Mechanical Properties of Isolated Cardiac Myocytes

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I. INTRODUCTION

Viewed from the perspective of organ function, there has been a long-standing interest in the separability of cardiac muscle length or ventricular volume-dependent effects (i.e., the Frank-Starling relation) from intrinsic inotropic mechanisms in the cardiac contractile response. Furthermore, at the cellular level there is continued interest in the mechanisms of passive and active force generation in the sarcomere. In fact, the resolution of both levels of interest has been severely limited in studies over the past decades by the complexity of multicellular cardiac preparations and our inability to measure or control a minimal number of the contractile-related parameters. Actually, the results of many of these studies strongly question the separability of geometrical and intrinsic factors of cardiac contraction (12, 71, 97, 99), but the issues are by no means closed.

For example, attempts to deduce cross-bridge mechanical kinetics from multicellular preparations (including geometrically linear preparations such as trabeculae and papillary muscles) have led to ambiguous results because of the viscoelastic nature of these preparations (93) and because no clear representation of the relation between passive and active elements of the tissue is evident (20). On the one hand, for simplicity, resting mechanical properties of these preparations have been assumed to be in parallel with active properties. This arrangement has been represented as a Maxwell mechanical analogue. Then, again, series elasticity in excised preparations has been largely attributed to end compliance of the preparations that is related to the attachment of recording devices so that a Voigt analogue is suggested.

Finally, tests of these assumptions reveal a considerable complexity of stress and strain distributions at the sarcomere level, reflecting significant structural and functional nonuniformity in both passive and actively expressed responses. Because contractile function is strongly sarcomere length and cross-bridge shortening dependent, this inhomogeneity further reduces the reliability of interpretations of cross-bridge function based on these global or whole tissue measurements.

Added to these concerns is the growing evidence that some phase of the contractile activation process may be diminished at short sarcomere length or with the shortening necessary to achieve sarcomere lengths below unattached rest length. Hence it becomes even more critical to know or to control sarcomere length at the sarcomere level.

On first impression, the isolated heart cell would seem to be ideal for mechanical studies. A preparation free of collagen and containing relatively few myofibrils in a single cohesive structure would allow almost direct access to the measurement of sarcomere length and cross-bridge contractile responses. At the present time, however, our ability to isolate a single heart cell for mechanical studies has simply reduced our problem from a state of megadimensional ambiguity to one of mere multidimensional ambiguity. Also, counterbalancing the structural simplification of the preparation are the inherent challenges of noninjurious cell attachment, the recording of microgram levels of force measurement, and individual sarcomere resolution. These challenges have kept our progress in studies of cardiac myocyte contraction at a slow pace; however, alternative mechanical measurements, such as shortening of unattached cells, auxotonic responses of cells attached to relatively compliant force transducers, stiffness measurements, steady-state activation of skinned cells, and labeled antibody staining of cytoskeletal and interme-
diately filaments, are leading to more detailed concepts of the contractile process in cardiac muscle. These developments are discussed in some detail in this review.

II. DEVELOPMENT OF ADULT MYOCYTE PREPARATION

During the past 20 years numerous attempts have been made, using several experimental approaches, to develop an excised single cardiac myocyte preparation that would tolerate normal Ca\(^{2+}\) concentrations and that could be used to evaluate electrical, metabolic, and contractile function at the cellular level. The vast literature on the progress of this development has been extensively reviewed by Dow et al. (28, 29), and recent methods of myocyte isolation have been evaluated by Powell (100).

During this period the isolation procedures have been modified such that myocytes can be isolated that remain quiescent and relaxed in normal Ca\(^{2+}\)-containing perfusates and are electrically excitable. As noted by Haworth et al. (47), a primary step in producing Ca\(^{2+}\)-tolerant cardiac myocytes was the recognition that a critical level of Ca\(^{2+}\) must be present in the digestion medium (~20 \(\mu M\)) for the first few minutes of coronary perfusion in order for the enzymes to separate the cells and break up the extracellular collagen. The addition of a variety of substrates to assist the cells in handling the Ca\(^{2+}\) loading that occurs with the isolation procedure also seems to be beneficial (7, 47, 84, 96, 125, 144).

Cell separation at the intercalated disks appears to be readily effected by collagenase so that the principal injury to the cells probably occurs in the separation of the cells from each other as they are freed from the collagen matrix. Gap junctional areas of the nexes may be particularly sensitive to mechanical damage, but electron micrographs of isolated cells show many intact gap junctions often with some adhering residue of an adjacent cell (38, 82, 85). The large number of cells that can be isolated from ventricular tissues [total yields of 65–79% in 1 mM Ca\(^{2+}\) (143) and up to 95% in 1.8 mM Ca\(^{2+}\) (8)] indicates that cells from which a gap junction has been removed can reseal the injured area (85) and that, as reported by Severs et al. (118), most gap junctions are internalized by endocytosis soon after cell isolation.

These preparations have been the subject of a wide variety of successful studies of cardiac cellular physiology, except that the cells, largely devoid of collagen, are extremely sensitive to external mechanical stress. These persisting attachment problems have resulted in an increasing number of studies that have utilized shortening in unattached cells as a measure of contractility.

III. MECHANICAL PROPERTIES OF CARDIAC MYOCYTES

One of the major objectives in studying the mechanical properties of the isolated cardiac myocyte, as in studies of whole cardiac tissue, is to deduce cross-bridge kinetics as a means of understanding the contractile process. However, our access to the cross bridges is still indirect. For example, to measure myocyte force or stiffness we must make some attachment to the cell, and this attachment may produce distorting strains or stresses that influence the contractile response. Also, to resolve the small forces developed by the isolated myocyte, the compliance of the force-sensing system may be appreciable relative to the length of the cell. Furthermore, the myocytes possess passive viscoelastic properties, the orientation of which with respect to the cross bridges is still unclear, so that the way in which these passive elements are represented in relation to the active contraction (series or parallel combinations) influences our interpretation of cross-bridge activity. On the other hand, when shortening in the unattached myocyte is measured, what is obtained is a measure of the maximum turnover rate of the cross bridges, but internal viscous loading and loading by longitudinal and radial elastic elements as well as external cell orienting or stabilizing forces against which the cycling cross bridges are working may not be well defined. In addition, the consequences of shortening on cross-bridge activation are only beginning to be understood. These problems leave our interpretation of cross-bridge dynamics in active cells still rather clouded.

It should be emphasized, however, that the variety of force-, stiffness-, and length-measuring techniques that have been utilized in isolated myocyte preparations in recent years has contributed substantially to our understanding of the myocardial contractile process. However, not unexpectedly, these studies have also extended the scope of parameters that must be dealt with in achieving this goal. The following discussion of these data attempts to put the definitive and ambiguous aspects of these studies in perspective.

