SPECIAL ARTICLE

Correlation of Myocardial Ultrastructure and Function

By Edmund H. Sonnenblick, M.D.

SUMMARY

The electron microscopic structure of heart muscle and the ultrastructural basis of cardiac contraction have been reviewed. The relation between muscle length and developed tension has been explained in terms of the structure of the sarcomere, which is the basic unit of contraction. Using the derived length-tension curve of the sarcomere, developed tension has been attributed to the overlap of thick and thin filaments within the sarcomere, lending support to the “sliding” mechanism in heart muscle.

It has been shown that initial sarcomere length is a function of ventricular filling pressure and that this relation explains the normal limits of the heart as a pump, including: (1) the Starling mechanism whereby increased diastolic volume (EDV) engenders an increased stroke volume (SV), (2) the upper limits to ventricular filling pressure and volume, and (3) the normal range to the ventricular ejection fraction (SV/EDV). Further, ultrastructure helps to define the processes which occur with acute and chronic ventricular dilatation. In this regard, the importance of sarcomere dispersion and “fiber slippage,” which may lead to disordered ventricular function, have been discussed.

Additional Indexing Words:
Electron microscopy
Ventricular function
Idiopathic hypertrophic subaortic stenosis
Heart muscle

A significant advance in our current concept of cardiac function derives from a realization that the performance of the heart as a pump depends in the first sense on the contractile activity of the heart as a muscle. With this realization has come the application of the tools of the muscle physiologist to the problems of cardiac function. One such tool is electron microscopy, which has now permitted a more direct analysis of the contractile machinery of heart muscle than has previously been possible. The basic philosophy of this approach is that the contraction of the entire organ will reflect the summated and integrated function of its individual contractile elements. Thus, in conjunction with quantitative studies of cardiac muscle physiology, the study of the ultrastructure of heart muscle has been undertaken over the past few years and has provided certain insights into the determinants of ventricular contraction. I would like to review four aspects of this work: (1) What is the structure of the contractile machinery of heart muscle? (2) How does the ultrastructure of heart muscle help to explain such basic physiological phenomena as the re-

From the Cardiovascular Unit, Peter Bent Brigham Hospital, and the Harvard Medical School, Boston, Massachusetts.

Work was supported in part by U. S. Public Health Service Grants HE-11306-01 and HE-5679-03S1, and the American Heart Association Grant-in-Aid 67-782.

Paper was presented at the Annual Meeting of the American Heart Association, San Francisco, California, October 20, 1967.

Circulation, Volume XXXVIII, July 1968 29
The structure of the myocardium as seen under the light microscope (upper portion of figure) and under the electron microscope (middle and lower portions of figure). In the lower portion of the figure the details of a single sarcomere are shown. Thick filaments of myosin which are 1.5 μ in length are confined to the central dark A band, while thin filaments of actin extend from both of the Z lines, which limit the sarcomere, through the I zone and into the lateral portions of the A band, where they overlap with thick filaments. At the center of the A band is a dark line flanked by two subtle, lighter zones. This area, which is 0.3 μ in width, forms a “pseudo H zone,” as the light zones are created by the change in the density of the thick filaments, while a true H zone is only seen at the center of the A band, when thin filaments are slid out from its center. Thus, H zone is only seen with sarcomere length greater than 2.2 μ. (From Braunwald, Eugene, Ross, John, Jr., and Sonnenblick, E. H.: The New England Journal of Medicine 277: 794, 1967, by permission of the publisher.)

ulation between developed tension and initial muscle length, that is, the biophysical basis of Starling’s law of the heart, where increased fiber length engenders increased force of contraction? (3) How does ultrastructure of heart muscle provide an explanation for such generalities of ventricular function as the relatively constant relation between stroke volume...
and diastolic volume in a broad range of species? (4) What is the relation of ultrastructure to ventricular dilatation and cardiac failure?

At the onset I would like to credit my collaborators in this work over the past several years, including Drs. Henry Spotnitz, David Spiro, and more recently, Dr. John Ross, Jr., as well as Drs. James Covell and Roger Taylor.

