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THE MEASUREMENT AND DYNAMIC IMPLICATIONS OF THIN FILAMENT LENGTHS IN HEART MUSCLE

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SUMMARY

1. The lengths of the thin filaments in amphibian and mammalian cardiac muscle have been determined from electron micrographs of serial transverse sections. Thin filament lengths in frog atrial trabeculae range from 0·8 to greater than 1·3 μm, with a maximum possible error of 0·14–0·15 μm. In rat atrial tissue the span is from 0·6 to more than 1·1 μm, whereas in rat papillary muscle the breadth of the distribution is much narrower, from 0·9 to greater than 1·1 μm. Double overlap of thin filaments should, therefore, exist over a wide range of sarcomere lengths. Thin filaments from opposite halves of a sarcomere accommodate each other by flexing up to an angle of about 2° and moving from the trigonal position among the thick filaments to the centre of the region between two thick filaments. Such rearrangement probably contributes to the internal resistance to shortening in the muscle.

2. Except for the variation in thin filament lengths, the over-all morphology of the cardiac sarcomere is generally similar to that found in skeletal muscle. Thick filaments in heart muscle are uniform in length, and their profiles change along their lengths. They are generally round in the M band, triangular adjacent to the M band, round again in the overlap region, and either round or triangular near the tapered tips.

The M bridges in rat cardiac tissue link neighbouring thick filaments to form a symmetric hexagonal array, whereas in the frog atrium, the M bridge connexions are incomplete and often form isolated triangular clusters.

3. Computed sarcomere length-developed tension curves were calculated using the thin filament length distributions and the assumptions basic to the sliding filament theory of muscle contraction. The curves for atrial tissue have plateau regions approximately as wide as the one-half micron variation in thin filament length.

4. Work done against the internal loads during systole may be stored as potential energy and released during diastole to produce sarcomeric re-extension.

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INTRODUCTION

According to the generally accepted sliding filament hypothesis of muscle contraction, the maximum amount of force that a muscle can develop is a function of the number of sites on the thick filaments that can interact with the thin filaments. This, in turn, depends upon the extent to which the thick filaments are overlapped by thin filaments. In amphibian skeletal muscle, where the dimensions of the thick and thin filaments are relatively uniform, with a variation of only a few per cent (Page & Huxley, 1963; Brown, Gonzalez-Serratos & Huxley, 1970), the amount of overlap can be accurately inferred from the sarcomere length. Similar inferences have been made for cardiac muscle, but recent preliminary data (Page, 1974; Robinson & Winegrad, 1977) indicate that although the length of thick filaments is uniform in both amphibian and mammalian cardiac muscle, there is considerable variability in the length of thin filaments. The situation in cardiac muscle is further complicated by the fact that the distribution of thin filament lengths is not even uniform in different parts of the same heart (Robinson & Winegrad, 1977). It is clear, therefore, that before any meaningful relation between sarcomere length and developed force in cardiac muscle can be considered, more precise knowledge of thin filament dimensions must be available. The major function of this study is to answer that need.

Another consequence of the variability of the length of thin filaments is the probability that double overlap of the thin filaments is a common occurrence. Precise information to provide a basis for understanding the functional consequences of double overlap has not been available, even though double overlap has frequently been considered to impair the interactions between thick and thin filaments. Morphological data in this study argue against that conclusion and suggest that double overlap may act as a positive factor in the contractile cycle by storing energy during contraction to facilitate the return to resting length during relaxation.

METHODS

The principle of the technique. Although the demonstration of an entire thin filament in a single longitudinal section may appear to be an aesthetically pleasing way to measure the length of the thin filament, there are important problems inherent in the use of longitudinal sections. Superposition of thin filaments within a single section can create the impression of a longer filament than actually exists. Although in many cases this can be recognized by tilting the specimen if the filaments are close together, tilting is not totally reliable, and the time involved for a complete examination of many filaments would be prohibitive. The use of very thin sections can reduce the chance of superposition, but it introduces the possibility of filaments leaving the plane of section.

These difficulties as well as those resulting from compression along the length of the filaments during sectioning are avoided by using serial, transverse sections. Furthermore, in transverse sections a large number of both thick and thin filaments can be studied, and their orientation to each other at every position in the sarcomere can be observed. No tilting is required if the sections are precisely perpendicular to the long axes of the filaments.

The lengths of the thin filaments of a myofibril were determined from the distance between the serial section in which they terminated and the section that contained the Z band, which demarcates the other end of the filament. Since the number of thick filaments in an A band free of lattice defects is constant, the ratios of the numbers of thin to thick filaments in each section define the thin filament density at each position along the sarcomere. From this information the percentage of thin filaments of each length can be estimated.

In order to minimize possible errors in the accuracy of the measurements, three requirements were set for data included in the final determination of thin filament lengths. (1) The Z disk of a myofibril must be entirely included within one section (Pl. 2). Since each section was approximately 100 nm thick and the Z disk is 50 nm thick, this requirement limits the error from shear within a myofibril to a maximum of 50 nm. (2) All thick filaments must show precisely transverse profiles in all sections without the use of any stage tilting; this ensures their perpendicular orientation to the plane of the section. (3) The maximum ratio of thin to thick filaments at the edge of the A band must be 2. A higher ratio would indicate the absence of good alignment of all thick filaments within the A band.

Tissue preparation. Atria from medium-sized frogs (*Rana pipiens*) and rats were isolated, and spread over polymerized Sylgard 184 (Dow Corning Corporation, Midland, Michigan, U.S.A.) in a chamber filled with Ringer or Krebs solution. Trabeculae that were 1–3 mm long and 10–200 μm in diameter in the case of frogs and 200 to several hundred μm in rats were dissected free after ties had been placed on the ends. Ventricular trabeculae and papillary muscles from young rats 16–19 days old were similarly dissected. It is difficult to preserve the *in vivo* dimensions during the fixation and dehydration procedures because of shrinkage, which may not even be uniform along the entire length of the thin filament. Errors of this nature were minimized by restraining the tissue during all procedures. The sarcomerere pattern was continuously observed in the light microscope with a 16× phase contrast objective (N.A. of 0·25) and periodically recorded on film. If any significant change in the sarcomere pattern occurred during the preparation of the tissue block, the specimen was discarded.