A. Attachment Procedures

The major limitation in attachment to intact cells is that the sarcolemma, without the protective collagen, is extremely sensitive to applied stress. A variety of cements have been tried to bond the cell to the stylus of mechanical transducers. Also, the cells will adhere to poly-L-lysine-coated surfaces to some extent, but cell damage or separation often occurs before maximum contractile activation occurs. Tarr et al. (131), using isolated frog atrial cells, and Shepherd and Kavaler (121), using single guinea pig ventricular myocytes, have had success with this coating procedure. The studies with frog atrial cells took advantage of the extreme length of frog atrial cells (200–300 \(\mu m\)) such that they were able to wrap the ends of these narrow (~5 \(\mu m\)) myocytes around poly-L-lysine-coated glass supporting beams. The calibrated deflection of one beam served as the force transducer. Intact cells survived this procedure well and remained quiescent and electrically excitable. The major disadvantage of this method was the neces-
sity to construct the glass beams such that a displacement of ~5% of cell length was necessary to obtain adequate resolution of the force responses. In the guinea pig study the cell was simply placed on and thus adhered to the coated surface of the two supporting glass beams. Sarcomere uniformity in these cells has not been reported.

Silicone rubber cements are useful, but a uniform stress distribution with attachment to the cell is difficult. A fibrin attachment procedure was reported to be successful by Copelas et al. (24). The compliance of the fibrin seemed to be tolerable (1 μm/mg), but the method has not been widely used.

Brady et al. (19) attached isolated cardiac myocytes of rats to a force transducer with single-barreled suction micropipettes and recorded electrically stimulated twitch tension as a function of cell length, external Ca^{2+} variations, and stimulus interval variations. They measured peak twitch forces of ~4 μN, but the success rate of such recordings was extremely low because of the high stress on the sarcolemma at the tips of the micropipettes.

Among the more promising attachment methods is a concentric double-barreled micropipette in which the inner pipette is recessed such that approximately four to five sarcomeres of each end of the cell can be drawn in against the inner pipette with mild suction. The glass surfaces are treated with a thin coat of a prototype barrelcement (Biopolymers) that provides sufficient adhesion to support full Ca^{2+}-activated force development in detergent-skinned myocytes. This attachment applies more of the supporting stress to the intercalated disk surface where stress transfer normally occurs (17). This technique has not yet solved the sarcolemmal stress problem with intact myocytes perfused with normal levels of extracellular Ca^{2+}. However, this method works well with detergent-skinned preparations and will support forces of maximal activation with sarcomere strain distribution only 1-2% in excess of that of unattached myocytes (K. P. Roos and A. J. Brady, unpublished observations).

A major problem with attachment to highly sensitive force transducers is that in cases where the force detection system is not submersible, some part of the force-sensing system must pass through a liquid-air interface. The surface tension of the meniscus around the connecting element through this interface can be of ~10 μN, whereas the myocyte forces of interest may be one to two orders of magnitude below this level. Thus extreme surface stability and reproducibility of the perfusate surface are required to measure myocyte forces with these transducers. Iwazumi (59) developed a flow controller system in which the chamber solution surface is monitored electronically and flow adjusted by means of servo-controlled solenoids to maintain a constant and reproducible solution level. This device, in conjunction with a novel electromagnetic force transducer, made possible the stable recording of tension from single myofibrils.

### B. Passive Mechanical Properties

#### 1. Comparative elastic properties of skeletal and cardiac cells

In attempts to determine whether the relatively high resting stiffness of cardiac muscle has an intracellular component, a number of studies have compared the stiffness of isolated cellular preparations of cardiac and skeletal muscle.

Thus comparing the elastic properties of isolated cells of the hamster gracilis and ventricular myocytes at sarcomere lengths 2.20 and 2.6 μm, Fish et al. (36) showed that resting stress values were three to four times greater in the cardiac cells at each sarcomere length. Also, an exponential dependence of stress on sarcomere length was found in which the slope of the natural log stress-sarcomere length relation in the cardiac and skeletal muscle cells was 7.48 and 5.77 mN/μm², respectively (where strain was measured as the relative displacement from control length, \(L - L_0/L_0\)). These values were significantly different at the 3% level. Thus passive mammalian isolated cardiac muscle cell stress is severalfold greater than skeletal muscle cells at similar lengths.

These data are supported by the higher cytoskeleton protein content of cardiac cells; for example, Maruyama et al. (78) report that 18% of the total myofibrillar protein is connectin in bovine heart, which is threefold greater than in skeletal muscle. Price (101) found 2% desmin, also in bovine heart, compared with a report of 0.35% in skeletal muscle (90). In a study of frog (Rana catesbeiana) glycerinated semitendinosus and atrial preparations in which the contractile filaments had been extracted in 0.6 M KCl, Matsubara and Maruyama (81) found that further treatment of the preparations with 1% NaOH or 2% sodium dodecyl sulfate to remove the remaining striated structures reduced the semitendinosus resting tension to ~20%, but the atrial preparation retained >50% of its resting tension at a sarcomere length of ~2.75 μm. They concluded that because the primary structure remaining after these treatments was probably a network of the large cytoskeletal filament connectin, the higher content of connectin in heart muscle may be responsible for the difference in resting tension.

#### 2. Relation of cellular to tissue elasticity in cardiac muscle

In studies of the passive mechanical properties of single isolated frog (R. catesbeiana) atrial fibers in which the cells were attached by wrapping around supporting glass rods, Tarr et al. (131) found that these cells had resting stresses 8- to 30-fold less than intact frog atrial trabeculae and could be readily extended to sarcomere lengths of 3.45 μm. In contrast, the passive force recordings in isolated mammalian cardiac myocytes (19,
32, 35) indicate that substantial resting tension is apparent at sarcomere lengths of 2.0-2.2 μm, and the myocytes become extremely stiff at sarcomere lengths of 2.4-2.6 μm (18, 35, 36). Nag and Zak (85) report that some collagen fibrils may be attached to enzymatically dissociated rat cardiac myocytes and might be responsible for some of the resting tension. However, other studies showed that these enzymatically dissociated cells are nearly free of collagen (18, 104) so that, in contrast to frog atrial tissue, the high resting tension of mammalian heart muscle must be, at least in part, a characteristic of the cardiac cell ultrastructure.

The relative contribution of passive cellular elasticity to total tissue elasticity is of interest both in terms of resistance to stretch and resistance to shortening. In the latter case a restoring force would be established that may be responsible for some of the steepness of the active length-tension relation and for the rapid restoration of initial length in cells shortened below their unattached rest length (sarcomere length ~ 1.85-1.93 μm) (see sect. 1V.B). With regard to a resistance to stretch, Kentish et al. (62) measured the passive length-tension relation in intact and detergent-skinned rat trabeculae and found a substantial reduction in resting tension after 30 min of perfusion with 1% Triton X-100. In the skinned preparation at sarcomere length of 2.20 μm, resting stress was ~40% of that of the intact preparation. These observations suggest that dissolution of the sarcolemma in detergent may isolate the intracellular elastic elements from the extracellular stroma.

