A Reexamination of the Influence of Muscle Length on Myocardial Performance

BRIEF REVIEWS

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"The constant interplay of the Frank-Starling mechanism (changing diastolic volume) and changing contractility determines the manner in which the heart varies its output relative to its input on a beat-to-beat basis and thus permits widely varying demands to be met."

THIS EXTRACT from a former Brief Review by Sonnenblick and Skelton provides a very neat summary of the essence of our understanding of how cardiac output is regulated. The interplay envisaged is between muscle length (which is governed by diastolic volume) and contractility (which can be altered by inotropic interventions), and these two factors are generally thought to influence myocardial performance through quite separate mechanisms. Muscle length and contractility or the inotropic state of the muscle have been regarded as amenable to independent manipulation as experimental variables, and the quest for the perfect index of contractility has rested squarely on the belief that some fortunate person would, one day, find a measure of mechanical performance that could be shown to depend on the inotropic state of the muscle and to be independent of muscle length. The conclusion reached in this article is that (in the isolated papillary muscle at least) there can be no such index of contractility because the inotropic state of the muscle is strongly influenced by its length.

The muscle literature over the past 15 years or more has included various papers that could be quoted in support of the above conclusion, but attention will be focused in this review on studies of the length-tension relation published during the past 3 years. Such a restricted coverage of the literature may be justified on two grounds: first, because some of these recent studies have involved technical innovations that take us into a new era of research on isolated preparations of cardiac muscle; second, because the ultrastructural basis of the length-tension relation was given a very thorough review by Sonnenblick and Skelton in 1974 and various related topics were discussed at the Ciba Symposium, "The Physiological Basis of Starling's Law of the Heart," held in 1973.

Excitation-contraction Coupling

As reference will be made from time to time to various links in the chain of events responsible for excitation-contraction coupling in cardiac muscle, it will be useful to begin with a very brief summary of these (for recent accounts, see References 10-12). Figure 1 is a schematic diagram of the main events, with various feedback loops omitted because, with one exception, they are of secondary importance in the context of this article. The exception is a possible influence of changes in muscle length during contraction on the activation of the contractile system. This will be mentioned briefly, but it has been considered in relation to the influence of initial muscle length on myocardial performance in a recent article by Lakatta and Henderson.

The end result of excitation-contraction coupling is the formation of tension-generating crossbridges between the overlapping parts of the thick (myosin) and thin (actin) filaments that make up the contractile system (see Fig. 3B). This is made possible by the binding of calcium to troponin, which is a regulatory protein present in the thin filament in association with tropomyosin and actin. At the low sarcoplasmic Ca²⁺ concentration present in the resting muscle, there is insufficient calcium binding by the troponin to remove the inhibitory influence it exerts (via tropomyosin) on the myosin-binding properties of the actin molecules. Excitation of the cell causes the sarcoplasmic Ca²⁺ concentration to rise and the additional binding of calcium to troponin results in unmasking of the myosin-binding sites on the thin filament: contraction then occurs as a result of crossbridge formation.

The amount of calcium bound by troponin determines the degree of activation of the contractile system. In cardiac muscle this can be altered by a variety of factors (inotropic interventions) which probably act in different ways on earlier events in the excitation-contraction coupling sequence but with the same end result: i.e., they alter the amount of calcium bound to troponin. Insofar as the concept of myocardial contractility or the inotropic state of the heart can be identified in terms of the events shown in...
Figure 1, it must therefore be equated with the amount of calcium bound by troponin as a result of excitation of the cell.

As a taste of possible things to come, it should be noted that the degree of activation of the contractile system may also depend on the phosphorylation of myofibrillar proteins, notably myosin and troponin, and this could be influenced for example by catecholamines through the mediation of cyclic AMP.

**Influence of Muscle Length on Tension Production:**

Physical and Activation Effects in Skeletal Muscle

Although the primary purpose of this article is to examine the influence of muscle length on myocardial performance as manifested by tension production of isolated preparations of cardiac muscle under isometric conditions, it is helpful to begin with a brief review of some relevant recent developments in the skeletal muscle field. Evidence has been accumulating that the dependence of tension production on muscle length in skeletal muscle results from two independent effects, which will be referred to subsequently as **physical** and **activation** effects (P and A in Fig. 1). The physical effects result from the fact that muscle length governs the shape of the fibers and the disposition of the structures inside, notably the sliding filament system, whereas activation effects result from dependence of the degree of activation of the contractile system on muscle length.