Muscles were fixed in 6% glutaraldehyde buffered with 0·1 M-cacodylate buffer at pH 7·3 either for 3 h at room temperature or overnight in the cold. After fixation, they were rinsed for several hours in buffer and post-fixed in 1% OsO_4 in the same buffer. This was followed by dehydration in alcohol and embedding in Araldite 502 or ERL 4206 (Spurr, 1969).

Test sections were cut with a diamond knife mounted on a Dupont-Sorval MT2-B ultramicrotome, and if the myofilaments were perpendicular to the plane of section a full series was cut. Xylene vapours were wafted over the ribbon to reduce compression (Satir & Peachey, 1958), and the problem of variability of thickness was controlled by using only those ribbons in which colour variation was minimal. The sections were light gold in colour, indicating an average thickness of about 0·1 μm and a possible range of approximately 0·09–1·10 μm (Peachey, 1958). Standardization of colour among ribbons from different blocks was made by referring to a colour slide made of a light gold ribbon and to the continuous interference colour and thickness scale for thin sections (Peachey, 1958). Portions of the ribbons with different numbers of sections were then mounted on the Formvar-coated grids so that the series could be easily numbered and catalogued. Low power light micrographs were taken to facilitate localization of specific regions of these grids in the electron microscope. After having been stained 10–30 min in 2% UrAc in methanol and for 3–10 min in 0·1% aqueous lead citrate, the grids were examined with a Zeiss EM9-S2 electron microscope that had been set for a low beam current.

Cellular regions were mapped by means of montages of low power electron micrographs of the muscle cross-section, and from these montages suitable areas for study at higher magnification were chosen.

Sampling and counting. Indications of a variation in the lengths of thin filaments from incomplete rosettes of thin filaments around thick filaments was observed in numerous regions of all of the ten rat and frog hearts that were examined. Serial sections were followed in eight regions of two atrial trabeculae from each of two frog hearts, and a complete analysis was performed on two regions from one of the muscles. Three rat atrial trabeculae from different hearts were studied. Serial sections were prepared from six regions of trabeculae from two of the hearts, and three full analyses were made. Six rat papillary muscles were observed, and two full analyses were made from the six regions of one muscle. A complete analysis involved the study of photographic prints of an appropriate region of serial transverse sections from at least the major portion of an entire sarcomere, if not the entire sarcomere. The requirements for a region to be studied included a flat Z band and at least 400 thin filaments.

RESULTS

Thin filament length variation

In a sarcomere with well aligned filaments and no shear, all filaments of the same length should terminate in the same serial transverse section, and the appearance of incompletely filled rosettes of thin filaments around each thick filament, that is, less than six thins around each thick, indicates that the thin filaments are not all of the same length, even within the immediate environment of a single thick filament. The presence of anything from 0 to 6 filaments around a single thick filament was routinely observed in sections taken from near the M band of cells from both rat and frog heart (Pl. 1). Within one myofibril the number in each rosette varied considerably, showing that the thin filament environment of thick filaments was not uniform. This heterogeneity has already been noted by Page (1974).

Since each transverse section was approximately 0·1 μm thick, thin filament lengths and their relative frequency were estimated at 0·1 μm intervals from the Z band by determining the density of thin filaments within a single myofibril. This density was expressed as the relative number of thin to thick filaments since the latter is constant in the A band. Although the cardiac bundles being studied had been stretched before fixation to decrease the likelihood of long filaments crossing the M band to the contralateral side of the sarcomere, the tissue resists excessive elongation and some crossover was always present, even at sarcomere lengths of 2·8 μm. Sections without thin filaments were never seen. Crossover leads to an underestimation of the length of the very long thin filaments because it is assumed that they arise from the nearer and not the farther Z band. The error fortunately is small, because only a very small fraction of the total thin filament population is long enough to cross over at the long sarcomere lengths. It does mean, however, that the absolute dimensions of the longest thin filaments have not been determined.

Accuracy of the technique

The accuracy of the measurement of thin filament length with serial transverse sections depends upon the correct estimate of: (1) over-all shrinkage of the embedded filaments relative to their lengths in the living state, (2) absolute thickness of sections, (3) variation of section thickness, (4) the amount of material lost between sections during cutting, (5) material lost from the section surface by irradiation, (6) waviness of the Z band within a single section thickness, and (7) the length of penetration of a thin filament into a section necessary for its detection. The limitations of the estimates of amounts of shrinkage of thin and thick filaments have been thoroughly discussed by Page (1974) and Page & Huxley (1963). They showed that the shrinkage of the thin filament is considerably greater in the I band than in the A band, but since all the thin filaments end in the A band the major effect of shrinkage of the thin filament would be to shift the entire histogram without changing the span of lengths.

In order to handle the problems of shrinkage and section thickness, an internal calibration for section thickness was derived from either the total number of sections

required for one half of the length of the thick filament, that is from the M bridge region to the tapered tip, or the number of sections for the total length of a thick filament. This estimate of section thickness was checked by an independent method. One of the rat atrial trabeculae that had been embedded for transverse sectioning was cut from its block and remounted to allow subsequent sectioning with the filaments parallel to the knife edge. The sarcomere length in these longitudinal sections, after a 6% shrinkage correction (Page, 1974) was 2·55 μm, which is in close agreement with both the 2·5 μm estimate from light micrographs of the living muscle, and the 2·55 μm estimate obtained from the transverse serial sections. A similar agreement of 1·55 μm between the lengths of the thick filament estimated from longitudinal and transverse sections adds further support for the relative accuracy of values derived from the transverse sections and for the use of 6% as the estimate for shrinkage during fixation. For the determination of lengths in longitudinal sections, calibration grids (Fullam, Inc., Schenectady, N.Y., 28,800 lines/in.) were photographed without any change in the lens settings. Averages of several sarcomere lengths and of calibration grid lines that had been measured directly from the negatives were used.