Comparing the length dependence of passive stress and stiffness of intact papillary muscle and trabeculae with excised single cardiac myocytes, Brady et al. (20) and Brady (16) found that at sarcomere lengths of ~1.9 μm the passive stress in trabecule and collagenase-isolated myocytes was similar. On the other hand, the slope of the natural log stiffness-length relation in trabeculae and papillary muscles was three to five times greater than in the excised myocytes. Similar conclusions were expressed by Fish et al. (36). In whole muscle preparations, Krueger (65) and Krueger and Toujioka (70) report two phases of the resting tension-sarcomere length relation with a steep component beginning around a sarcomere length of 2.6 μm. They attribute much of the more compliant component below a sarcomere length of 2.69 μm to intrinsic cellular elements and the steeper portion to extracellular elements.

These results suggest that the major component of passive stress in whole tissue is probably borne by the extracellular collagen at the longer sarcomere lengths (65, 105). However, extrapolating these relations to sarcomere lengths < 1.9 μm suggests that at the shorter lengths a compressive stress or stiffness of the myocytes may become a significant or even the dominant factor in total tissue elasticity, particularly if the elements of the strutlike structure of the extracellular collagen matrix go slack below unstressed muscle length (13). This possibility is suggested by the fact that reextension after active shortening in isolated myocytes is as rapid or more rapid than in multicellular preparations (64, 66, 87, 104, 111, 113). Krueger (66) emphasizes the fact that myocyte relengthening after active shortening occurs in a fast and a slower phase. The relative contributions of elastic recoil and cross-bridge detachment processes to these phases remain unclear, but as Krueger (66) points out, the magnitude of the restoring forces is sufficient to influence ventricular filling in the whole heart by a sucking action during early diastole.

3. Structural basis of cardiac myocyte resting tension

The relatively high resting stiffness of the cardiac cell has led to correlative mechanical and ultrastructural studies aimed at the identification of the cellular structures responsible for the high resting tension. Brady (16) and Brady and Farnsworth (18) found that the stiffness at a sarcomere length of 2.0 μm measured with 5-Hz sinusoidal length perturbation (and at 70 Hz (unpublished observations)) was relatively unchanged with membrane skinning in 1% Triton X-100 detergent but fell to ~20% of control after nearly complete A-band extraction in 0.47 M KCl (pH = 6.2). In 0.6 M KI, after I-band filament extraction, stiffness fell to ~10% of the intact value. These data indicate that the A-band filaments are probably directly involved in bearing resting stress in cardiac muscle.

Similar myosin disassembly studies in single skeletal muscle fibers (48, 49, 58) also gave results implicating A-band filaments in the support of resting stress. A recent study in detergent-skinned cardiac myocytes (110) showed that in attached cells stiffness declined as the A band was extracted in high salt, whereas in unattached cells, cell length shortened with A-band extraction. The studies with unattached cells indicate that in the intact cell some longitudinal elements of the cell must be normally stressed at rest length and that this stress is relieved as the A-band filaments are disassociated, i.e., the cell shortens as the A band disassembles.

The high-salt filament-extraction data strongly indicate that longitudinal elements, related to the integrity of the A band, are responsible for a major component of the relatively high resting tension characteristic of cardiac muscle. These data also point to differences in passive cytoskeletal elastic structure rather than membranous elements or contractile filament interactions, since all the membranous systems were disrupted in detergent and perfusate free Ca²⁺ was <10⁻⁸ M [2-10 mM ethylene glycol-bis(β-aminoethy] ether)-N,N',N"-tetraacetic acid (EGTA)-Ca²⁺ buffer], and the ATP concentration was buffered in the millimolar range.

Among the more exciting candidates for this stress-bearing element is titin (referred to as connectin in some studies). For example, fluorescent labeling of titin and immunolabeling of antibodies to titin epitopes indicate that this megadalton filament (molecular mass = 2.4-2.6 MDa) (44) is distributed between the M and Z lines in both skeletal and cardiac muscles and thus is in a position to bear resting tension (65, 78, 80, 86, 139-143). Wang (140) reports that 10-15% of the skele-
The possible contribution of microtubules to cardiac resting tension (116) is more difficult to assess, since the development of this tubular system appears to parallel sarcomereogenesis but tends to decline with the maturation in adult cells. Cartwright and Goldstein (23) report that microtubules run helically around myofibrils in rat neonatal soleus muscle. The number of microtubules was maximal (1 microtubule/μm²) at day 5 but declined to 0.36 microtubules/μm² cross section in adults. Colchicine disrupted microtubules, which correlated with the loss of organization of the myofibrils in myoblasts and regenerating skeletal muscle. Goldstein and Entman (40) found 0.2 microtubules/μm² in dog and guinea pig papillary muscle, but in functional cells of the adult rat heart, Samuel et al. (116) found tubulin to be only 10 μg/100 mg (0.01%) of total protein. Furthermore, the reorganization of tubulin distribution in response to L-thyroxine was not blocked by colchicine in the cardiac myocytes (115). These data indicate that although the microtubules may contribute significantly to the passive mechanical properties of cardiac cells during myofibrillogenesis, they probably play a minor role in this regard in normal adult myocytes.

Other candidates for resting cell stress in intact cells where shortening increases cell diameter are the radially stressed cytoskeletal filaments. For example, at the Z line there are reports of desmin and vimentin (43, 103, 138), vinculin (91, 119), filamin (63), and spectrin (83) and at the M line, skelemins (102). These elements may be stressed as the cell changes length at constant volume or where the contractile filament lattice spacing is altered. Some evaluation of the relative magnitude of this radial component is indicated in the work of Roos and co-workers (107, 111) in which they report that in unattached myocytes placed in varied osmotic strength media the cells decreased in both length and width in hypertonic media. On the other hand, in hypotonic solutions, cell length increased little above its control value (sarcomere length = 1.83–1.93 μm) while most of the volume increase occurred by an increase in diameter.

Krueger (66) evaluated the distribution of stress in cardiac myocytes by recording the sarcomere patterns in propagating asynchronous contractions. Krueger noted that, in the traveling wave, a section of 10–15 sarcomeres ahead of the traveling wave underwent a pre-lengthening of ~0.1 μm. From these patterns and passive stiffness data from hamster (36) and rat (16) myocytes, Krueger calculated that the passive longitudinal component of restoring force in the contracted regions was ~0.5 mN/mm².