Figure 2 shows length-tension data obtained by Gordon et al. from very careful measurements made during fused tetanic contractions of single fibers taken from frog semitendinosus muscles. (Only their data for the ascending limb of the length-tension relation will be considered as this covers the range of sarcomere lengths of particular interest in relation to cardiac muscle.)

Very similar results were obtained by Edman in an independent study of the same preparation with less sophisticated methods. Gordon et al. found that tension declined toward zero at a sarcomere length of about 1.3 μm, but they noted that substantial tensions had been observed at shorter lengths by previous workers though this was generally in association with irreversible shortening of the muscle fiber. The situation at very short muscle lengths was clarified subsequently by Taylor and Rüdel, who showed that when frog single muscle fibers shorten to sarcomere lengths less than 1.6 μm the innermost myofibrils became wavy, probably as a result of a failure of activation. This interpretation was supported by their observation that the myofibrils did not become wavy when the fibers were stimulated in the presence of caffeine, a potentiator of calcium release from the sarcoplasmic reticulum. In the presence of caffeine they observed tension development at sarcomere lengths down to about 1 μm, as illustrated by Figure 2B and the dotted line in Figure 3A. In relation to a later discussion of possible reasons for the difference between the length-tension curves represented by the dotted and solid lines in Figure 3A, it should be noted that although Rüdel and Taylor were able to observe that myofibrils in the core of their muscle fibers contracted when they were stimulated in the presence of caffeine, they had no means of knowing whether the fiber was maximally activated.

The only experimental situation in which one has any sort of guarantee that maximal activation has been achieved is in studies of skinned (i.e., sarcolemma-free) muscle fibers. Schoenberg and Podolsky have obtained length-tension curves for skinned frog muscle fibers, and they observed tensions at a sarcomere length of 1.0 μm that were 30–70% of the value observed in the plateau region (2.0–2.2 μm). The exact value depended on the procedure used when analyzing records that showed an initial rise of tension when the bathing Ca²⁺ concentration was raised and then a further slow climb. They felt that the...
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Figure 2 Length-tension data for single frog skeletal muscle fibers (redrawn from the original figures with permission). Tension production was measured in fused tetanic contractions and has been expressed as a percentage of the value in the plateau region (2.0-2.2 \( \mu m \)). Sarcomere length was obtained from measurements of the striation spacing in the living fiber. A: data from four fibers (different symbols) over the ascending limb of their length-tension relation. Other measurements in the same study had shown the presence of a tension plateau at sarcomere lengths between 2.0 and 2.2 \( \mu m \). B: data from two fibers (solid and open symbols) in 3 mM caffeine (squares), 2 mM caffeine (triangles), and no caffeine (circles), with the dashed curve from panel A for comparison.

The true value was between these limits, and their results are represented by the solid line in Figure 3A.

As it should be possible to achieve maximal activation of the contractile system in a skinned fiber by providing a suitable chemical environment and enough calcium to saturate the troponin, the length-tension curve obtained from the skinned fiber must be presumed to reflect purely physical effects of length on tension development that are inherent in the sliding filament system. It is important to note, however, that the solid line in Figure 3A may not reflect the intrinsic properties of the sliding filament system in an intact fiber because of various constraints imposed by the presence of the sarcolemma: for example a skinned fiber does not show the constant volume behavior seen in an intact fiber.

From considerations of the behavior expected of the sliding filament system (Fig. 3B), two kinds of physical effect have been postulated to account for the decline of tension production at sarcomere lengths less than 2.1 \( \mu m \): those that interfere with tension generation (e.g., double overlap of the thick filaments by thin filaments from opposite ends of the sarcomere at lengths less than 1.8 \( \mu m \)), and those that produce opposing forces (e.g., resistance to overlapping of the thin filaments at lengths below 1.95 \( \mu m \) and longitudinal compression of the thick filaments at sarcomere lengths less than 1.65 \( \mu m \)). Physical effects of the second kind can be regarded as an internal load on the tension-generating system and they provide intrinsic restoring forces that return the fiber to its slack length during relaxation.