From the visibility of thin filaments in longitudinal sections it seems likely that the presence of as little as 0·01 μm of thin filament in a section is adequate to produce a clear transverse image. Consequently a maximum error of about 0·09 μm could be made in estimating the length as a result of imprecise knowledge about where the filament ends. This error could be reduced by comparing the relative electron density of the thin filaments in a given section, but the relatively small additional accuracy from this measurement did not warrant the large additional effort.

The loss of material between sections is relatively small with soft embedding material like metacrylate (Winegrad, 1965) and the loss is likely to be even less and more uniform with the harder embedding material used in these experiments. Any loss in material during sectioning will, however, be compensated for by the use of the thick filament and the sarcomere length for calibration. The same argument holds for the effect of loss of material during irradiation.

The major uncertainty in estimating the lengths of thin filaments by the method of serial transverse sections is therefore due to the imprecision with which the position of the ends of the thin filament in a given section can be determined. The waviness of the Z band within a single section may introduce an error up to 0·05 μm, and the need for only about 0·01 μm length to be seen in a traverse section provides an error of up to 0·09 μm at the other end. The total maximum possible error is about 0·14 μm. In a population of 400 thin filaments that presumably have been sectioned in a random manner the average error should be considerably smaller.

The distribution of thin filament lengths

The frequency of lengths of thin filaments in frog and rat atria and rat right ventricular papillary muscle expressed in terms of numbers of section thicknesses is shown in the histograms in Text-fig. 1 and 2. The span is more than 0·6 μm in both frog and rat atrial trabeculae and more than 0·3 μm in rat papillary muscle.

The distributions of thin filament lengths were essentially the same for different

regions within the same fibre bundles and from corresponding regions of two different hearts from the same species of donor animal. The spans of filament lengths were not the same in the rat atrium and papillary muscle nor were they the same in rat atrium and frog atrium. In the frog atrium, filaments varied from 0·8 μm

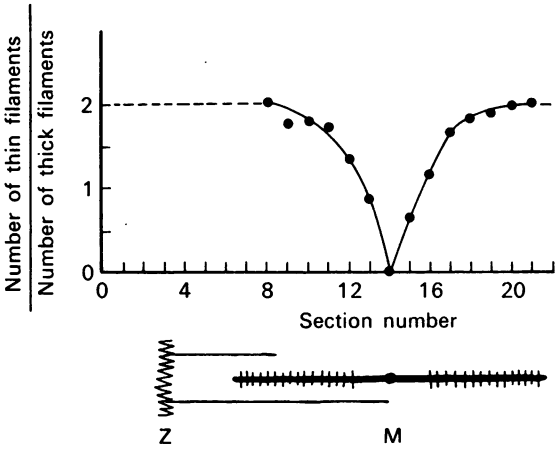


Fig. 1

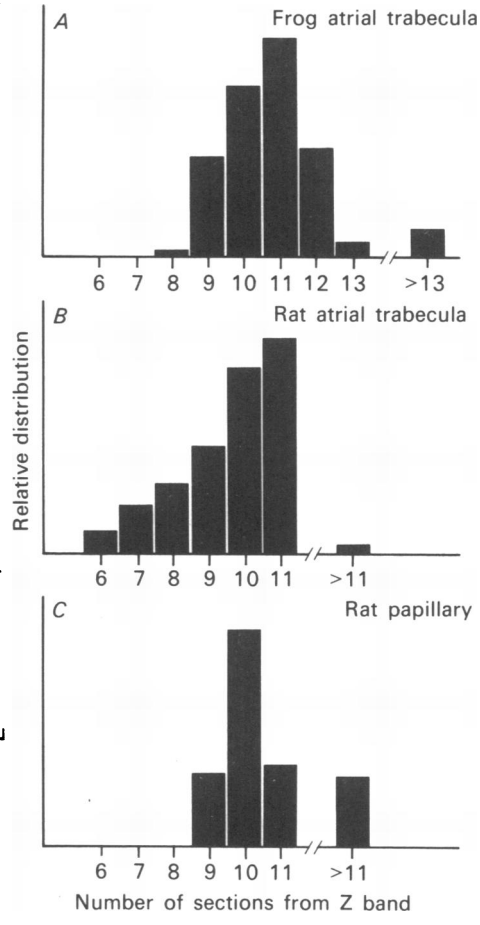


Fig. 2

Text-fig. 1. The ratio of the number of thin filaments to the number of thick filaments in a single sarcomere of a single myofibril in rat atrial tissue as a function of serial section number. Counts were made within the area corresponding to the area of the flat Z band, which in this case is in section number 3. The ratio in section 14, which contains the M band, is close but not quite equal to zero. A few very long thin filaments cross the M band, even though the muscle was stretched and held during preparation.

Text-fig. 2. The distribution of thin filament lengths as a function of the number of sections from the flat Z band. *A*, frog atrial trabeculae; *B*, rat atrial trabeculae; *C*, rat papillary muscle. Each section is approximately 0·1 μm thick. The thin filament lengths have been corrected for shrinkage.

to greater than 1·3 μm, with a mean between 1·0 and 1·1 μm. The rat atrium had a similarly broad span but the shape of the distribution was skewed toward the smaller lengths, producing a slightly smaller mean for the rat. As thin filaments

in the rat papillary muscle varied much less in length, the distribution, but not the mean, differed from those in atria.

Double overlap and thin filament accommodation

From the serial transverse sections it is also possible to follow the course of individual thin filaments and their positions relative to each other. This is of particular interest in the case of double overlap of thin filaments, where thin filaments from one half sarcomere have crossed the M band to the contralateral half and thus have raised the filament density in the latter region, in view of the suggestion that such an arrangement might interfere with the interaction between thick and thin filaments.

Thin filaments followed serially into double overlap show the conventional trigonal location in the rosette of six before double overlap and the accommodation of these ipsilateral thin filaments upon the addition of thin filaments from the contralateral half sarcomere. A rearrangement of thin filaments begins two to three sections before the introduction of the contralateral thin filament into the rosette. A total transverse movement as large as 10 nm is involved, but since it occurs over 300 nm, the maximum angle of bending of the thin filament in the course of the accommodation is about 2°. The thin filaments are, therefore, relatively inflexible within the A band, although not as much as the thick filaments, and a significant amount of energy is probably necessary to produce the observed strain.