**Ultrastructure of Myocardium**

It is well to start with a description of the basic structure of heart muscle. In the upper portion of figure 1 is shown a section of heart muscle as it appears under the light microscope. The myocardium is composed of a number of interconnecting, branching fibers, or cells, which are 5 to 10 μ in diameter. The fibers are delimited, but attached end to end, by modified cell membranes which are termed “intercalated disks,” and the cell membrane along the longitudinal portions of the cell is called the “sarcolemma.” Due to the fact that electrical resistance between cells across the intercalated disk is very low, the myocardium is considered to be a functional, although not an anatomic, syncytium. When studied under the electron microscope with its greater resolution, much finer detail of the fiber is revealed. This is shown in the middle portion of figure 1, which represents a three-dimensional reconstruction of the cell as taken directly from electron micrographs. The fiber contains within it a number of irregular rodlike structures which run the length of the fiber and are termed “fibrils.” The fibrils in turn are composed of a regular repeating structure termed the “sarcomere,” which creates the striped, or cross-banded, appearance of the fibril. The sarcomere is the basic unit of contraction in all forms of striated muscle—including the myocardium. The sarcomere in turn is composed of the two sets of contractile proteins, termed “myofilaments,” the thicker ones composed of myosin, and the thinner ones, primarily of actin. The myofilaments themselves are relatively rigid, and repetitive reactions between multiple sites along these two sets of filaments are thought to lead to force development, and thus, to shortening of the sarcomere. To recapitulate, the myocardium is composed of fibers, or cells, connected at their ends by modified cell membranes termed “intercalated disks.” The fiber contains numerous fibrils formed by a series of connected sarcomeres, and these are composed of the contractile proteins, termed “myofilaments.” Located between the fibrils are numerous mitochondria which supply the energy for contraction. Surrounding the myofibrils is a fine network of microtubules termed the “sarcoplasmic reticulum,” which is intimately related to the mechanism of activation and relaxation of the contractile system. This interesting system is beyond the scope of this discussion, however.

The specific banding of the sarcomere is very useful, since it signifies where within the sarcomere the myofilaments are located. As shown in the lower portion of figure 1, the sarcomere is delineated by two dark lines termed “Z lines.” In the center of the sarcomere is a dark area, termed the “A band,” which is flanked by two lighter zones termed “I bands.” The bands of the sarcomere are well explained by the disposition of myofilaments within it. Thus, thick filaments of myosin run the length of the A band. Thin filaments, composed primarily of actin, extend from the Z line, through the I band, and end near the center of the A band. Thus, in the lateral portions of the A band, an overlap of thick and thin filaments is present. It is now established for heart and skeletal muscle that these two sets of filaments are constant in length, both at rest and during the course of contraction. The thick filaments are approximately 1.5 μ in length, while the thin actin filaments are 1.0 μ long. With activation, a repetitive interaction occurs between sites along these filaments leading to the displacement inward of the thin filaments so that the sarcomere and, hence, the muscle, shorten. This process forms the basis of the “sliding model” for muscle as shown for skeletal muscle and supported for heart muscle as well. While it appears clear that sliding takes place between myofilaments during contraction, the detailed nature of the force gen-

*Circulation, Volume XXXVIII, July 1968*
Sarcomere Length and Tension Development

With this brief structural background, it is possible to relate some aspects of sarcomere activity to physiological function. One important property of heart muscle and other forms of muscle which form the biophysical basis of Starling’s law of the heart is the relation between initial muscle length and developed tension. The cat papillary muscle from the right ventricle has been used for these studies since it forms a linear, metabolically intact segment of heart muscle that can be rigorously controlled. For comparison to skeletal muscle, the sartorius muscle of the frog has been employed.