Skeletal muscle fibers have a well defined slack length, which corresponds with a sarcomere length of about 2.1 \( \mu m \) in intact fibers and about 1.95 \( \mu m \) in skinned fibers, and they cannot be shortened passively below slack length. Any attempt to reduce the length of a resting fiber below this length results in buckling of the fiber or its connections to the recording equipment unless special tricks are employed. The cause of this behavior appears to be a resistance to overlapping of the ends of the thin filaments in the middle of the sarcomere. A fiber can take up a length less than its slack length by active shortening because the shearing force generated by the formation of crossbridges overcomes the resistance to overlapping of the thin filaments. Thus, when a buckled fiber is stimulated, there are two phases to its contraction: first there is active shortening against zero external load, which takes up the slack in the system; then there is tension development under "isometric" conditions at the shorter sarcomere length reached as a result of active shortening.

Figure 3 A: comparison of length-tension curves for single frog skeletal muscle fibers under different experimental conditions. Solid line: skinned fibers under conditions of maximal activation. Dashed line: intact fibers during fused tetanic contractions (see Fig. 2A). Dotted line: intact fibers during fused tetanic contractions in the presence of 3 mM caffeine (see Fig. 2B). Tension has been expressed as a percentage of the maximal value obtained. B: diagram of the sliding filament system to show key dimensions (\( \mu m \)) at the sarcomere length (2.1 \( \mu m \)) at which all the side projections from the thick filament are just overlapped by thin filaments. Note: the accuracy with which these dimensions can be estimated is about \( \pm 0.02 \mu m \).
Figure 3A shows that at sarcomere lengths below about 1.6 μm tension production falls off more steeply in intact fibers (even in the presence of caffeine) than it does in skinned fibers. This could be due entirely to activation effects of the kind shown to be responsible for the difference between the two broken curves or it could be due partly to consequences of enclosing the contractile system within the sarcolemma. This structure and the surrounding connective tissue must be stretched circumferentially to an increasing extent at short sarcomere lengths because of the constant volume behavior of the cell; it could therefore provide an additional “internal” load during contraction and augment the intrinsic restoring forces that return the fiber to its slack length during relaxation. That would be a purely physical consequence of the presence of the sarcolemma, but as noted previously it may impose other more subtle constraints on the behavior of the sliding filament system.

What does emerge clearly is that in fused tetanic contractions of frog skeletal muscle fibers it is only at sarcomere lengths less than about 1.6 μm that activation effects contribute to the dependence of tension production on muscle length. Under conditions in which activation of the contractile system is less likely to be complete—for example, in twitches of frog muscle at room temperature and in twitches and unfused tetanic contractions of mammalian skeletal muscle at body temperature—there is evidence that length-dependence of activation may be the dominant factor determining tension production over the entire ascending limb of the length-tension relation, which may extend up to sarcomere lengths of nearly 3.0 μm.

In the foregoing analysis of the length-tension relation in skeletal muscle and the one that follows for mammalian ventricular muscle, heavy reliance is put on measurements of sarcomere lengths which are in fact average values obtained from many sarcomeres. If the sarcomere length varies much along the length of the fiber or from one fiber to another in a multicellular preparation, then it would be imprudent to attempt any detailed analysis of the length-tension data. Unfortunately none of the papers under review includes stringent tests for uniformity of sarcomere spacing within the preparation.

Cardiac Muscle: Application of New Techniques to the Isolated Papillary Muscle Preparation

Studies of the length-tension relation of cardiac muscle are compromised to some extent by the lack of a suitable single-cell preparation. Papillary muscles from the right ventricles of the hearts of small mammals provide the best available preparation from the mechanical point of view, but until recently they were regarded as too thick or too nonuniform to permit measurements of sarcomere lengths in the living muscle. Various attempts have been made to relate length-tension data to sarcomere lengths measured in muscles after fixation (e.g., see References 17, 18, 31), but shrinkage artifacts and uncertainties about the exact mechanical status of the muscle fibers at the time of fixation have been a constant problem. Within the past 5 years several groups have found it possible to measure sarcomere lengths in thin preparations of living muscle. Pollack’s group has been very successful in developing light diffraction methods that allow sarcomere length to be monitored during contractions of rat papillary muscles, and Julian’s group has measured sarcomere lengths by taking photomicrographs of the striation pattern during contractions of both rat and rabbit papillary muscles. Direct measurements of sarcomere length have also been made in frog atrial trabeculae by light diffraction methods and by using Nomarski optics. It is one of the drawbacks of isolated preparations of cardiac muscle that one end (at least) must be anchored by a clamp, and it has long been suspected that the adjacent damaged region(s) might interfere with measurements of the mechanical properties of the muscle. It has now been shown that in isometric contractions the central region of the muscle shortens at the expense of the damaged ends, and the extent of the shortening in contractions at Lmax (the muscle length at which maximum tension is developed) was found to be about 7% in one study and 11% in the other. Both showed that the amount of internal shortening did not decrease in isometric contractions at lengths less than Lmax even though the tension developed was substantially less. Thus the damaged ends of the preparation behave as series elastic structures with very strange properties, which may result from the behavior of the partially damaged region between the crushed end and the healthy muscle: the unhappy consequences of this for users of simple analog models of cardiac muscle will be abundantly clear.

