   Photomicrographs of an atrial trabecula first at zero resting tension, then gradually stretched and finally partially released. Upper left and middle: two different optical sections through trabecula at zero resting tension. Upper right, lower left, and middle: resting tension gradually increased. Lower right: resting tension decreased. Note change in width of A band. Numbers beneath figures indicate average sarcomere length. Calibration bar equals 25 μm.

applied to the surface of the trabecula, was proportional to the increase in the length of the sarcomeres. At the damaged ends the sarcomeres frequently lengthened proportionally less than the tissue.

The width of the A band changed as the muscle was stretched from its resting length (Figs. 3, 4). It increased from 1.1 to 1.5 $\mu$m as the sarcomere length increased from 1.9 to 2.3 $\mu$m, and then it remained approximately constant at 1.5 $\mu$m until sarcomere lengths exceeded 2.6 $\mu$m. The relation between the width of the A band and sarcomere length was independent of the amount of tension that was present; that is, the A band was the same width in thick trabeculae without resting tension as in thin trabeculae with the same
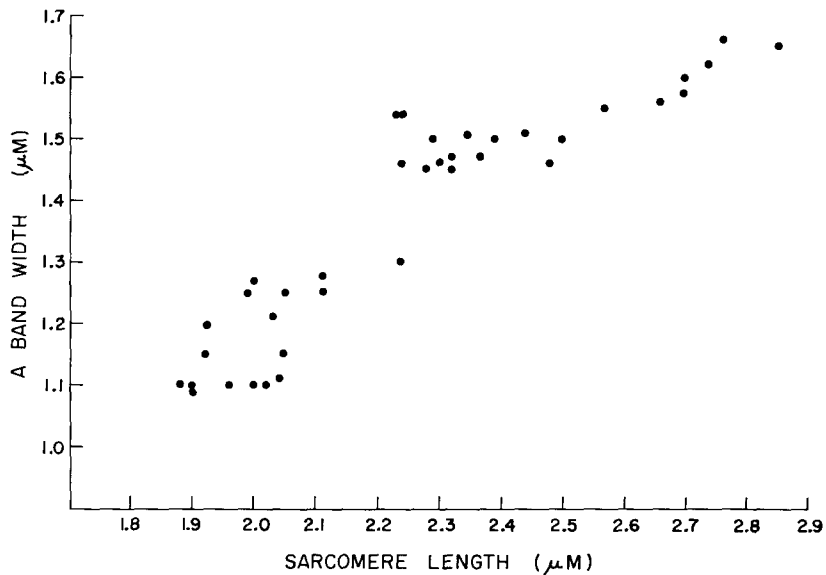


FIGURE 4.   Plot of width of A band as a function of sarcomere length in a series of atrial trabeculae.

sarcomere length produced by the presence of resting tension. This variation of the width of the A band has also been found in single frog skeletal muscle fibers (Huxley and Niedergerke, 1958). It does not necessarily indicate a shortening of the thick filaments as a discrepancy between the length of the thick filaments and the width of the A band at shorter sarcomere lengths can be produced by the limited resolving power of the light microscope (see Huxley and Niedergerke, 1958, for a detailed discussion). In sarcomeres which had been passively stretched beyond 2.6 $\mu$m the width of the A band increased to as much as 1.7 $\mu$m. The broadening of the A bands was completely reversed when the sarcomeres were allowed to shorten (Fig. 3).

The resting tension increased considerably as the trabeculae were length-

ened (Fig. 5). It was difficult to stretch sarcomeres beyond 2.6 $\mu$m, and the tissue generally tore before 3.0 $\mu$m could be produced. No long-term hysteresis in the resting length-tension relation occurred as long as sarcomere length did not exceed 2.6 $\mu$m but in most bundles stretching sarcomeres to 2.7 $\mu$m or more shifted the resting length-tension curve to the right along the length axis. Short-term changes such as stress relaxation and its opposite were not rigorously followed for technical reasons.