In figure 2, active tension expressed as a percentage of maximum tension has been plotted as a function of initial muscle length. L\textsubscript{max} has been taken as that muscle length at which active tension is maximum. When plotted in this fashion, the relation between active tension and initial muscle length is generally the same for heart muscle as represented by the papillary muscle from the right ventricle of the cat, and skeletal muscle, as represented by the sartorius muscle of the frog.\textsuperscript{1} However, the maximum force of contraction of heart muscle is less than that of skeletal muscle. Recently we have found that this lesser maximum force development of cardiac muscle can be explained largely by two factors: (1) the less than maximum contractility of heart muscle and (2) the large amount of noncontractile mass of the myocardium.\textsuperscript{9} Using cross sections of the cat papillary muscle, only 58 ± 2% (SEM) of the tissue was found to be contractile mass, the remainder of the tissue being composed of mitochondria, nuclei, intercellular spaces, and membranes. In contrast, the rat soleus was found to be 90 ± 3% contractile tissue on cross-section analysis. With maximum potentiation of contraction, the maximum isometric force of contraction of heart muscle averaged 10.8 ± 0.4 g/mm\textsuperscript{2}. Correcting this latter value for the noncontractile mass, maximum force of contraction produced by the contractile mass of heart muscle alone averaged 18.6 ± 0.6 g/mm\textsuperscript{2}. This compares well with a tetanic force of contraction of similarly treated skeletal muscle (rat soleus) which averages 21.1 ± 0.8 g/mm\textsuperscript{2}. Thus the force-generating capacity of heart and skeletal muscles is basically similar.

When initial muscle length is decreased along what has been termed the “ascending limb of the length-tension curve,” developed tension decreases. Similarly, when the muscle is stretched beyond L\textsubscript{max}, it passes onto the descending limb of the curve, and developed tension again declines.

Although the active tension-length relations, when normalized, are the same for heart and skeletal muscle, the resting tension curves reveal considerable differences. Thus, in skeletal muscle, resting tension is very small at L\textsubscript{max} and rises appreciably as the descending limb of the active length-tension curve is approached (fig. 2). In contrast, resting tension in heart muscle is quite substantial at L\textsubscript{max} and is present along the major portion of the

\[ L_{\text{max}} \text{ (cat papillary muscle)} = \text{average length} \]

\[ L_{\text{max}} \text{ (skeletal muscle)} = \text{average length} \]

\[ \text{Tension} = \text{maximal tension} \]

\[ \text{Resting tension} = \text{minimal tension} \]

\[ \text{Active tension} = \text{tension generated by contraction} \]

\[ \text{Resting tension (cardiac)} = \text{minimal tension for cardiac muscle} \]

\[ \text{Resting tension (skeletal)} = \text{minimal tension for skeletal muscle} \]

\[ \text{Muscle length} = \text{length of muscle} \]

\[ \text{Tension - % of maximum} = \text{percentage of maximum tension} \]

\[ \text{Active tension} = \text{tension generated by active contraction} \]

\[ \text{Passively stretched muscle} = \text{muscle stretched without active contraction} \]

\[ \text{Stimulated muscle} = \text{muscle activated by electrical stimulation} \]

\[ \text{Contractile mass} = \text{portion of muscle that actively contracts} \]

\[ \text{Noncontractile mass} = \text{portion of muscle that does not actively contract} \]

\[ \text{SEM} = \text{standard error of the mean} \]

\[ \text{g/mm}^2 = \text{grams per square millimeter} \]

\[ \text{L_{max}} = \text{maximum length} \]

\[ \text{L_{resting}} = \text{resting length} \]

\[ \text{L_{active}} = \text{active length} \]

\[ \text{L_{noncontractile}} = \text{noncontractile length} \]

\[ \text{L_{contractile}} = \text{contractile length} \]

\[ \text{T_{max}} = \text{maximum tension} \]

\[ \text{T_{resting}} = \text{resting tension} \]

\[ \text{T_{active}} = \text{active tension} \]

\[ \text{T_{passively stretched}} = \text{tension generated by passively stretched muscle} \]

\[ \text{T_{stimulated}} = \text{tension generated by stimulated muscle} \]

\[ \text{MUSCLE LENGTH - TENSION RELATION} \]

\[ \text{Figure 2} \]