The filaments never come very close to each other in spite of the additions to the rosette. Those that move go from the trigonal position in the thick filament lattice to the space between two thick filaments without assuming positions that obviously block access of thick filaments to other thin filaments. The rearrangement of thin filaments caused by their greater density produces a lattice resembling the pattern found in some striated muscles, such as insect flight muscle (Toselli & Pepe, 1968), that normally have a ratio of thin to thick filaments higher than 2:1 (Pl. 3). Many thin filaments are arranged in straight lines in a pattern that itself is characteristic of double overlap in frog cardiac fibres. The lines of thin filaments are often much longer than those found in double overlap in frog skeletal muscle (Huxley, 1965).

The over-all morphology of cardiac myofilaments

Thick filament morphology

The disappearance of all thick filaments in a given region in one section as well as the maximum ratio of thin to thick filaments of 2 at the A–I junction indicates that the thick filaments are well aligned and uniform in length in both the frog and rat hearts (Text-fig. 1). Very occasionally, the absence or misalignment of a thick filament produces a defect in the thick filament lattice, but short thick filaments are never seen.

The profile of a cardiac thick filament changes along its length (Pls. 1, 4) and in over-all morphology, resembles the thick filaments of skeletal muscle (Pepe, 1975). It is generally round in the M band, triangular adjacent to the M band, and round again in the overlap region. At the junction of the A and I bands, the tapered ends are often triangular again.

M bridge configurations

The connexions among the central bare zones of thick filaments at the M band are different in rat and frog atria. In the rat, each thick filament is connected to each of its six neighbouring thick filaments, at least at one level along the M band, as indicated by the highly symmetrical pattern of M bridges seen in the transverse sections (Pl. 1). In the frog atrium, on the other hand, thick filaments are rarely connected to all six neighbouring thick filaments, and the M band pattern is striking in its lack of regularity. Here, the M bridges usually lie tangential to the thick filaments, often with a curve or a bow, and form isolated, generally triangularly shaped clusters of filaments. The pattern is similar to that seen in frog ventricular muscle (Page & Neidergerke, 1972). In some regions of activated frog atrial trabeculae long lines of thin filaments and M bridges are parallel without any mutual crossing (Pl. 3). Because of their incompleteness, the M bridges of frogs should offer less resistance to shear than the more complete M bridge system of the rat heart (Winegrad, 1974).

DISCUSSION

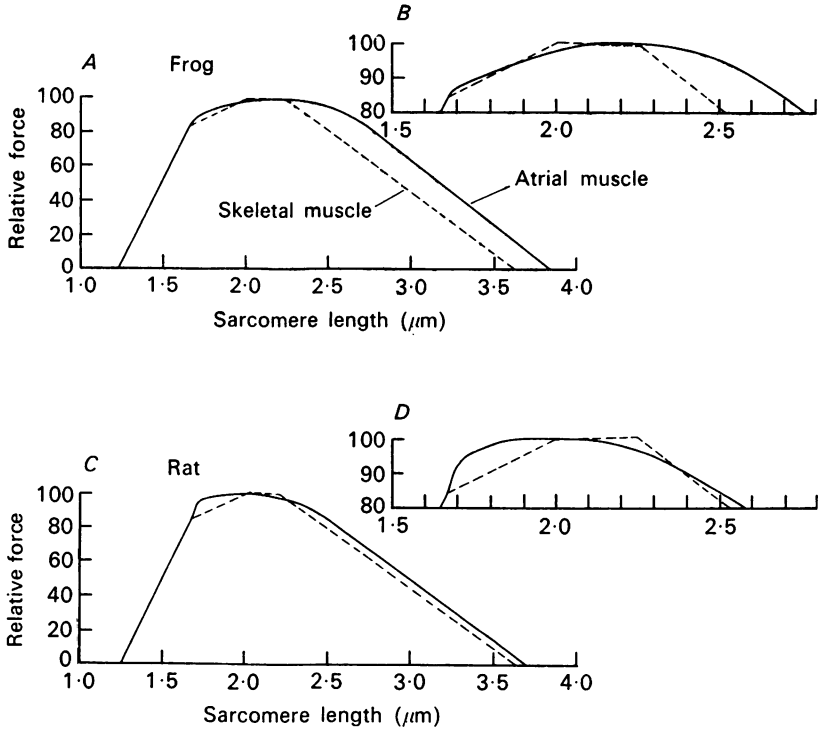
Effect of variable thin filament lengths on the sarcomere length–tension relation

The lengths of thin filaments in frog atria and in both atria and ventricles from rats vary considerably even within a single sarcomere of an individual myofibril. In rat ventricle the range of lengths is about 0·3 μm while in mammalian and amphibian atria the span exceeds 0·6 μm. Consequently, there is no single value for the extent of overlap of thick and thin filaments, even within one sarcomere. In accounting for the relation between sarcomere length and force generation according to the generally accepted sliding filament hypothesis of muscle contraction, the distribution of lengths of thin filaments must therefore be considered.

If developed force in cardiac muscle depends, as it seems in tetanized skeletal muscle, primarily on the degree of overlap of thin and thick filaments (Gordon, Huxley & Julian, 1966), then developed force will not have the simple relation to sarcomere length in this tissue that it does in fast skeletal muscle, where the variation in thin filament lengths is less than 0·1 μm (Page & Huxley, 1963; Brown *et al.* 1970). Composite, calculated sarcomere length-developed tension curves for atrial muscle were made by constructing an individual curve for each thin filament length based on the principles described by Gordon *et al.* (1966), weighting it by the appropriate factor from the length distribution, and summing all the weighted curves (Text-fig. 3).