One of the consequences of these recent revelations is that the definition of Lmax and the calculation of tension developed have become areas of possible confusion. The cardiac muscle literature contains many examples of length-tension curves of the kind shown in Figure 4A, where tensions have been plotted against muscle length. The dashed line identifies Lmax, the length at which tension—i.e., total tension minus resting tension—is maximum (Fig. 4B). Measurements of sarcomere lengths in living muscle allow tension measurements at rest and during contraction to be plotted against the corresponding sarcomere lengths, and Figure 4C shows how the data represented by Figure 4A would appear if displayed in this way. It is important conceptually to recognize that Figure 4A shows the behavior of the preparation as a whole, whereas Figure 4C is concerned only with fibers in the central region. The muscle as a whole contracts “isometrically” (dashed line, Fig. 4A), but the central region contracts auxotonically (dashed line, Fig. 4C) and this complicates the calculation of tension developed. If resting tension is considered to be borne by structures in parallel with the contractile system (e.g., the sarcolemma), then the shortening of the central region would result in a transfer of tension (1, Fig. 4C) from the parallel elastic elements to the contractile system. The value obtained for tension developed (DT) would then be greater than that calculated by the traditional method (DT, Fig. 4D). However, there is an increasing body of evidence that resting tension may originate, at least in part, in the sliding filament system. If this is the case then there would be no particular reason for preferring DT over DT as the value.
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The Length-Tension Relation of Mammalian Ventricular Muscle

As a result of work published from three laboratories since 1975, it is possible to compile a diagram like Figure 3 for rat ventricular muscle. The solid curve in Figure 5A shows the length-tension curve obtained by Fabiato and Fabiato3 from skinned single fibers that were maximally activated. It is very similar to the corresponding curve in Figure 3A and again it must be presumed to reflect the dependence of tension production on muscle length due to purely physical or geometric factors intrinsic to the sliding filament system when released from any constraints imposed by the sarcolemma. It is of interest to note that a steeper decline in tension was observed at sarcomere lengths below 1.5–1.6 μm in a more recent study8 in which polyvinylpyrrolidone was added to the bathing solution to prevent the swelling that otherwise occurs in skinned muscle fibers. At greater sarcomere lengths the curve was as shown in Figure 5.

The dashed lines show the length-tension curves obtained by plotting peak tension developed in twitches against the sarcomere length in the central region of intact rat papillary muscles at the peak of the twitch, as determined in two ways: by light diffraction methods4 (right dashed curve, Fig. 5A) and from photomicrographs of the striation pattern5 (left curves). The length-tension curves obtained in these two studies of the rat papillary muscle differ markedly, though there was good agreement over the sarcomere lengths observed in resting muscles at Lmax (Table 1). Julian and Sollins5 noted that when they calculated tension developed as DT (Fig. 4) their data closely resembled those obtained from frog muscle by Gordon et al.16 (dashed curve, Fig. 3), but Krueger and Pollack4 observed a much steeper decline of tension with muscle length and they pointed out that “peak active force decreased by 75% over the same range of sarcomere lengths...
in which force decreased by only 7% in truly isometric contractions of frog muscle. 37 Although the experimental conditions used by the two groups differed in various respects (e.g., 20°C in the former and 30°C in the latter), there is no obvious explanation for the difference in their experimental findings. However, this discrepancy must not be allowed to obscure the fact that the dashed curves fall well to the right of the solid curve in Figure 5A. In other words, tension falls off more steeply in the intact muscle than can be explained by the properties of the sliding filament system. The reason(s) for this could be that as the muscle length is reduced there is: (1) a reduction in the duration of the twitch; (2) increasing "deactivation" due to active shortening; (3) increasing extrinsic restoring forces; (4) decreasing activation of the contractile system. Each of these possibilities will be considered in turn.