In several preparations spontaneous activity developed in a small number of cells in the trabecula, and this revealed some additional mechanical properties of the tissue (Fig. 6). Sarcomeres in the spontaneously active cells shortened to as little as 1.5 $\mu$m and at the same time sarcomeres in apparently
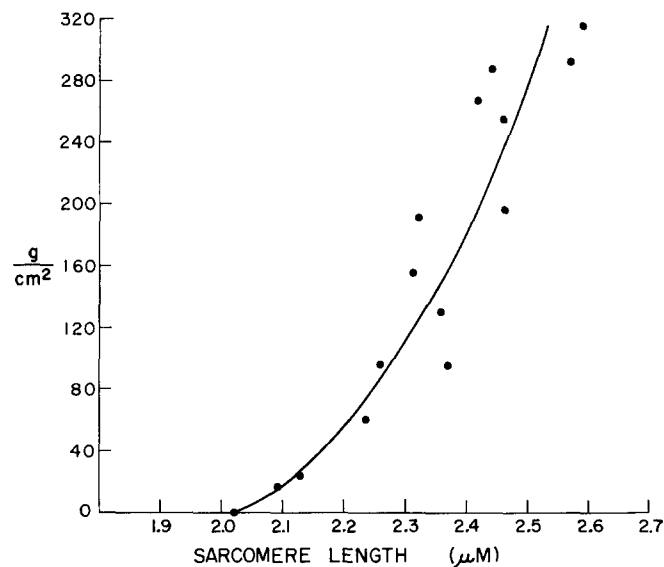


FIGURE 5.    Plot of a typical sarcomere length-tension curve in a resting atrial trabecula.

resting cells in series with the active ones lengthened to as much as 3.2 $\mu$m, which was considerably longer than any sarcomere could be passively stretched even by a resting tension that exceeded the maximum active tension developed by the trabecula. The sarcomere lengths of adjacent cells in the spontaneously active region often differed considerably, sometimes by as much as 1.7 $\mu$m, and there was never any waviness in any cells adjacent to the heavily contracted ones as might have been expected if passive shortening had been occurring (Huxley and Gordon, 1962). These observations indicated that there was loose mechanical coupling between adjacent cells or adjacent groups of cells but rather tight mechanical coupling between cells in series. The width of the A band in the cells with the very long sarcomeres was 1.5 $\mu$m, 0.2 $\mu$m narrower than the A bands in the sarcomeres that had
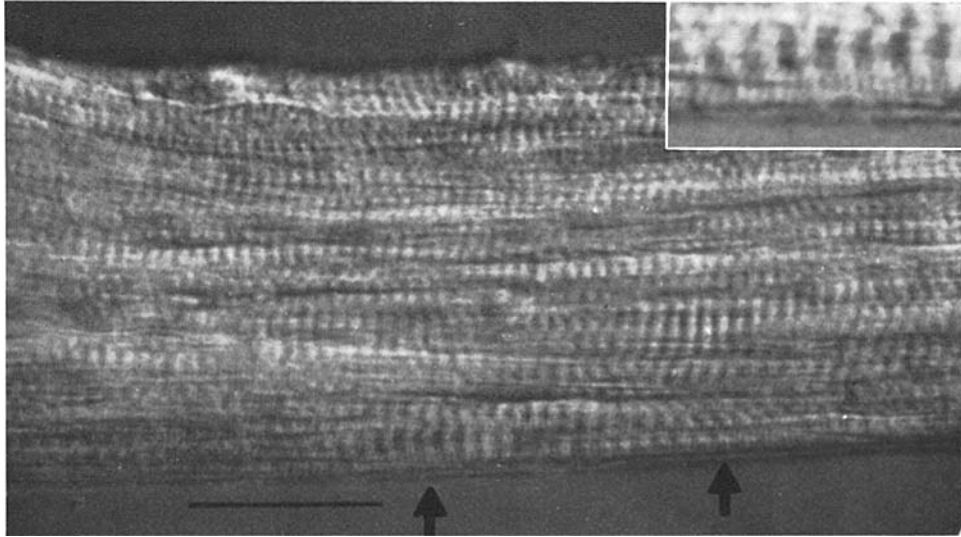
Figure 6.   Photomicrograph of an atrial trabecula in which a few fibers were contracting spontaneously. In the region of the arrows note: (a) sharply contracted cells with contraction bands (sarcomere length about 1.5 $\mu$m) next to and in series with sarcomeres as long as 3.2 $\mu$m; (b) the absence of any waviness in fibers; (c) A bands 1.5 $\mu$m wide in the very long sarcomeres. The insert in the upper right-hand corner of the figure is an enlargement of the portion of the photomicrograph above the left arrow to show adjacent fibers with long and short sarcomeres. Calibration bar equals 25 $\mu$m.