The actual mechanism of the rising phase of the sarcomere length–tension curve at short sarcomere lengths is not clear but separate calculations were performed for two possibilities. In one case, net production of force declines as a thin filament slides past the bare zone of thick filaments into the contralateral side of the overlap region. In the second case, it is the double overlapping of thin filaments itself that resists sarcomere shortening, presumably due to flexure of the thin filaments as they accommodate each other and shift positions in the filament lattice. The second set of calculations was performed using the assumption that all ipsilateral filaments

are opposed in the contralateral half sarcomere by filaments of average length. This simplifying assumption was made since the data do not indicate the nature of the matching of thin filaments across the M band. The figures shown were derived with the first of the two assumptions, although both sets of computations gave similar results. Their flat shape indicates that as a consequence of the variable length of thin filaments there is very little change in the potential for tension production throughout the putative working range of cardiac sarcomere lengths



Text-fig. 3. Calculated sarcomere length-developed tension curves. The derived relation is the composite of individual sarcomere length–tension curves weighted by the appropriate factor from the distribution histographs (Text-fig. 2). Each of the individual curves was constructed for a given thin filament length using the inferences of Gordon *et al.* (1966), concerning the relation between developed force and filament configuration. *A*, frog atrial trabecula and, *B*, enlargement. *C*, rat atrial trabecula and, *D*, enlargement. Interrupted line (-----) represents curve for skeletal muscle.

(in contrast to skeletal muscle). Sliding of myofilaments with changes in length should therefore have little direct effect on force generation in the intact heart. The broad zone with only a very shallow slope in the length–tension relationship of calcium-activated, mechanically skinned cardiac fibres (Fabiato, 1975*b*) is consistent with this conclusion. On the other hand, the isolated but intact rat ventricular muscle bundle has a plateau in its length–tension relation that is even narrower than that of frog skeletal muscle (Julian & Sollins, 1975; Julian, Sollins & Moss, 1976) in spite of the broader range of thin filament lengths in the rat ventricle

(Text-fig. 2). Modulation of force in the intact cardiac tissue may thus involve another step, such as activation, in the contractile cycle (Fabiato & Fabiato, 1975*a*).

The breadth of distribution of thin filament lengths can be correlated with a rough estimate of the compliance of the tissue (Text-fig. 2). The stiffer papillary muscle had a narrower span than the more compliant atrial bundles. This raises the possibility that the cell might actually set the breadth of the distribution according to its needs. The resultant plateau in the sarcomere length–tension curve would then be adequate for the extent of shortening that the cell must undergo during contraction in the intact heart.

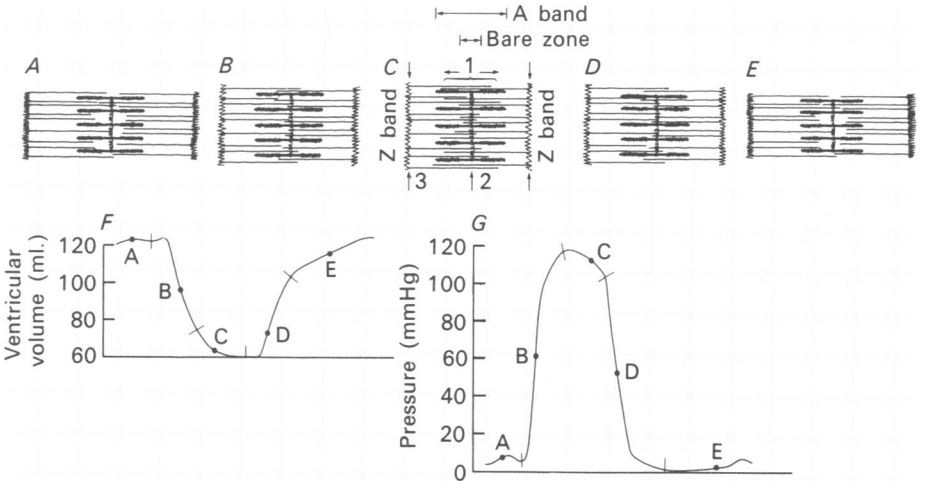
Dynamics of double overlap

As the sarcomere shortens and thin filaments begin to cross the centre of the A band the ratio of thin to thick filaments in regions of double overlap will gradually increase to more than 2:1. To accommodate the greater density of contractile material, the ipsilateral thin filaments begin to move from the trigonal points in the thick filament lattice to positions between adjacent thick filaments. The new packing pattern is still hexagonal but there are now planes in which the thin filaments are present in linear arrays as in the pattern in insect flight muscle, where the ratio is also greater than 2:1 (Toselli & Pepe, 1968). These regions are very easily identified in transverse sections. The distribution of such linear arrays, often quite long, might be influenced by the irregular M bridge distribution in the frog heart.

In the frog muscle, where these shifts of position have been most carefully examined, the accommodation of thin filaments to the greater density involves a very small angles of flexure (2°), suggesting that the thin filament, at least in the A band is relatively stiff. From morphological considerations, there is no reason to believe that the rearrangement of thin filaments has impaired the possible interactions between thick and thin filaments. Contractile work must be performed, however, in order for the thin filaments to bend into the more highly packed configuration, and some of the energy involved in producing this strain and in increasing the separation between filaments should be stored in the filament lattice during active shortening. The stored potential energy would then be available at the termination of active shortening for the extension of the fibre toward its resting length. This notion is consistent with several general observations. Double overlap cannot be produced in the totally resting fibre, even with applied longitudinal compression (Huxley & Gordon, 1962; Gonzalez-Serratos, 1966; Brown *et al.* 1970), and it has never been observed in resting cardiac muscle (Winegrad, 1974; S. Winegrad, unpublished results). Also, in isolated skeletal muscle fibres there is an internal resistance to shortening in response to increases in osmotic strength of the bathing medium at the sarcomere length where double overlap would be expected to occur (Simmons, 1971).