1. Reduction in twitch duration. The time to peak tension is appreciably reduced at short muscle lengths,37 38 and it is possible that progressive curtailment of the rise of tension could account for the steep decline of peak tension with muscle length. In order to counter this argument, maximum rate of rise of tension was also measured in recent studies of the length-tension relation of cat papillary muscle.38 39 This is a complicated parameter to interpret because it must depend on both the rate of increase in the number of crossbridges and the kinetics of crossbridge movement,35 but it does have the advantage over peak tension that it is unaffected by changes in the time course of the twitch. The length-tension curves for peak tension and for maximum rate of rise of tension were found to be the same under a variety of experimental conditions,38 39 so shortening of the twitch per se is unlikely to be the explanation of the steepness of the length-tension relation.

2. "Deactivation" due to active shortening. It is a well documented property of both skeletal and cardiac muscle that shortening during a twitch accelerates the decay of mechanical activity.30 31 This is a quite different phenomenon from the "inactivation due to shortening" described by Taylor and Rüdel.22 Inactivation was recognized by the appearance of wavy myofibrils in the core of skeletal muscle fibers when they shortened to sarcomere lengths less than 1.6 /um in tetanic contractions and it is probably due to a failure either of inward propagation of the excitatory event along the transverse tubular system or of the calcium release mechanism when the muscle is stimulated at a sarcomere length less than about 1.6 /um.23-24 "Deactivation," on the other hand, is produced by active shortening of the muscle in a twitch: it is a property of both skeletal and cardiac muscle, but especially of the latter, and if it involves activation mechanisms at all it is more likely to be due to accelerated disappearance of calcium from the sarcoplasm (and therefore from troponin) than to impairment of the calcium release mechanism.41-42 However, a more likely explanation of this behavior seems to be that it is an inherent property of the crossbridge mechanism of tension generation.49

The possibility that deactivation due to shortening may have contributed to the steep decline in tension3, 5 shown by the dashed curves in Figure 5A has been ruled out quite conclusively by recent studies5 7 in which muscle pullers have been used to prevent shortening of the central region of the preparation during a contraction. Pollack and Krueger6 adopted Hill's ploy43 of preventing internal shortening by applying a carefully judged stretch to the muscle during the rise of tension: they used an exponentially increasing stretch and by adjusting its time of onset, time constant, and magnitude they were able to hold the sarcomere length in the central region of a rat papillary muscle constant to within 1-2% throughout most of the twitch. This allowed them to measure tension production under essentially isometric conditions at sarcomere lengths from about 2.3 /um down to slack length (1.9 /um, Table 1). Tension production at shorter sarcomere lengths was studied by allowing active shortening early in a contraction and then using the muscle puller to prevent further shortening of the sarcomeres during the remainder of the contraction. Julian et al.7 used the "length clamp" method developed by Gordon et al.44 to control the length of a region of the muscle between two externally applied markers. This allowed them to hold the length of the central region of a rabbit papillary muscle constant to within 1% throughout the entire twitch. When internal shortening was reduced to a minimum, the peak tension measured at Lmax by both groups was almost doubled, but it still fell off steeply at shorter sarcomere lengths. Julian et al.7 found that peak tension fell by over 60% when the sarcomere length was reduced from 2.3 /um to 2.0 /um in rabbit papillary muscle (dotted curve, Fig. 5B). In rat papillary muscle7 peak tension remained fairly constant from 2.3 /um down to 2.15 /um, but at shorter sarcomere lengths it fell linearly to reach zero at about 1.6 /um (dashed curve, Fig. 5B). Thus, as shown by the length-tension curves in Figure 5A and B, tension declined steeply whether or not there was internal shortening, so the steepness of the curves cannot be attributed to deactivation due to shortening of the active muscle.