The relation between tension and initial muscle length for cat papillary muscle and the sartorius muscle of the frog. Length has been normalized relative to that length where actively developed tension is maximum (L\textsubscript{max}). Active tension is normalized relative to the tension developed at L\textsubscript{max}. Resting tension is the tension produced by the passively stretched muscle. Active tension is the tension the stimulated muscle will generate when stimulated. (Modified from Ross, John, Jr., Sonnenblick, E. H., Covell, J. W., Kaiser, G. A., and Spiro, David: Circulation Research 15: 14, 1964, by permission of the publisher.)
ascending limb of the active length-tension curve. Since the intact ventricle must depend on a filling pressure to establish an initial fiber length, this fact is essential if the heart is to function along the ascending limb of the curve, which it does, as will be seen. However, skeletal muscle operates primarily at the apex of this curve and has its initial length adjusted primarily by the mechanical setting of joint position; thus, a stiff resting length-tension curve in skeletal muscle would serve little physiological purpose. The underlying basis of this difference in resting stiffness between heart and skeletal muscle is not entirely clear, but could result from the greater amount of connective tissue in heart muscle. The smaller diameter of cardiac muscle cells (5 to 10 μ) relative to skeletal muscle cells (50 to 100 μ) would create a relatively larger surface area, or sarcolemma, for a given amount of muscle in myocardium. Whether the sarcomeres of heart muscle themselves have resistance to stretch is not known. Thus, the exact reasons for the increased passive stiffness of heart muscle requires further study.

In recent studies, sarcomere structure has been analyzed in relation to the length-tension curve, and it has been shown that changes in sarcomere length and disposition of myofilaments within the sarcomere serve to explain the active length-tension curve. This has been accomplished by determining the relation between active tension and length in the cat papillary muscle: subsequently, muscle length has been set at a predetermined point and maintained constant; then the nonactivated muscle has been fixed in the myograph with glutaraldehyde. Glutaraldehyde has been shown to have the distinct advantage of permitting fixation without inducing contraction. The tissue has then been processed for study under the electron microscope. From such sections, multiple sarcomere lengths have been measured and average sarcomere length determined.10

It has been found that along the greater part of the ascending limb of the length-active tension curve, average sarcomere length is proportional to muscle length. The same general relation has been demonstrated for the frog sartorius muscle.1 From these relations it has been possible to convert the relation between muscle length and active tension into a relation between sarcomere length and active tension. This is shown in figure 3. At the apex of the sarcomere length-tension curve, sarcomere length measures 2.10 to 2.25 μ, if one includes the entire apex to the curve. When muscle length is decreased to the point where active and resting tension extend toward zero, sarcomere length decreases to about 1.5 μ. This decrease in the sarcomere length is accomplished solely by a decrease in the width of the I bands, while the A band remains constant in width. As muscle length is increased beyond Lmax, actively developed tension again declines, and the muscle passes onto the descending limb of its length-tension curve. Along this portion of the curve the

![Sarcomere Length-Tension Curve](http://circ.ahajournals.org/)

**Figure 3**

The relation between tension development and sarcomere length obtained from the cat papillary muscle. The lower curve represents resting tension, while the upper curve represents active tension. These curves represent the average normalized results of 27 muscles; this representation is possible, since muscle length is proportional to average sarcomere length along the greater portion of this curve. (Modified from Sonnenblick, E. H., Spiro, David, and Cottrell, T. S.: Proc Nat Acad Sci 49: 193, 1963, by permission of the publisher.)
close relation between sarcomere length and muscle length does not hold in heart muscle, since sarcomere length increments are no longer adequate to explain the increases in muscle length which are produced. Furthermore, resting tension rises precipitously at this point, and sarcomere lengths in excess of 2.60 μ are rarely produced. Thus, in overstretched heart muscle (lengths > L_{max}), something else in addition to increases in sarcomere length must have ensued, and from this observation it has been suggested that "slippage of muscle fibers" occurs along the descending limb of the cardiac sarcomere length-tension curve.\(^8\)\(^{,10}\) The potential significance of such "slippage" in cardiac dilatation will be discussed subsequently.