This evidence indicates that both radial- and longitudinal-oriented intracellular elastic structures play a role in setting the resting sarcomere length and that the elastic moduli of these radial and longitudinal components are likely rather different. The distributions of cytoskeletal filaments are the probable bases of these elastic elements, and since these distributions are complex, it becomes extremely important to know their functional relation to the contractile filaments to interpret active force developments and shortening in terms of cross-bridge dynamics and Ca²⁺ activation.

4. Sarcomere uniformity

1) Unattached Cells. The distribution of sarcomere patterns in isolated cardiac myocytes has been assessed in intact unattached cells at rest (108, 109, 112), in varied osmotic and ionic stresses (107), during isotonic shortening in electrically initiated contraction [26, 67, 87, 111, 113, 114 (the latter two at image rates of 50–500/ s)], and under voltage-clamp conditions (75). A remarkable degree of regional sarcomere uniformity is noted in these unattached cells both at rest in modified media and during active isotonic contraction. At rest in unattached myocytes the major sarcomere length deviation is noted near the nuclei, resulting in an overall sarcomere length variation of ±6% (108). In the electrically stimulated unattached cells (113, 114), both the general uniformity and regional differences were maintained
during active shortening but the dispersion became greater during relaxation due to regional variations in the onset of relaxation. Using laser diffraction and video imaging, Krueger et al. (67) reported a similar uniformity of shortening and of relaxation as well. They did note, however, that although no buckling of myofibrils was evident at short sarcomere lengths (<1.5 μm), the first-order diffraction band did not go to zero as expected at these lengths as the Z bands approached the A band. Using computerized enhancement of charge-coupled device (CCD) linear-array images, Roos et al. (111) also noted clear imaging of sarcomeres at sarcomere lengths < 1.6 μm. At this point it is unclear why both the laser diffraction and the CCD imaging resolved I bands at a sarcomere length where the Z line would be expected to be firmly against the thick filaments. Krueger and London (68) suggest that the cross bridges at the end of the thick filament might add 0.29 μm of variability to the A-band length by way of being folded back on the thick filament stalk at short sarcomere lengths. For example, in the extended muscle the end cross bridges would appear more similar to I-band densities because of the absence of the thick filament stalk. In effect, then, as far as shortening is concerned, the A bands would appear to be only 1.4 μm long rather than 1.6 μm. Roos et al. (111) also reported sarcomeres visible by CCD imaging down to a length of 1.4 μm.

Another explanation might involve A-band shortening as suggested by Pollack (98) and recently shown in skeletal muscle (92). They report that in active shortening of toe muscles (lumbricalis digitorum IV) of the frog (R. temporaria) the A bands shorten by 15-20% at sarcomere lengths less than L0. It is interesting that in their electron micrographs of these shortened fibers both the M line and the H bands become obscured. Whether this loss of filament definition may be related to an alteration in cytoskeletal filament distribution (e.g., titin, connectin) has not been established.

II) ATTACHED CELLS. In stressed passive cells attached with single-barreled micropipettes and in myocytes impaled with microtools, stress distribution is highly nonuniform near the points of attachment, often with substantial skewing of the sarcomere pattern across the width of the cell. In CCD imaging of single-barreled micropipette-supported preparations, Roos (personal communication) reports that a stretched cell with a mean sarcomere length of 2.47 μm had a range of sarcomere lengths of 1.7-2.8 μm, a standard deviation (SD) > 8%, and a median skewed toward 2.6 μm (the number of sarcomeres measured was ~500). This deviation compares to an SD of ~6% in the unattached cell. In a cell at a mean sarcomere length = 2.23 μm, SD was >8%, sarcomere length range was 1.7-2.6 μm, and the median was 2.2 μm. In this cell, axial skewing was relatively minor, but sarcomere misalignments across the width of the cell (radial skewing) spanned the entire length of the cell.

In these modes of attachment, uniform radial support is poor so that the isolated cardiac myocyte offers little advantage over multicellular preparations in terms of control of sarcomere length. On the other hand, attachment to detergent-skinned myocytes with double-barreled micropipettes (18) distributes the applied stress much more evenly over the face of the intercalated disk. In preliminary studies in skinned myocytes, sarcomere length SD values were ±6-7%, with little radial or axial skewing at cell extensions 90% above rest length compared with a SD of ±5-6% in unattached cells (1,000-2,000 sarcomeres) (K. P. Roos, personal communication).

In any case, these observations indicate that in order to take advantage of the greater structural simplicity of single isolated heart cells in mechanical studies, appropriate attachment procedures must be employed to maintain the inherent sarcomere uniformity of the myocytes.

C. Contractile Responses in Active Cardiac Myocytes

1. Force measurements in skinned myocytes

Early studies of Ca2+ activation of isolated cardiac myocytes made use of the fact that skinned myocytes adhere well to glass microtools. For example, Bloom (9, 10) and Bloom et al. (11) excised small clusters of cardiac cells (3-5 cells wide by 2-3 cells long) from the mouse ventricle by mechanical disaggregation and, in the latter work, measured contractile force by means of microtools impaling the preparations. These preparations were, in effect, skinned, and they responded with spontaneous contractions in micromolar levels of EGTA-buffered Ca2+ perfusates. The sarcotubular network appeared to be intact as evidenced by a contracture response to 1 mM caffeine. Spontaneous irregular active contractions occurred in most preparations, and their amplitude increased with perfusate Ca2+ in the range of 1-10 μM. Maximal steady-state contracture responses were ~4 μN, which is similar to tonic responses (3-4 μN) in mechanically skinned cells (35). Sarcomere length and uniformity were not reported in these studies.

De Clerck et al. (25) measured force and shortening in mechanically skinned rat cardiac myocytes also using a microtool attachment. The cell did not tolerate normal Ca2+ levels, but contraction could be initiated by ionophoretically released Ca2+ from an extracellular pipette. Loads could be electronically clamped at predetermined levels with a servo-controlled system, but a shortening of ~5 μm occurred during the clamping process. The contractile responses were limited by diffusion from the pipette, but "isometric" contractile forces also of ~4 μN and maximum unloaded shortening velocity averaged only 0.35 cell lengths/s. The resulting force-velocity relations resembled those of intact cardiac tissue but were too irregular to deduce the basic nature of the relation, possibly due to the nonuniformity of activation by the electrophoretic procedure.