3. Extrinsic restoring forces. The sarcolemma has been

Table 1 Sarcomere Lengths in Resting Rat Papillary Muscle

<table>
<thead>
<tr>
<th>Source</th>
<th>Material</th>
<th>Lmax (um)</th>
<th>Slack length (um)</th>
</tr>
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<tbody>
<tr>
<td>Fabiato and Fabiato</td>
<td>Skinned fibers</td>
<td>~</td>
<td>1.9</td>
</tr>
<tr>
<td>Krueger and Pollack</td>
<td>Intact muscle</td>
<td>2.22</td>
<td>1.93± 0.14*</td>
</tr>
<tr>
<td>Pollack and Krueger</td>
<td>Intact muscle</td>
<td>2.35± 0.06*</td>
<td>1.97†</td>
</tr>
<tr>
<td>Julian and Solins</td>
<td>Intact muscle</td>
<td>2.1-2.3</td>
<td>1.9-2.0</td>
</tr>
<tr>
<td>Grimm et al.</td>
<td>Fixed muscle</td>
<td>2.2-2.25‡</td>
<td>1.9†</td>
</tr>
</tbody>
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* Mean ± standard deviation.
† Estimate by reviewer.
‡ Estimate by Page27.
mentioned already as a possible source of extrinsic restoring forces in frog skeletal muscle fibers at sarcomere lengths below 1.6 μm. To account for the difference between the broken and solid curves in Figure 5, the sarcolemma would have to begin loading the tension-generating system at sarcomere lengths above 2.0 μm, but measurements of the slack length in rat papillary muscle give no indication of this (Table 1). Grimm et al.\(^3\) fixed resting muscles at various lengths after determining their length-tension curves, and measurements on the fixed material after correction for shrinkage\(^2\) showed that a minimum sarcomere length of 1.9 μm was reached at 85% \(L_{\text{max}}\), the length at which resting tension disappeared. Matching results were obtained from living muscle by Pollack and Krueger,\(^6\) who noted that “resting sarcomere length could not be brought below 1.9 to 2.0 μm no matter how much the muscle length was reduced.” Julian and Sollins,\(^2\) on the other hand, reported sarcomere lengths of 1.5-1.6 μm at slack length (75% \(L_{\text{max}}\)), but they obtained a value of 1.8-1.9 μm in their later work on rabbit papillary muscle.\(^1\) These measurements, taken together, indicate a sarcomere length in the slack muscle of about 1.9 μm, which was also the value obtained by Fabiato and Fabiato\(^3,4\) in skinned ventricular muscle fibers. It follows that extrinsic restoring forces cannot account for the difference between the solid and broken curves in Figure 5 at sarcomere lengths above 1.9 μm (because the restoring forces will be zero above slack length); however, a contribution from restoring forces at shorter sarcomere lengths cannot be excluded.\(^8\)

The cause of the resistance to passive shortening of cardiac muscle fibers below slack length is not clear at present. Attempts to shorten muscles passively to less than slack length resulted in waviness of the myofibrils and disappearance of the clear zone (H zone) in the central region of the sarcomeres.\(^2,3,4\) Because of the similarity of these findings to those in skeletal muscle,\(^28\) it seems likely that the resistance to shortening may be due to the thin filaments meeting in the middle of the sarcomere. Unfortunately there are no accurate data available for the thin filament length in rat ventricular muscle; however, it is worth noting that Page’s values\(^32\) for cat and frog heart muscle (thin filament lengths of 1.0-1.09 μm) are not consistent with this hypothesis.

4. Activation of the contractile system. By exclusion, decreasing activation of the contractile system is left as the most likely explanation for the decline in tension production in contractions at sarcomere lengths between 2.1-2.2 μm and 1.9 μm—the range over which a substantial fraction of the total decline occurs (Fig. 5). The relative importance of activation effects and extrinsic restoring forces at sarcomere lengths below 1.9 μm cannot be decided from studies of the kind reviewed so far. Possible mechanisms underlying length-dependence of activation will be discussed later.

Effects of Inotropic Interventions on the Length-Tension Relation

The cardiac muscle literature includes many studies of the effects on tension production of inotropic interventions and changes of muscle length, but only in recent years has it been recognized that length and inotropic state may not be independent regulators of tension production.\(^30,32,45-47\)

The approach used in recent work from our laboratory\(^30,32\) has been to obtain length-tension curves for a muscle in different inotropic states, to normalize these curves by expressing tension production as a percentage of that observed at \(L_{\text{max}}\), and then to test whether the normalized curves are superimposable. Figure 6 shows an example in which the inotropic state of a cat papillary muscle was altered by varying the calcium concentration in the bathing solution. If muscle length and inotropic state are independent regulators of tension production, then the normalized length-tension curves should be superimposable. Figure 6B shows that this was not the case when the inotropic state was varied by changing the bathing Ca\(^2+\) concentration, and similar shifts to the left in the normalized length-tension relation have been seen when muscles were potentiated by catecholamines,\(^49\) paired pulse stimu-
and increases in the rate of stimulation. Essentially identical results were obtained when maximum rate of rise of tension was used instead of peak tension as a measure of tension production. It should be noted, however, that these studies do suffer from the drawback that overall muscle length rather than sarcomere length in the central region served as the independent variable, and it remains to be seen whether similar results will be obtained when the effects of inotropic interventions on the length-tension relation are examined at the sarcomere level.