The relation of sarcomere length to tension development may be explained by the disposition of myofilaments within the sarcomere. This was first suggested from experiments with skeletal muscles,\(^6\)\(^,7\) and these observations form the basis of the "sliding filament" model for muscle. Subsequent studies with skeletal muscle preparations have lent support to these initial views,\(^1\)\(^,11\)\(^,12\) and the same principles have now been affirmed for heart muscle.\(^1\)\(^,8\)\(^,13\) These general relations are shown in figure 4, using sarcomeres taken along the length-tension curve of the frog sartorius.\(^1\) On the left in figure 4 are sarcomeres taken at various muscle lengths, while on the right, the disposition of the myofilaments within these sarcomeres is shown diagrammatically. The

---

**Figure 4**

Changes in sarcomere length and band pattern as muscle length (frog sartorius) is altered. In panel A are shown the sarcomeres themselves; in B, the diagrammatic disposition of myofilaments drawn to scale. The sarcomere in the middle (3) is taken at L_{max} (Modified from Circulation Research 15 (suppl. II): II-14, 1964,\(^8\) by permission of the publisher.)
middle sarcomere (no. 3) is taken from a skeletal muscle fixed at the apex of the length-tension curve and measures 2.2 μ. Thin filaments are 1.0 μ in length; thick filaments of the A band, 1.5 μ. Thus, as seen on the right, thin filaments extend in 1.0 μ from each Z line. Since the center 0.2 μ of the thick filaments in the A band are without reactive sites, a sarcomere length of 2.2 μ provides for the optimal placement of thick and thin filaments so as to provide for the largest number of interactions between the two sets of filaments and, thus, the greatest force development. If muscle length is then increased as shown above in nos. 4 and 5, the thin filaments which are constant in length are slid out from the A band, and a lighter area in the center of the A band is created, which is termed the "H zone." Only thick filaments are seen in the H zones, and the H zone widens as sarcomere length is increased (no. 5). The width of the A band, reflecting the length of the thick filaments, remains constant. Accordingly, the degree of overlap between thick and thin filaments thus decreases, and force development falls proportionally. This decrease in force, which is proportional to the decrease in overlap of the two sets of filaments, is fundamental to the "sliding filament" model for muscle. As muscle length is decreased below the apex of the length-tension curve, the I bands become narrower, and the thin filaments now slide into the center of the sarcomere (fig. 4, nos. 1 and 2). Three phenomena occur which may lead to this fall in force development: (1) at sarcomere lengths of 2.0 μ or less, thin filaments moving into the opposite half of the sarcomere may interfere with one another directly or disrupt bond formation in the opposite half of the sarcomere and thus reduce force development; (2) the filaments are polarized and may actually repel one another, thereby providing a restoring force which would counter active force development; and (3) since the sarcomere volume is constant, the filaments move further apart laterally as the sarcomere shortens, leading to less opportunity for force generating interactions at bridges along the filaments, as well as directional distortion of bonds. Any or all of these explanations may be applicable, and, at present, there is no information to permit a choice between them. Thus, along both the ascending and descending limbs of the length-tension curve, the disposition of myofilaments within the sarcomere may be used to explain force generation in accord with the sliding filament model, although the detailed mechanisms involved await definition. Furthermore, in overstretched heart muscle where sarcomeres are no longer stretched in proportion to muscle, slippage of fibers appears to occur as well; nevertheless, the same "sliding" principles apply in heart and skeletal muscle.

From the sarcomere length-tension curve some of the generalities and limitations of ventricular performance can now be derived. As shown in figure 5, a projected course of sarcomere shortening which might occur under the afterloaded conditions of the intact heart has been plotted within the

![Figure 5](https://circ.ahajournals.org/)

The limits of sarcomere shortening under afterloaded conditions within the constraints of the sarcomere length-tension curve. A is the starting point for contraction, P represents the load, and L, the extent of shortening.
The effects of altering initial muscle length from 2 to 1 on the shortening of the afterloaded sarcomere. While the isometric force of contraction is only increased modestly ([from $P_2$ to $P_1$]), the extent of shortening ($\Delta L$) is substantially augmented from 13 to 20%.