In studies of contractile responses in mechanically skinned rat ventricular cells, Fabiato and Fabiato (35)
found a damped series of contractile oscillations (0.1–1 Hz) occurred in partially activated myocytes in a critical range of Ca\(^{2+}\) concentrations (pCa 6.4–5.7) and at sarcomere lengths < 3.10 \(\mu m\). These oscillations appeared on the rising phase of the contracture between 50–100% of the final tension response. They characterized these oscillations as local stress-equilibrating adjustments of the force-sarcomere relations of the sarcomeres such that longer sarcomeres stretch shorter or weaker sarcomeres in series and thus increase the weaker activation levels, whereas the stronger sarcomeres reduce their force by shortening. On the basis of a viscoelastic model of these localized oscillations, they calculate a sarcomere maximum velocity of \(\sim 10 \mu m/s\) (4–5 sarcomere lengths/s), which is similar to that of intact cardiac muscle (95) and that later reported in unattached cells (\(\sim 9 \mu m/s\)) by Krueger et al. (67), as well as 3.42 cell lengths/s reported by Roos et al. (109) and 3.6 \(\mu m/s\) reported in cat myocytes (61). These damped oscillations during the rise of tonic activation are distinct from the oscillations that occur at much lower Ca\(^{2+}\) (pCa = 7.4) (33) that are attributable to Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the sarcotubular network.

These studies show that the tonically activated skinned cardiac myocyte is capable of force development and shortening comparable to twitch responses in intact tissue.

2. Active force development in intact cells

In studies of isolated intact frog atrial myocytes (\textit{R. catesbeiana}) (127, 131), contractile force was determined from the deflection of a force beam measured with a video system and a calibration of the beam compliance. This attachment procedure was possible because the cells are 200–300 \(\mu m\) long; however, only about a 50 \(\mu m\) segment of the cell was used for sarcomere length measurements. The cells averaged \(\sim 5 \mu m\) wide and thus contain only a few myofibrils in cross section. The cells were electrically excitable so that twitch responses could be recorded along with a video record of sarcomere length. They found that frog intact atrial cells develop twitch contractile forces of \(\sim 150–250\) nN, depending on the compliance of the supporting beams (132). Force beam compliance ranged from 0.028 to 0.128 \(nm/nN\), resulting in sarcomere length changes of 10–20% for contractions of this magnitude (128, 132). Contractile strength and shortening velocity continuously increased with length even to sarcomere lengths > 3 \(\mu m\) (129). The strength of the twitch and the shortening velocity could also be graded by both the amplitude and the duration of the stimulus current, indicating a graded release of activating Ca\(^{2+}\) in the cell (130). With the recording of contractile responses after a variety of quick releases, auxotonic force-sarcomere length relations could be measured so that auxotonic force-velocity relations could be deduced (129, 130).

At an initial length of 3.0–3.2 \(\mu m\), these cells shortened at nearly constant velocity to a sarcomere length of \(\sim 1.6 \mu m\). Earlier, Manring et al. (76) and later Nasser et al. (87) found similar shortening characteristics in both trabeculae and rabbit myocytes and referred to heart muscle in this regard as a “shortening machine.” These data were explained as resulting from the balance of the time-dependent rise in contractile activation of the cell and the shortening-dependent deactivation of the contractile process (12, 15, 31).

Using a similar frog atrial myocyte preparation and a high-velocity perfusion technique, Shepherd and Kavalier (121) found contractile force and shortening in sarcomeres to respond maximally to step changes in Na\(^+\), Ca\(^{2+}\), and caffeine in 800 ms and concluded that there is no evidence for Ca\(^{2+}\)-induced Ca\(^{2+}\) release in frog atrial cells, i.e., that the Ca\(^{2+}\) source must be external and that caffeine must have a sarcolemmal action because its augmentation of contraction is as fast as the contractile effects of Na\(^+\) and Ca\(^{2+}\) changes.

In an isolated guinea pig myocyte preparation (120), a cell was stimulated with a patch-clamp electrode. The measured contractile forces at 35\(^\circ\)C averaged 5.3 mN/mm\(^2\) (1.2 \(\mu N\)) at a sarcomere length of 1.81–1.88 \(\mu m\) with shortening up to 8% in the central region of the cell. No measurement of sarcomere uniformity was reported in these experiments. In the measurements of shortening in unattached cells, using a Sr\(^{2+}\)-perfusate (122), they concluded that, in contrast to the frog atrial myocytes, activator Ca\(^{2+}\) must come from internal stores and that the sarcotubular network Ca\(^{2+}\) release requires Ca\(^{2+}\) entry through channels.

If these measurements are put in perspective with respect to multicellular preparations, then the contractile forces reported for the isolated guinea pig myocytes are somewhat disappointing. For example, Yeatman et al. (146) reported a stress of 42 mN/mm\(^2\) in papillary muscle at 37\(^\circ\)C. They reported a Q\(_{10}\) of \(\sim 0.9\) so that at 35\(^\circ\)C the papillary force would be 43 mN/mm\(^2\). Thus the guinea pig myocyte (122) generated forces only 12% (5.3 mN/mm\(^2\)) of those of intact tissue, not compensating for extracellular space. If the extracellular space is assumed to be 20% of tissue volume, then the active force is only 10% (54 mN/mm\(^2\)). In the guinea pig myocyte study, the beam compliance was 0.88 mN/\(\mu m\), giving a beam deflection of 0.62–2.9 \(\mu m\) or 1.0–4.5% of the 65-\(\mu m\) cell length. The highest recorded force was 3.3 mN in contracture (120), which is comparable to the twitch tension recorded in isolated rat myocytes at room temperature by Brady et al. (19). Although the cells did not have an appreciable preload (sarcomere length \(\sim 1.8 \mu m\)), it is doubtful that the small twitch contractions in the guinea pig preparation were entirely due to the short initial length and the high compliance of the supporting beams. Another factor may relate to a low Ca\(^{2+}\) load in the short cell. For example, Nichols (88) found that in cat papillary muscle at 30\(^\circ\)C, changes in diastolic length affected subsequent twitch tension. Because stretch enhanced subsequent twitches more than release, Nichols suggested that the diastolic length phenomenon may be related to the surface-to-volume ratio of the cells and that ionic exchange sites may be more...
accessible in the stretched cell. Thus excised cells may be less Ca\(^{2+}\) loaded than intact preparations, perhaps because they tend to be shorter after being freed from the collagen matrix. Another concern is that the low contractility may be indicative of a major nonuniformity of sarcomere dimensions during active contraction.

These observations indicate that there are still considerable reservations concerning the functional state of attached isolated cardiac myocytes.