Since the volume of the lattice in living muscle remains constant as the sarcomere length changes (Huxley, 1953; Elliott, 1965), other components of the filament lattice such as the M bridges (Matsubara & Elliott, 1972) and the Z band (Franzini-Armstrong, 1973; Goldstein, Schroeter & Sass, 1977) might resist shortening of the cell, but this study does not directly address these possibilities. In addition to

internal loads of the myofibril, forces that resist shortening probably arise from components at higher levels of organization, such as linkages between cells (Robinson & Winegrad, 1978) and connective tissue (Winegrad, 1974). Consequently, the myofibril may be considered in terms of an oscillator in which a significant fraction of the actively generated shortening energy is conserved as potential energy by the various elasticities and converted back to the kinetic energy of sarcomere lengthening during relaxation (Text-fig. 4). The question of restoring forces and 'negative ventricular pressure' to assist in early diastolic filling of the ventricles has been discussed for many years without resolution. There is no doubt that the filling of the isolated heart is assisted measurably by restoring forces, but since cardiovascular



Text-fig. 4. Diagrammatic representation of the changes in structural organization of a ventricular sarcomere and their functional implications for the cardiac cycle. The filament configurations in *A–E* are the assumed states of the myofibrils during different stages of the cardiac cycle, as indicated by the volume and pressure records in *F* and *G*. Some of the energy from the active phase of shortening (*A–C*) is stored as potential energy in the form of: (1) doubly overlapped thin filaments, (2) stretched M bridges, and (3) stretched Z bands. The directions of forces are indicated by the arrows in *C*. Since the filament lattice maintains a constant volume, a decrease in diameter is accompanied by an increase in length of the sarcomere. The elongation of the sarcomere past the equilibrium position of double overlap, shown in *A*, has been attributed to atrial pressure, particularly during atrial systole. (Data for the diagrams of ventricular pressure and volume have been taken from Berne & Levy, 1967.)

physiologists have failed to measure negative pressure in the ventricle during early diastole, the argument has been made that the heart never shortens sufficiently in the intact animal to produce significant restoring force. This objection, however, is inconsistent with the observations in the intact heart that during systole the sarcomere shortens by 15–30 % from a resting length which, even in the dilated heart, is no more than 10 % greater than the minimum length at which double overlap begins (Sonnenblick, Covell, Spotnitz & Spiro, 1967). In the intact heart most of the ventricular filling occurs in a brief period at the beginning of diastole when restorative forces should be most prominent. The rate at which the ventricle

fills resembles the curve of a critically dampened oscillator coming to rest. There actually is a simultaneous decline in intraventricular pressure and increase in ventricular volume during early diastole (Berne & Levy, 1967), an observation incompatible with an entirely passive role of the ventricle during filling.

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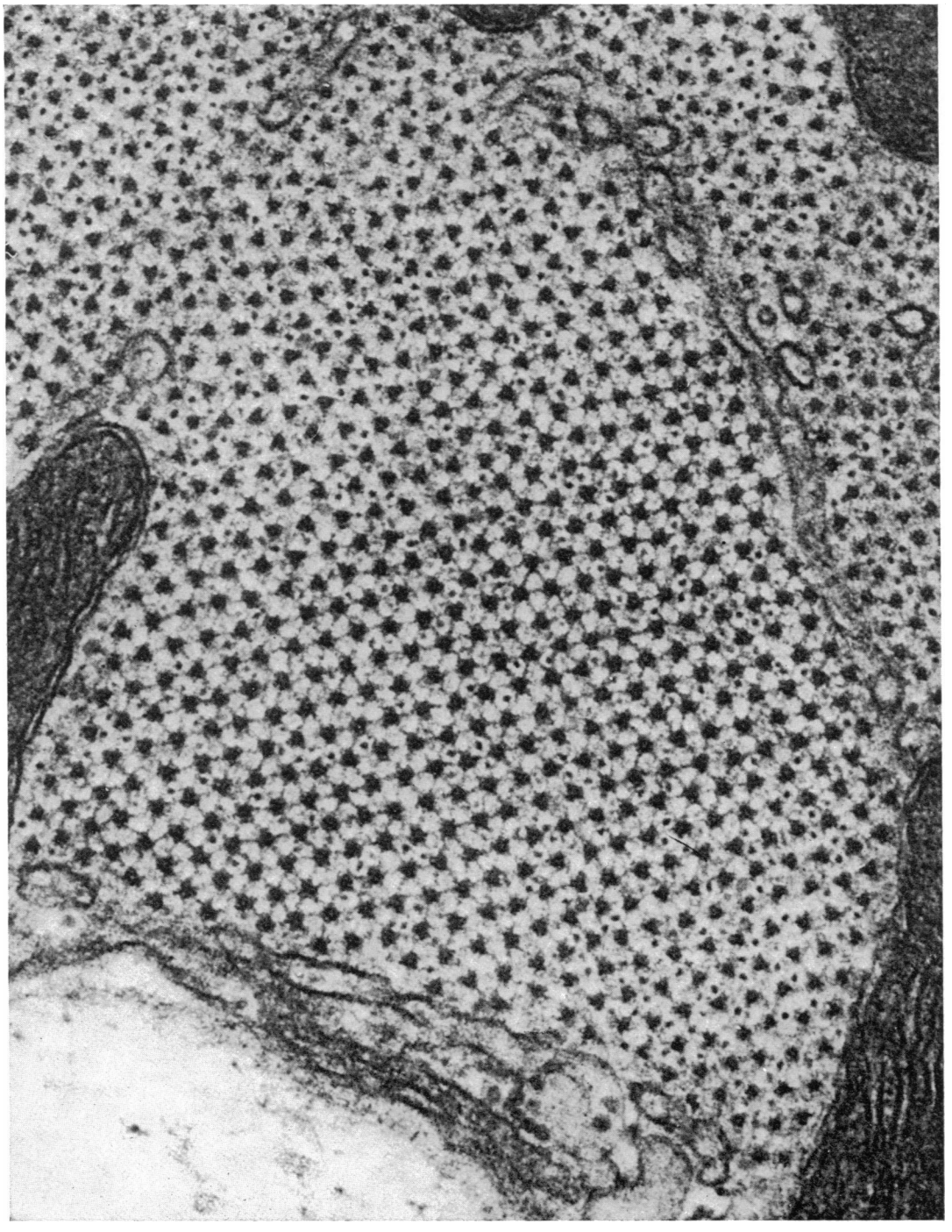