constraints provided by the sarcomere length-tension curve. The sarcomere starts shortening at point A on the resting tension curve. During the isometric portion of contraction (A to P), force is developed, and when the force developed equals the load (or the blood pressure [P] in the intact heart), the sarcomere shortens ($\Delta L$) and continues to shorten until it reaches the active tension curve. Relaxation then ensues. Were there no constraints on shortening, the sarcomere could shorten about 30%, that is, from 2.2 to 1.5 \( \mu \) (insert, fig. 5). However, the limits of muscle loading under normal conditions will allow the sarcomere to shorten only about 12 to 18%. These, then, are the theoretical limits to sarcomere shortening. From figure 6 one may also note that, although an increase in sarcomere length from 2.0 to 2.2 \( \mu \) will only induce a modest increase in force of contraction, that is, from $P_2$ to $P_1$, a substantial increase in $\Delta L$ (from $\Delta L_2$ to $\Delta L_1$) still occurs. This increase in $\Delta L$ then provides a basis for the Starling phenomenon.

Sarcomere Shortening and Ventricular Ejection

The fiber shortening in the wall of the heart, which is necessary to explain normal ventricular ejection, may also be predicted on a theoretical basis. Stroke volume (SV) is the difference between the end-diastolic volume (EDV) and the end-systolic volume (ESV). Holt and associates\textsuperscript{14} have noted that the ejection fraction of the ventricle, that is, the SV/EDV, is quite constant in a broad number of animal species ranging in size from the rat to the elephant. By use of angiographic techniques which yield an answer which is about 10% greater than dye volumes, the ejection fraction in man and the dog has been shown to vary normally between about 50 and 65\textsuperscript{%}.14-16 The explanation for this similarity of ventricular ejection fraction independent of heart size is not to be found in the properties of the heart as a pump, but becomes readily apparent when viewed in terms of the shortening properties of the sarcomere. As is shown in the diagram of the left ventricular wall in figure 7, changes in the radii and circumferences of the left ventricle can be calculated between end-diastolic volume (EDV) and end-systolic volume (ESV), the difference being the stroke volume (SV). In figure 8 the calculated changes in midwall circumference have been expressed as percentages and plotted as functions of ejection fraction (SV/EDV). Both of these values, of course, are

\[ EDV - SV = ESV \]

\[ r_m = \frac{r_o + r_i}{2} \]

\[ C_m = 2\pi r_m \]

A diagram of the left ventricle as a thick-walled structure. EDV = end-diastolic volume; SV = stroke volume; and ESV = end-systolic volume; $r_m = $ mid-wall radius; $r_i = $ inner radius; $r_o = $ outer radius; and $C_m = $ midwall circumference.
without dimension and can thus apply to a ventricle of any size. Furthermore, since sarcomeres are arranged in series around the ventricle, a percentage change in circumference can also be used to express an anticipated change in sarcomere length. The vertical dashed lines in figure 8 represent the entire range of normal ejection fractions reported in the literature from angiography, ranging from 50 to 65%. Of interest, this ejection fraction requires a sarcomere shortening of 12 to 17%, a range which corresponds well with that predicted from the afterloaded sarcomere shortening from the sarcomere length-tension curve (fig. 5). Such an estimation of SV/EDV is, of course, independent of absolute values and would thus apply to any size heart. Thus, on a theoretical basis, the shortening capabilities of the sarcomere would serve to explain ventricular ejection. What follows lends support to this view.