3. Displacement recordings in unattached stimulated cells

The measurement of shortening in electrically stimulated isolated cardiac myocytes has been successful, with considerable new information regarding relations between cell function and metabolism. A wide variety of methods have been devised to record myocyte contractions, including 1) direct cell transmittance changes (32), 2) video recording of cell motion (32, 45, 46, 57, 68, 87), 3) laser diffraction (61, 89, 117, 145), 4) phase-lock detectors (26, 27, 64), 5) the more complex CCD and video imaging systems (109, 112), and 6) edge detectors in which the motion of a contracting isolated cell can be measured in a variety of contractile states (22, 37, 72-75, 123). The edge-detection methods rely on some adhesion of the isolated cell to a stable surface or a perfusion micropipette so that a constant reference of cell position is obtainable. Because sarcomere length uniformity appears to be well maintained during shortening in unattached myocytes, the problem of sarcomere length uniformity is of less concern than in attached preparations. On the other hand, the load, both internal and external, against which attached cells contract in all of these methods is not known. Thus even though shortening can be graded by the stimulus and environmental factors in these preparations, without a knowledge of the loading of the unattached cells the interpretation of the contractile state of these cells in relation to activating Ca\(^{2+}\) may be ambiguous or, at least, may require the measurement of a number of parameters to deduce the basis of changes in contractility. These concerns are outlined next.

IV. INDEXES OF CONTRACTILITY

A complete description of the mechanical component of the contractile process with which to relate the underlying biochemical processes requires measurements of force, stiffness, and shortening characteristics under a variety of loading and perturbational conditions. With the currently available techniques there is a limitation in the extent of these parameters that can be monitored. Problems with attachment of isolated cardiac myocytes to recording and control devices puts a constraint on any characterization of the myocardial contractile process such that the most extensive data at hand have come from shortening responses. The limited information that is available regarding force development indicates that isolated cardiac myocytes retain at least some of the relatively high passive stiffness characteristic of cardiac muscle and that they are capable of active stress generation, both in tonic and in twitch responses, comparable to intact preparations. However, the sensitivity of the collagen-free sarcolemmal membrane to applied stress and the absence of adequate cell attachment methods that maintain the inherent sarcomere uniformity during stress development leave little more than a few, more or less, auxotonic force recordings under very limited experimental conditions. Certainly a sarcomeric force-velocity relation that is necessary to relate the contractile process to biochemical kinetics cannot be characterized. The recording of isometric and afterloaded force responses and the establishment of the functional relation between active and passive forces as a function of sarcomere length remain among the most important experimental challenges.

In the face of such limitations in contractile force measurement, a great deal of progress has been made in quantifying the shortening responses of the isolated myocytes under a wide variety of conditions. The more opportune challenge, then, is to understand the intrinsic factors that control myocyte shortening in terms of externally applied parameters, such as Ca\(^{2+}\) levels, stimulus patterns, and pharmacological agents. Some of the advances and concerns related to the measurement of shortening in unattached cells are discussed in the next section.

A. Shortening as an Index of Contractility

The active shortening response of an unattached myocyte reveals the maximum degree of shortening and the shortening velocity of the minimally loaded cell manifested under the conditions extant in the internal and external environment of the cell. Both myocyte shortening and shortening velocity are readily measurable with current techniques. Thus, to the extent that these two parameters are indicative of the state of the contractile process, there is a measure of functional parameters that can be modified by experimental conditions. The parallels that have been demonstrated between myocyte shortening responses and those of intact tissue confirm that the isolated myocyte is a useful model of cardiac tissue. These features have been recently reviewed by Allen and Kentish (1).

A number of major concerns need emphasis in the use of unattached cell responses as an index of contractility and, in particular, in the use of this index to infer cytosolic and sarcocytolic network Ca\(^{2+}\). In this regard, Krueger (66) emphasizes the point that myocyte shortening and relengthening reflect different components of internal loading and contractile processes so that a measure of maximum shortening alone may not be a sufficient measure of contractility. Krueger indi-
cates that the ratio of the rates of shortening to re-
lengthening provides additional information that may help to differentiate between passive and active compo-
nents of the shortening event. For example, interven-
tions that increase the elastic loading of the shortening
cell, e.g., compression of cross bridges or increased
stress in the cytoskeleton, as may occur in anoxic heart
tissue (39, 121), would slow the shortening rate but accel-
erate relengthening. In contrast, isoproterenol in-
creases both shortening and relaxation rates in rat and
rabbit myocytes (45) as well as increasing the extent of
shortening. Thus in assessing the mechanism of an in-
tervention the rate of cell length change should be in-
cluded along with the measurement of maximum length
change.

There are further concerns when the basis of short-
ening is examined in terms of the Frank-Starling
length-tension relation. The steep ascending limb of the
length-tension curve, relative to skeletal muscle, im-
plies that the ability of the cell to generate force must be
affected by some property of cardiac contraction at
short sarcomere length or by the shortening process it-
self, which is required to achieve a sarcomere length
below rest length (~1.85–1.90 μm) (42). A question is,
What characteristics of the contractile process do mea-
surements of maximum unloaded shortening and maxi-
mum shortening velocity reveal in altered contractile
states relative to changes in cross bridges and/or Ca2+
kinetics? To put some of these questions in perspective,
the next section reviews some of the issues relative to
the functional basis of the ascending limb of the cardiac
length-tension relation.

B. Shortening Related to Frank-Starling Relation

Because isolated unattached myocyte preparations
are not normally preloaded, except by possible adhesion
to the supporting surface and by the viscous loading of
the perfusion media, initial length factors affecting con-
tractility may not be a variable parameter. Exceptions
might appear where Ca2+ loading or unloading affects
the initial cell length; however, these variations could be
monitored during the interventions under study. On the
other hand, when a muscle shortens in a twitch from an
initial length near the peak of the ascending limb of the
length-tension relation, its capacity to manifest exter-
nal force declines as the muscle becomes shorter. Conse-
quently, when shortening in unattached cardiac myo-
cytes is used as an indicator of contractility, the under-
lying length-dependent factors may affect the inter-
pretation of the mechanism of an intervention, de-
pending on which process or processes are responsible
for the ascending limb. There are consistent data indi-
cating that the ascending limb of the length-tension re-
lation in cardiac muscle is significantly steeper than in
skeletal muscle (for reviews see Refs. 1, 60, 133). The
higher resting tension, restoring forces, the absence of
tetanization, the relatively low level of Ca2+ activation
during a twitch in cardiac muscle, and an apparent
length dependence of activation all are factors that may
affect the determination of this steep length-tension
curve. Therefore it is helpful to consider what is known
about the basis of force-length relations in cardiac
muscle.

Specifically, the following are some of the mecha-
nisms that have been proposed to influence the ascend-
ing limb of muscle: 1) interference with cross-bridge at-
tachment from thin filaments passing through the M
zone of the sarcomere at short sarcomere lengths (41, 56);
2) internal passive elastic restoring forces that in-
crease with cell shortening or with an increase in cell
diameter (41); 3) buckling of the ends of thin filaments
of the adjacent halves of the sarcomere as they meet in
the M region (21); 4) compression of attached cross
bridge as sarcomere shortening occurs, thus creating a
cross-bridge-dependent restoring force [in skeletal mus-
cle (4)]; 5) shortening deactivation that may occur as a
result of quick releases during activation or from the
necessary shortening required to reach sarcomere
lengths less than rest length (sarcomere length ~ 1.80–
1.85 μm) before the isometric phase of contraction (12,
14, 30); 6) a length-dependent activation process in
which Ca2+ bound to troponin C (TnC) is released when a
cross bridge becomes detached during active shortening
with a consequent reduction in the number of activation
sites as shortening occurs (2, 3, 52); and 7) the influence
of diastolic length on intracellular Ca2+ levels (88).
These mechanisms are considered in some detail.