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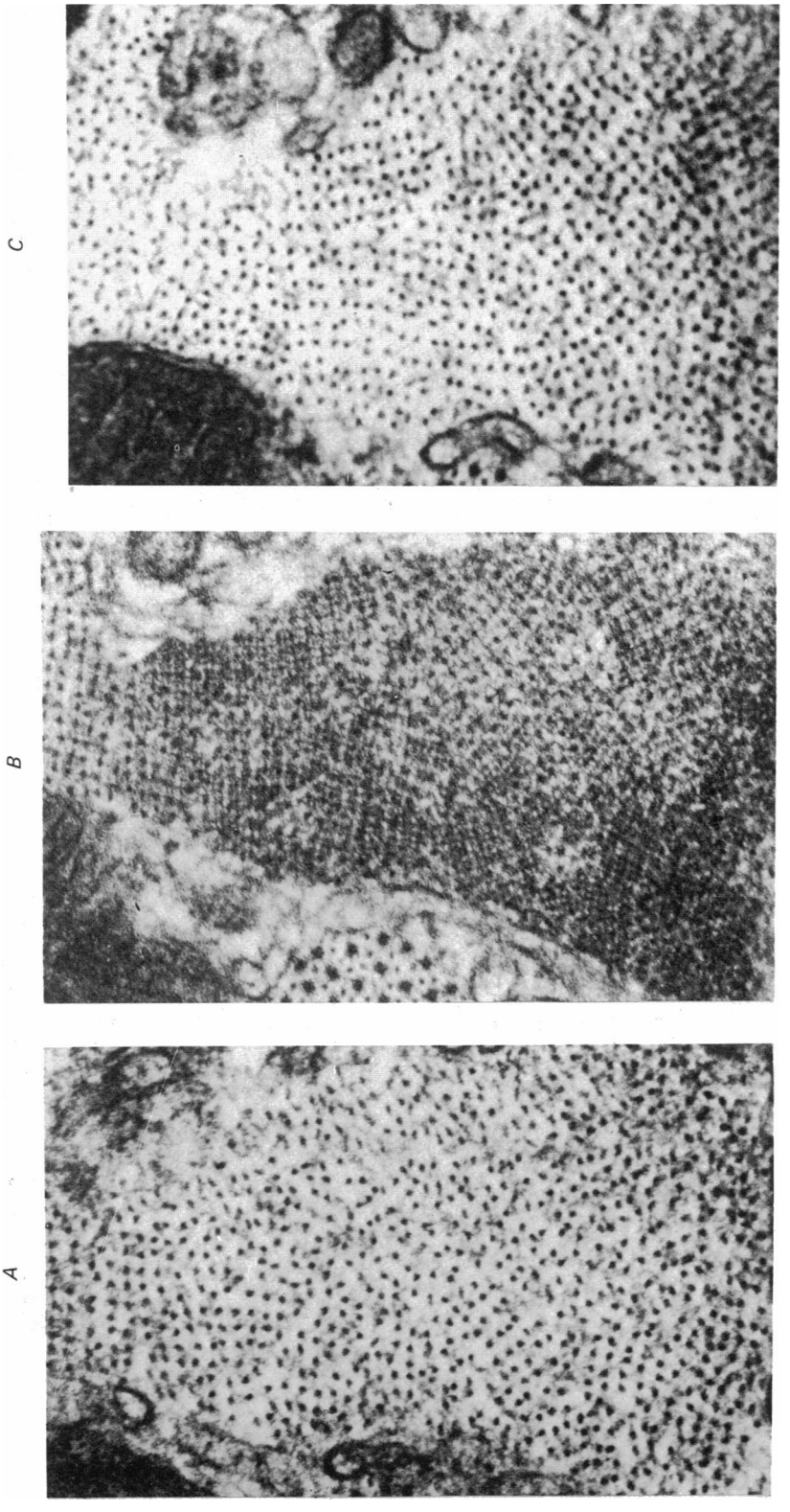
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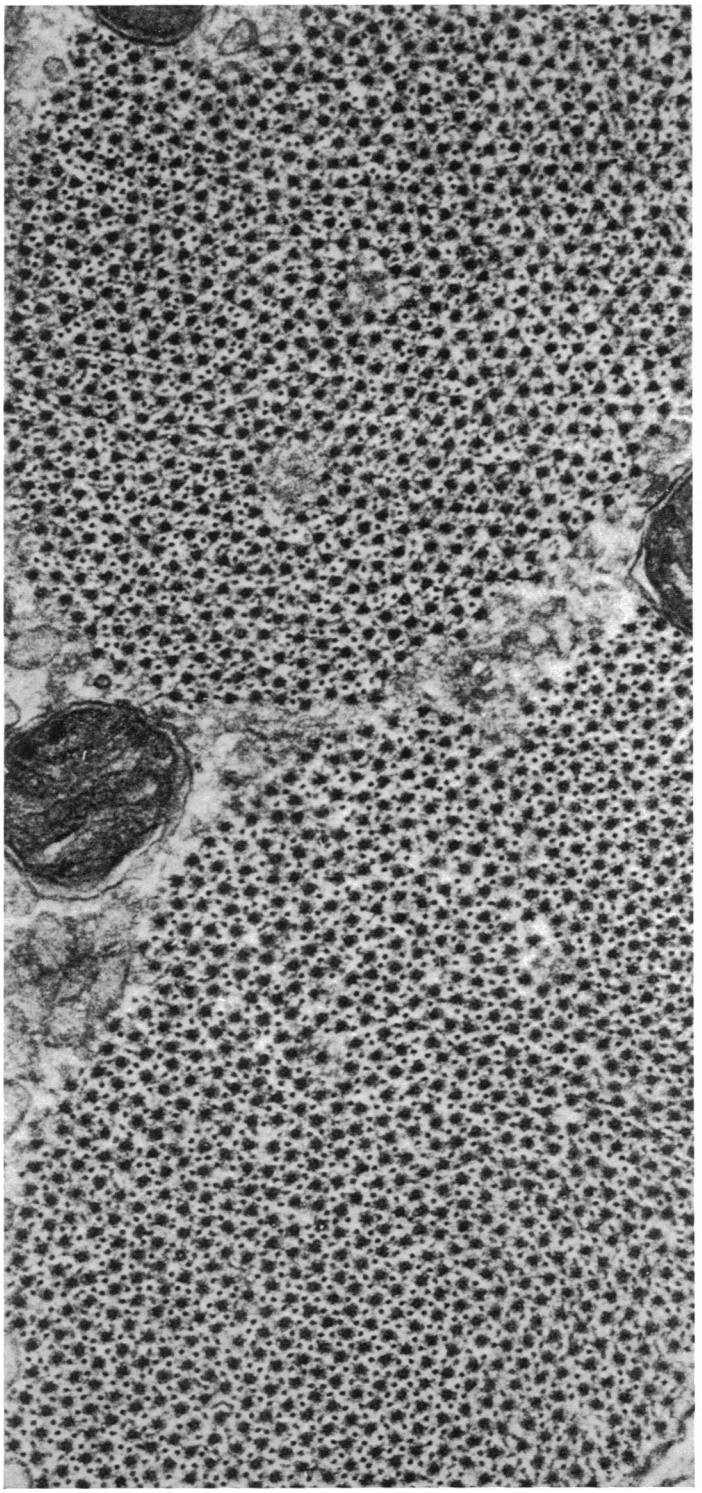