A shift to the left of the normalized length-tension curve as a result of potentiation of the muscle shows that the inotropic intervention was more effective at short muscle lengths than at L_{max}. The explanation which has been offered for this behavior is that the degree of activation of the contractile system produced by excitation of the muscle decreases progressively as the muscle length is reduced below L_{max}—in other words, activation is length-dependent. An alternative explanation is possible (at least in principle) on the basis of increasing restoring forces at short muscle lengths: i.e., if the tension observed at short muscle lengths is small because most of the tension generated is required to overcome extrinsic restoring forces, then the increased activation of the contractile system brought about as a result of an inotropic intervention will lead to a proportionately greater increase in the tension (measured externally) at short muscle lengths than it will at longer lengths where much less of the tension generated is used to oppose restoring forces.

This hypothesis in its simplest form can be tested as follows. If it is assumed that the difference between the decline of tension in a skinned fiber (solid curve, Fig. 5) and in the intact muscle is due entirely to restoring forces, then the magnitude of the latter can be calculated at each muscle length as shown by the inset diagram in Figure 7. When this is done for the data illustrated by Figure 6A, a different length-tension curve is obtained for the internal load at each Ca^{2+} concentration (Fig. 7). It is therefore necessary to postulate that the internal load varies with the inotropic state of the muscle, and this severely weakens the credibility of the restoring force hypothesis as the sole explanation of the shift in the length-tension relation shown in Figure 6B: however, a partial contribution cannot be excluded on the basis of the evidence available.

A further feature of the length-tension relation that is not easily explained by the restoring force hypothesis is its time-dependence. When the length of a cat papillary muscle is changed between one beat and the next, there is an immediate change in peak tension developed (AB', BA'; Fig. 8) followed by a further change in the same direction (B'B, A'A) that takes several minutes. If the muscle length is changed in a series of steps between consecutive beats, it is possible to obtain a series of points along paths AB' and BA'. This procedure gives a length-tension curve that is less steep than the steady state curve AB. Julian and Sollins obtained their length-tension data for rat papillary muscles in this way, whereas the procedure used by Krueger and Pollack would have given results closer to the steady state curve. If time-dependent changes in tension production are a marked feature of rat papillary muscle, then it is possible that this difference in experimental protocol may account for some of the difference between the length-tension curves shown by dashed lines in Figure 5A.

The essential point, however, in relation to the restoring force hypothesis is that time-dependence of the length-tension relation (and the fact that the time-dependence is influenced by the experimental conditions) cannot be reconciled with the concept of a purely passive internal load, but these properties of the muscle are compatible with the hypothesis that activation of the contractile system is influenced by muscle length.

**Length-Dependence of Activation: Possible Mechanisms**

If the degree of activation of the contractile system depends on the amount of calcium bound by troponin, then length-dependence of activation could be due to effects of muscle length on (1) the affinity of troponin for calcium, or (2) the rise in sarcoplasmic Ca^{2+} concentration brought about by excitation of the cell. A smaller rise in Ca^{2+} concentration at short muscle lengths could occur because less calcium is released (due to impairment of the release mechanism or to diminished loading of the store with calcium), or because the calcium that is released disappears more rapidly from the sarcoplasm. These possible mechanisms are identified by the numbers 1-4 in Figure 1. Other possibilities include length-dependence of the phosphorylation of myofibrillar proteins or of the

**Figure 7 Test of the restoring force hypothesis.** Each line shows the restoring force that would be required to account for the decline in tension production with muscle length shown by the equivalent length-tension curve in Figure 6A. The number alongside each curve indicates the bathing Ca^{2+} concentration (mM Ca^{2+}). The assumptions made in the calculation are (1) that in the absence of restoring forces tension production would have declined with muscle length as it does in skinned rat ventricular muscle fibers (Fig. 5), and (2) that the deviation of the observed length-tension curve from this expected curve is due only to the effects of restoring forces. The calculation is illustrated in the inset diagram, where restoring force (RF) equals expected tension minus observed tension (DT). According to the hypothesis, the three lines shown should be superimposed.
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control of actin by the troponin-tropomyosin system, but there is no evidence for either of these at the present time.

Endo has reported results for skinned skeletal muscle fibers that are consistent with an effect of muscle length on the affinity of troponin for calcium, but there is no comparable information available for cardiac muscle. There is also evidence for length-dependence of the rise in sarcoplasmic Ca\(^{2+}\) concentration in skeletal muscle fibers from experiments in which aequorin has been used as a calcium indicator, but technical difficulties have so far precluded the use of this method in cardiac muscle cells.