1. Thin filament interference of cross-bridge attachment

The early suggestion that the ascending limb of the
length-tension relation might be due to the interference
of cross-bridge attachment from thin filaments of oppo-
site ends of the sarcomere (41, 56) has been discussed
extensively by Jewell (60), Gordon and Pollack (42), and
Allen (5). The conclusion is that in maximally activated
muscle fibers the plateau of tension development ex-
tends down to nearly 1.6 μm where the thick filaments
begin to interact with the Z lines. Indeed Fabiato and
Fabiato (33) showed a decline to only 85% maximum
force development in tonically activated skinned cardiac
myocytes at a sarcomere length of 1.6 μm compared with
40% in phasic Ca2+-induced Ca2+ release responses.
Thus this portion of the ascending limb appears to be
due more to incomplete activation or to the develop-
ment of internal forces that resist sarcomere shortening
rather than an interference with cross-bridge attach-
ment.

2. Restoring forces

The fact that neither cardiac muscle nor isolated
myocytes will retain a sarcomere length less than ~1.8
μm after active shortening indicates that some restor-
ing force must be developed with shortening that re-
stores the rest length during relaxation. The source and
that in the intact cell the restoring force is related, in part, to the active force generation process such as the compression of attached cross bridges.

In this regard, it would be helpful to have a measurement of stiffness during controlled shortening and relengthening to evaluate the nature of the stressed elements (cross bridges and elastic structures) extant during the shortening and relengthening states.

3. Buckling of thin filaments

Brown et al. (21) observed the appearance of waves in the contractile filaments of passive semitendinosus fibers of R. pipens compressed in gelatin. Krueger et al. (67) saw similar wavy patterns in isolated ventricular myocytes under similar compressed conditions. In the skeletal muscle study, Brown et al. (21) found that rather than forming a double-overlap pattern in the M zone in the compressed fiber, the ends of the thin filaments appeared to abut together, forcing the sarcomere to bend laterally and causing the wavy appearance. They suggested that a repulsive force due to the charges on the thick and/or thin filaments in the resting state may resist thin filament entrance into the M-zone region, whereas with activation thin filaments can be drawn past each other. On the other hand, the implication may be that in the passive sarcomere thin filament alignment between the two halves of the sarcomere is sufficiently precise to orient the end of the filaments into a relatively rigid end-to-end encounter. With active shortening, force exceeds the repulsive force or with cross-bridge attachment the thin filaments become misaligned enough to allow them to pass. Evidence for this process might be seen in a careful analysis of the slope of the myocyte shortening record in the vicinity of sarcomere length - 1.6 μm. On the other hand, the varied thin filament length in cardiac muscle (106) might smooth this transition such that only a subtle change in slope would occur.

4. Compressed cross bridges

From another point of view, the steep ascending limb of the length-tension relation may reflect a component due to compressed cross bridge during shortening. In active skeletal muscle, Allen and Moss (4) found that the stiffness-to-force ratio increased at short sarcomere lengths, indicating that an increasing number of cross bridges must be in compression as sarcomere length decreased. If substantial cross-bridge compression does occur in shortening myocytes, then inotropic interventions that change the metabolic state of the cell, and thus the cross-bridge cycling rate by way of pH or adenosine triphosphatase changes, might be expressed differently between the isometric and isotonic modes of contraction. The additional measurement of stiffness during shortening would help resolve this issue.
5. Shortening deactivation

When active muscle is allowed to shorten, its ability to generate force is diminished even though the initial length is restored. In skinned cells in which activation can be maintained, the original force can be regenerated at the initial length but the redevelopment of force is relatively slow [half time = 410 and 280 ms in 8 and 15 μM Ca\(^{2+}\), respectively (2)]. This shortening deactivation has been well demonstrated in both intact cardiac and skeletal muscle (12, 14, 30).

However, shortening in unloaded cells to ~1.6 μm can occur even in low-Ca\(^{2+}\)-perfused experiments. The shortening rate is Ca\(^{2+}\) dependent, but the Ca\(^{2+}\) level does not determine the extent of shortening in skinned cells (62). Therefore the deactivation process is not a simple function of length change or activating Ca\(^{2+}\).

6. Length-dependent activation

Along similar lines, there is a growing body of evidence that contractile activation is length dependent, particularly in twitch contractions of intact muscle. These data indicate that Ca\(^{2+}\) binding to TnC is reduced at short sarcomere lengths. Questions that arise are whether there is a common mechanism between shortening deactivation and the length sensitivity of Ca\(^{2+}\) activation and how this deactivation and length-dependent activation relate to the steep ascending limb of the length-tension relation and, with regard to all these factors, what state of contractility is measured by unattached cell shortening when contractile states are changed by various inotropic agents.

From studies in glycerinated cardiac muscle of cows, in detergent-skinned trabecuclae preparations of rats, and from the substitution of skeletal muscle TnC for cardiac TnC in skinned trabecuclae of hamsters, the following points concerning myocyte shortening and Ca\(^{2+}\) activation are evident. 1) Calcium is released from TnC when cross bridges detach with shortening steps (2, 50, 55). 2) In heart muscle the released Ca\(^{2+}\) correlates best with the force change rather than with the magnitude of the length change as in skeletal muscle (2, 51). 3) With re-lengthening the Ca\(^{2+}\) may rebind to TnC at least in steady-state activations as in skinned or glycinated preparations (82). 4) Calcium is bound to TnC in rigor states, and the amount bound varies with sarcomere length (50). 5) Substitution of skeletal muscle TnC for cardiac TnC increases force development at short sarcomere lengths, indicating that the cardiac TnC regulation of contractile force is more length dependent than skeletal TnC regulation (6).

With regard to deactivation, then, these data indicate that as unattached myocytes shorten, Ca\(^{2+}\) binding to TnC is likely to be much reduced relative to an isometric state at each length, since in shortening muscle few cross bridges are attached and thus TnC activation would be low. These effects may have several important consequences relative to the method of assessment of cardiac contractility. First, cytosolic Ca\(^{2+}\) would be expected to be higher in unattached shortening, as cross bridges detach during sarcomere shortening causing the release of Ca\(^{2+}\) from TnC. Second, the contraction may be of shorter duration, since sequestering by the sarcotricular network would be faster. Third, if the cell becomes afterloaded at some point during the shortening process, then the developed tension would be reduced, since some of the Ca\(^{2+}\) released from TnC would be sequestered by the sarcotricular network. Indeed, the latter process may be the primary basis for the steep ascending limb of the cardiac length-tension relation as measured by contractile force after shortening to a preset length or by the final sarcomere length after active shortening with various afterloads from an initial non-slaek rest length. Thus the ascending limb of the Frank-Starling relation may be predominantly determined by the loss of activating Ca\(^{2+}\) bound to TnC as shortening occurs (1).