been stretched to 2.9 $\mu$m. Optical sectioning of the trabeculae during the period of spontaneous activity confirmed both the localization of the spontaneous mechanical activity and the absence of any obvious passive shortening.

The atrial trabeculae were stimulated at different resting sarcomere lengths by two massive platinum electrodes to determine the relation between resting sarcomere length and developed tension. Although the maximum tension was developed when the resting sarcomere length was about 2.3 $\mu$m, the results of these experiments are hard to interpret as sarcomere length changed by as much as 10–15% during the contraction. At least a substantial portion of this shortening was due to lengthening in the damaged ends of the muscle. A rigorous description of the relation of developed tension to sarcomere length, therefore, requires the removal of the large series compliance of the damaged ends of the muscles, and such studies are now under way.

DISCUSSION

*Relation of Sarcomere Length-Tension Curve to Pressure-Volume Curve of Heart*

The length of thin filaments in a sarcomere of histologically fixed frog heart

muscle is 1.93 $\mu$m (Page, 1974) and if a correction factor of 6% for shrinkage during fixation is applied, as has been shown to be necessary in skeletal muscle (Page and Huxley, 1963), the length of the thin filaments in vivo becomes 2.05 $\mu$m. In about 75% of the atrial trabeculae and in both ventricular strips the shortest sarcomere length that was observed in the resting state was at least 2.05 $\mu$m and in no trabecula was it ever less than 1.89 $\mu$m. Double overlap of thin filaments therefore was at most a very uncommon occurrence in the resting heart, and in view of the variability of thin filament length and the nature of the correction for shrinkage it is possible that double overlap never occurred. It is almost certain that double overlap of cross bridges on the thick filament, which would require sarcomere lengths less than about 1.90 $\mu$m, never occurred. The values of sarcomere lengths in the absence of resting tension, 1.89–2.30 $\mu$m, correspond approximately to the range within which there is essentially complete overlap of cross bridges. This raises the possibility of some type of interaction between cross bridges and thin filaments in the resting state (see below) as the existence of such interactions would make the configuration of the filaments relatively unstable in the presence of non-overlapped cross bridges.

In their classical study of the sarcomere length-tension relationship in frog skeletal muscle, Gordon et al. (1966) showed that maximum active tension was developed at sarcomere lengths between 2.00 and 2.25 $\mu$m in accordance with predictions based on the dimensions of the thick and thin filaments. Since the lengths of the filaments in frog heart are very similar to those in frog skeletal muscle the configuration of filaments in most resting frog hearts is optimal for developing force even in the absence of any resting tension. Filament configuration therefore is unlikely to be the sole basis of the pressure-volume relationship in intact hearts or the length-tension relationship in isolated myocardial strips as heart muscle normally operates not on the plateau of these curves but on the portion with a positive slope (Frank, 1895; Starling, 1918; Abbott and Mommaerts, 1959).

*Connective Tissue Control of Resting Sarcomere Length*

It appears likely that the resting myocardial cells are responsible for very little of the stiffness or shearing force of the trabeculae. The major reasons for these conclusions come from observations of the sarcomere patterns in trabeculae where only a few cells were contracting. A contracting cell was able to stretch another cell in series to a longer sarcomere length than a large passive tension on the whole trabecula could achieve and therefore the major stiffness of the tissue is most likely due to connective tissue rather than myocardial elements. The same contracting cell, however, appeared to be unable to influence the sarcomere length of an adjacent cell.