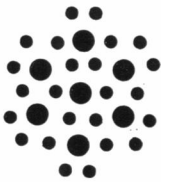

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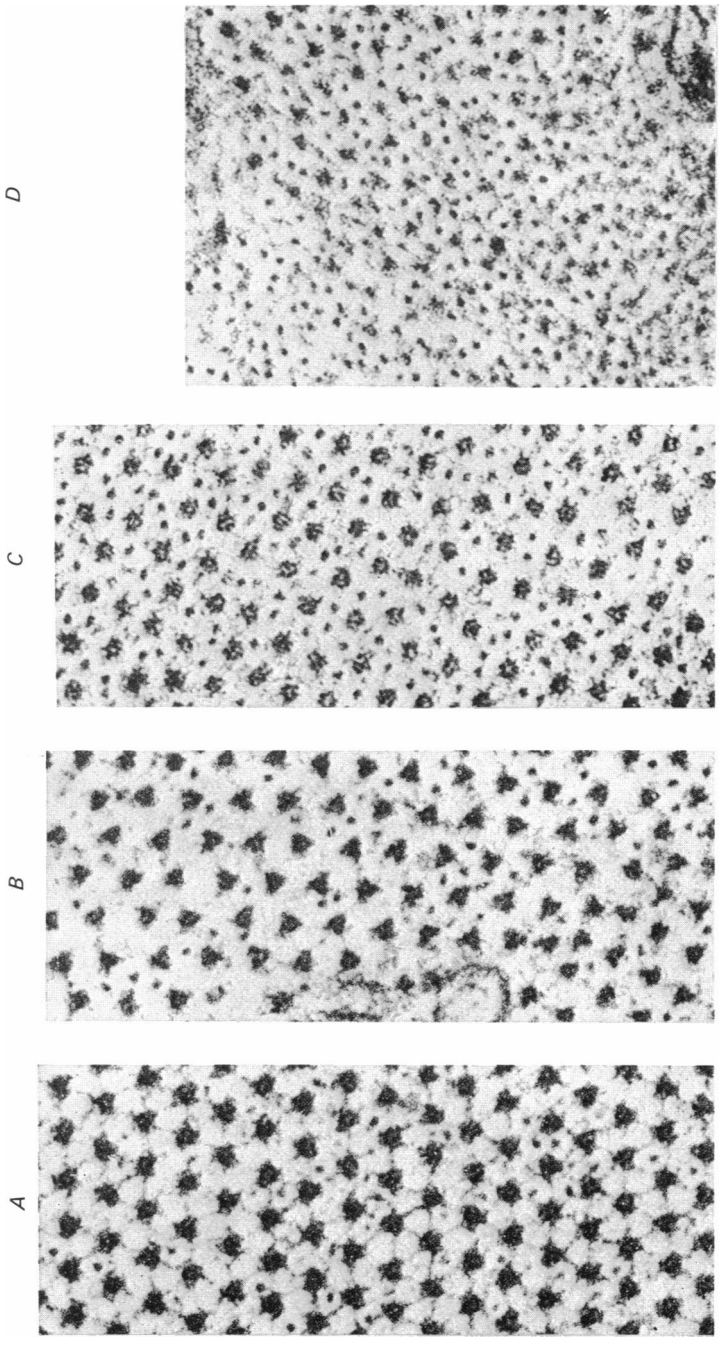

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EXPLANATION OF PLATES

PLATE 1

Transverse section through a portion of a rat atrial trabecula. Some long thin filaments extend through the central region of the A band where the hexagonally arrayed thick filaments are interconnected by M bridges. Mitochondria, sarcoplasmic reticulum, plasma membrane, and cell coat can be seen near the myofilaments. × 74,300.

PLATE 2

Serial transverse sections through an area suitable for counting in a single sarcomere of a stretched rat atrial trabecula. The flat Z band (*B*) is included between sections containing only I band (*A*, *C*). × 102,000.

PLATE 3

A transverse section through an activated frog atrial trabecula showing, in the lower region, a basically hexagonal pattern characteristic of a ratio of thin to thick filaments of 2:1 (lower diagram). In the upper region, a higher degree of double overlap has produced some linear arrays of thin filaments similar to those seen in insect flight muscle, where the filament ratio is 3:1 (upper diagram). The full complement of thin filaments from a 4:1 ratio is rarely seen since the activated muscle was restrained from shortening. × 80,400.

PLATE 4

Transverse sections of rat atrial trabeculae. Cross-sectional profiles of the thick filaments vary with position along the filament: *A*, round in the M band; *B*, triangular in the regions of the bare zone adjacent to the M band; *C*, round in the crossbridge region; and *D*, round or often triangular near the tips. × 150,000.