With regard to the question of what aspect of contractility unloaded shortening measures, the relation of unloaded shortening responses to cytosolic Ca\(^{2+}\) during inotropic interventions becomes complex to interpret. For example, Allen and Kentish (2) showed that free Ca\(^{2+}\) increased with shortening in skinned cardiac myocytes, which is consistent with the hypothesis that binding of Ca\(^{2+}\) to TnC is reduced after detachment of cross bridges from an actin sites. However, increased buffering of the free Ca\(^{2+}\) under these conditions did not alter the development of force after shortening. Furthermore, Hofmann and Fuchs (52) showed that, indeed, Ca\(^{2+}\) binding to TnC in glycerinated heart fibers was reversibly dependent on sarcomere length, i.e., Ca\(^{2+}\) binding to TnC decreased with shortening but increased again with relengthening. Thus with alteration of the inotropic state of a cell, the measurement only of unattached cell shortening may not accurately reflect either sarcotricular network Ca\(^{2+}\) or cytoplasmic Ca\(^{2+}\), i.e., considerably more Ca\(^{2+}\) might be released by the sarcotricular network and cytosolic Ca\(^{2+}\) might be greater than indicated by the magnitude of shortening. An independent measure of cellular Ca\(^{2+}\) is still necessary to evaluate the activation processes.

7. Contractility related to diastolic length

In cat papillary muscle, Nichols (88) found twitch force to vary with time after a diastolic length change. Also, the increment in twitch force was greater in response to diastolic length increases than the decrement with similar decreases in diastolic length. This protracted dependence of force development on stepwise length changes suggests that contractile activation can be altered by diastolic length by mechanisms other than simple changes in myofilament overlap. In fact, Allen and Kentish (2) measured intracellular Ca\(^{2+}\) transients as a function of diastolic length in ferret ventricular...
muscle and found that the transient responses underwent a slow decline after a reduction in diastolic length, paralleling the slow decline in tension. They attribute this length dependence to possible stretch dependent conductance channels that may influence diastolic intracellular Ca\(^{2+}\) levels.

These observations emphasize the extreme lability of Ca\(^{2+}\) levels in the cardiac myocyte relative to both length and time. Hence interpretations of activation kinetics based on shortening must include these variables.

V. SUMMARY AND CONCLUSIONS

A wide variety of techniques have been developed to monitor the mechanical responses of isolated cardiac myocytes. The most successful are those that measure shortening in unattached cells. Because of their relative ease of implementation, edge-detector methods of following cell displacement have become most widespread. Laser diffraction techniques have been applied to the single heart cells, and sophisticated sarcomere imaging systems capable of 2-ms time resolution of shortening responses have also been developed.

Active force has been recorded in intact single cells from frog atria; however, the compliance of the force transducers was relatively high (~5% \(L_o\)). (There is an obvious trade-off between transducer sensitivity, which affects noise and drift and compliance.) Some success has been reported with the use of intact rat myocytes supported by suction micropipettes and in guinea pig ventricular myocytes adhering to poly-L-lysine-coated glass beams. With the rat preparation, contractile stress was comparable to that of ventricular muscle, but few cells survived the attachment. In guinea pig myocytes, contractile stress in electrically induced twitches was only ~10% of the active stress developed by mammalian trabeculae or papillary muscles at the same temperature (35°C), but, as with the frog atrial transducer, the compliance of the supporting beams was relatively high. Sarcomere uniformity has not been evaluated in these intact preparations.

For attachment to the relatively short mammalian cardiac myocytes, the more promising methods that better preserve sarcomere uniformity include double-barreled micropipettes coated with a barnacle adhesive; however, for nonsubmersible transducers, a continuing limitation is the problem of solution surface stability. Unfortunately, the more severe limitation to effective attachment to intact cells is still the extreme sensitivity of the sarcolemma to mechanical stress. The challenge remains to develop an attachment to the intercalated disk such that cell stress can be transferred to the supporting transducers along the normal stress-bearing cellular interface.

The ultrastructural and passive mechanical data strongly indicate that although the extracellular collagen limits the extension of cardiac muscle beyond the peak of the active length-tension relation, there is also a substantial cellular component of resistance to extension. Furthermore, this cellular component is related to the cytoskeleton rather than to membranous elements in the cell. The more likely candidates for the longitudinal resting stress-bearing element are titin (connectin) and desmin. Titin is the most likely candidate, since it is present in both skeletal and cardiac muscle in relative large quantities, and immunolabeling and stiffness data show that titin extends from the M line to the Z band. These data also show that there is a close association of titin with the thick filaments in both cardiac and skeletal muscle and that passive stiffness falls as myosin is extracted and the titin attachment to myosin is disrupted in high salt.

It remains to be established whether the influence of passive stress applied to the thick filaments by titin has any regulatory effect on active force development.

It is apparent that the basis of the ascending limb of the Frank-Starling length-tension relation may be a composite of several factors, each of which may influence the magnitude of shortening in unattached cells when contractility is changed. The sensitivity of activating Ca\(^{2+}\) bound to TnC on cross-bridge attachment may play a predominant role in the length-tension relation. The development of restoring forces with cell shortening appears to be significant, but their effect may be more in restoring diastolic length than in influencing the length-tension relation. The relative contributions of other factors (cytosolic viscosity, cross-bridge compression, diastolic Ca\(^{2+}\)) remain to be evaluated.

Obviously, contractility during varied inotropic interventions measured by shortening does not measure the same properties of the contractile system as determined by isometric force development. A complete definition of the contractile process requires measurements of both modes of contraction and a determination of their complex interaction. With these data, an understanding of the basis of the steep ascending limb of the length-tension relation and its relation to inotropically altered Ca\(^{2+}\) activation may be forthcoming.

The isolated cardiac myocyte represents the most promising preparation available at the present time to aid in the understanding of the cardiac contractile process. The isolation procedures have been sufficiently refined to provide an actively contractile unit that seems reasonably representative of intact tissue. The challenge now is to further develop attachment methods, force transducers, and sarcomere monitoring and control devices with which to relate the mechanical parameters of contraction to the myofilament components of the cell and biochemical kinetics. Among these challenges is the necessity to understand the functional relation between the passive stress-bearing elements of the cytoskeleton and the contractile filament system.

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