It is difficult to explain the variability of sarcomere length either with

bundle diameter or at high resting tension by a simple model of relatively uniform myocardial cells in parallel but poorly coupled with a connective tissue compliance. The connective tissue in a trabecula is however a three-dimensional network with connecting longitudinally and transversely oriented components (Fawcett and Selby, 1958; Johnson and Sommer, 1967; Staley and Benson, 1968). Such a network would determine the dimensions of the myocardial cells by the amount of longitudinally and transversely directed forces, the former tending to shorten the fibers and the latter to elongate them. A larger relative amount of longitudinally oriented connective tissue, as appears to exist with thinner trabecula (in view of the relatively constant thickness of the connective tissue sheath regardless of bundle diameter), could produce shorter cells. Any nonuniformity of the compliance of the various portions of the network would produce a progressively larger range of sarcomere lengths with increasing passive tension on the entire trabecula. This heterogeneity of sarcomere length with increasing resting tension could also result from the way in which the units of the connective tissue network were linked together.

### Activity in the "Resting" Heart

The width of the A band equaled the length of the thick filament (Page, 1974) only at sarcomere lengths between 2.3 and 2.6 $\mu$m and 3.2 to 3.3 $\mu$m (the latter in trabeculae with the spontaneously contracting cells). Huxley and Niedergerke (1958) attributed the analogous narrowing of the A band at short sarcomere lengths in skeletal muscle to the limits of resolution of the light microscope, and their arguments apply equally well to these studies of heart muscle, but with optics having a similar resolving power they did not observe in skeletal muscle the increase in the width of the A band in sarcomeres between 2.6 and 2.9 $\mu$m that was seen in frog heart. The wider A band in moderately long sarcomeres of frog heart indicates therefore something special about the behavior of cardiac muscle. The most probable explanation for the large width of the A band is misalignment of thick filaments (preliminary electron micrographs support this interpretation), but for misalignment of thick filaments, which are not connected to the sarcolemma, to occur in response to a force applied to the ends of a trabecula some interaction between thick and thin filaments must be present. Data from X-ray diffraction studies (Matsubara, 1974) and the heat measurements of heart muscle (Gibbs et al., 1967) also support the notion of interactions between thick and thin filaments in resting muscle. The absence of misalignment of thick filaments at very long sarcomere lengths should be due to the limited opportunity for interactions when the overlap of filaments is small, and the absence at shorter sarcomere lengths could be due to a length dependence of the probability of interaction. D. K. Hill (1968) has produced evidence in

resting skeletal muscle in favor of weak interactions which become more prominent at greater sarcomere lengths. A length-dependent interaction of thick and thin filaments would be useful in the heart where it would oppose cardiac enlargement. The existence of tension-generating links in the resting heart has already been proposed by Bartelstone et al. (1965) on the basis of changes in diastolic compliance which they felt they had demonstrated.

*Note Added in Proof* Since the submission of this manuscript the resolving power of the optical system has been approximately doubled by using a Neofluor oil immersion lens with a numerical aperture of 1.25. This system has been used to measure the width of the A band at different sarcomere lengths to determine the validity of the explanation offered by Huxley and Niedergerke (1958) for the decreasing width at sarcomere lengths below 2.3 $\mu$M. With the better optical system the width of the A band remained at approximately 1.5 $\mu$M down to sarcomere lengths of 2.0 $\mu$M, but the increase in the width of the A band at sarcomere lengths greater than 2.6 $\mu$M was not changed. These observations strongly support the suggestion by Huxley and Niedergerke that the apparent decrease in the width of the A band at shorter sarcomere lengths is due to the limitations of resolution. The broadening of the A band at longer sarcomere lengths however cannot be due to problems in resolution, and misalignment of thick filaments remains the most attractive explanation.

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