Direct observation of papillary muscles during contraction has given no indication that length-dependence of activation at sarcomere lengths below 2.1–2.0 μm results from a failure of excitation-contraction coupling in the core of the muscle fibers, as observed in skeletal muscle fibers at sarcomere lengths below about 1.6 μm. Julian and Sollins reported waviness in the striation pattern in greatly shortened muscles (sarcomere lengths of 1.4–1.5 μm), but they noted that there was very little if any active tension recorded at these lengths. It has also been pointed out that inward propagation of the excitatory events along the transverse tubular system is probably less crucial in excitation-contraction coupling in cardiac muscle, where the diameter of the fibers is about 1/10 of that in skeletal muscle. However, Fabiato and Fabiato have obtained evidence for length-dependence of the calcium release mechanism. They found that in skinned rat ventricular muscle fibers the length-tension curve for phasic contractions elicited by small changes in bathing Ca\(^{2+}\) concentration in a weakly (calcium) buffered solution lay well to the right of the curve obtained for tonic contractions (solid line, Fig. 5). They concluded from this and other experiments in which contractions were produced by using caffeine to release calcium from intracellular stores that “decreasing sarcomere length below optimum results in partial inhibition of the process of Ca\(^{2+}\)-triggered release of Ca\(^{2+}\) from the sarcoplasmic reticulum of cardiac muscle cells. This mechanism may contribute to the explanation of the Frank-Starling law of the heart.” This interpretation of their results does of course rely on the assumption that phasic contractions of the skinned fiber are a realistic model for twitches of the intact muscle.

A smaller rise in sarcoplasmic Ca\(^{2+}\) concentration when the muscle is stimulated at short lengths may reflect a diminished release of calcium, but it could also result from a more vigorous uptake by the sarcoplasmic reticulum or other sinks for calcium (e.g., mitochondria or extrusion from the cell). The decreases in time to peak tension and the overall duration of the twitch at short muscle lengths may possibly reflect an earlier return of the sarcoplasmic Ca\(^{2+}\) concentration to its resting level. However, it has already been argued that the identical results obtained when peak tension and maximum rate of rise of tension were used as indices of tension production show that curtailment of the twitch at short muscle lengths does not account for the steepness of the length-tension curve. Thus, while muscle length may influence the activity of calcium sinks, notably the sarcoplasmic reticulum, this does not appear to be a major factor responsible for length-dependence of activation.

The explanation that has been offered for the slow change in tension production that follows a change in muscle length (Fig. 8) is that there is length-dependence of the loading of the stores from which calcium is released by excitation of the cell. The magnitude and time course of this slow change were shown to be influenced by alterations in the bathing Ca\(^{2+}\) concentration, rate of stimulation, presence of verapamil (an inhibitor of transsarcomemal calcium fluxes), all of which would be expected to affect the loading of the store. Furthermore, length-dependent changes have been reported in the action potential that would be consistent with this hypothesis.

There are various detailed arguments that could be offered for and against each of the mechanisms proposed as possible explanations for length-dependence of activation in cardiac muscle, but this is an area where speculation is currently running well ahead of the experimental evidence. Because of the lack of suitable techniques or the special problems of working with cardiac muscle, most of the arguments depend rather heavily on circumstantial evidence, but that state of affairs should be rectified within the next few years as a result of further work with skinned fibers and the use of calcium indicators in studies of intact muscle cells. For the present the most likely explanation of the two phases of the increase in tension production after a sudden increase in muscle length appears to be the following: the immediate increase (AB', Fig. 8) is due mainly to an effect of length on the calcium release mechanism which allows the first action potential after the stretch to release more calcium than it would have at the shorter muscle length (mechanism 2, Fig. 1); the subsequent slow increase (BB', Fig. 8) is probably due to a progressive further increase in the amount of calcium released by each action potential as a result of increased loading of the store with calcium (mechanism 3, Fig. 1).
Conclusions

1. Length-dependence of activation accounts almost entirely for the dependence of tension production on muscle length over the ascending limb of the length-tension relation in isolated papillary muscles.

2. If the inotropic state of the muscle is equated with the degree of activation of the contractile system, then muscle length influences inotropic state and a change of muscle length must therefore be regarded as an inotropic intervention.

3. If these results are applicable to the intact heart, then diastolic volume and inotropic state cannot be regarded as independent regulators of cardiac output.

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