**Relation of Sarcomere Function and Cardiac Performance**

One approach to establishing the relation between sarcomere function and the performance of intact heart has been to obtain the relation between filling pressure and volume in the arrested heart, and then to fix the heart by coronary perfusion with glutaraldehyde at known pressures and volumes. Following appropriate preparation, average sarcomere lengths have then been determined at the endocardial, midwall, and the epicardium of the anterior free wall of the left ventricle. In figure 9, the filling pressure at which the dog and cat left ventricles were fixed is shown on the ordinate, and the average sarcomere length from the middle of the left ventricular wall is shown on the abscissa. Each point represents the average value from an individual heart. At zero filling pressure, which occurs with an intraventricular volume averaging 12 cc, sarcomere length is 1.90 μ. As filling pressure is augmented, sarcomere length increases, and as sarcomere length approaches 2.2 μ, filling pressure rises quite precipitously. Despite the fact that there is a ten-fold difference in the weight of the ventricles of the cat and the dog, the identity of the filling pressure-sarcomere length relations is quite apparent. Thus, the filling pressure of the

---

**Figure 8**

The relation of percentage change in midwall circumference or midwall sarcomere length to ventricular ejection fraction (SV/EDV).

**Figure 9**

The relation of left ventricular filling pressure to sarcomere length for the left ventricle of the dog and the cat. The upper curve represents the relation between tension development and sarcomere length as taken from the cat papillary muscle. (From Spotnitz, H. M., Sonnenblick, E. H., and Spiro, David: Circulation Research 18: 49, 1966, by permission of the publisher.)
heart, with its correlate of intramyocardial tension, is the general determinant of initial sarcomere length independent of heart size.

Further, in the upper curve of figure 9 the relation between sarcomere length and tension as derived from the cat papillary muscle has been plotted, and certain points of interest may be noted. First, the upper limit of normal for the left ventricular filling pressure, above which stroke volume rises very little for increased end-diastolic volume, is about 12 to 15 mm Hg. This filling pressure is associated with a sarcomere length of about 2.2 μ and, in turn, with the apex of the sarcomere length-tension curve. Furthermore, the normal ventricle, independent of absolute size, appears to operate along the upper portions of the ascending limb of the sarcomere length-tension curve. Second, diastolic sarcomeres only extend down to 1.90 μ, providing evidence for the view that a substantial elastic restoring force exists in the ventricular wall. Thus, a major limit to ventricular performance is clearly the sarcomere. If filling pressure is increased beyond normal limits, one pushes the sarcomere onto the descending limb of its curve.8, 13, 17

**Acute Ventricular Dilatation and the Sarcomere**

What happens then to sarcomere length and structure if the ventricle is acutely dilated? Two events occur which have already been seen in the isolated muscle. First, sarcomeres longer than 2.2 μ appear, as the descending limb of the sarcomere length-tension curve is approached, and an H zone may be perceived for the first time within the central portion of the A band, as thin filaments are slid out of the center of the sarcomere. However, these H zones are not as easy to discern in heart muscle as they are in skeletal muscle (fig. 4, no. 4), and the reason for this is not readily apparent.8, 10 Moreover, it should be emphasized that H zones only appear following acute massive ventricular dilatation. Second, “fiber slippage” seems to occur after ventricular dilatation, a factor already alluded to relative to the stretched isolated muscle. In this setting, “fiber slippage” could be defined as displacement of fibers in opposite directions relative to one another so that increments in tissue length could be greater than the change in length of the individual cell and its constituents. Thus, with repeated dilatation of the arrested ventricle, “stress-relaxation” ensues, that is, one finds that the intraventricular pressure falls for any intraventricular volume. Under these circumstances, the sarcomeric lengths determined are not long enough to explain fully the observed increase in ventricular volume at these elevated filling pressures and volumes. Indeed, “fiber slippage” appears to be the primary factor in chronic dilatation in the intact heart, as will be discussed in a subsequent section. Further, unless cardiac tissue is fixed at the elevated filling pressure pertaining in life, sarcomeres will shorten down on fixation, and any excessive extension of sarcomere that might have existed in vivo would not be perceived.

**Limits of Sarcomere Function in the Intact Contracting Heart**

In association primarily with Dr. John Ross, Jr., we have also been interested in establishing the limits of sarcomere function in the intact contracting heart.18, 19 This has been accomplished by arresting hearts in either diastole or systole by the programmed injection of glutaraldehyde directly into the coronary...
Table 1
Comparison of Experimentally Found and Predicted Changes in Ventricular Circumference with Ejection Fraction of 58%

<table>
<thead>
<tr>
<th></th>
<th>% Change Found</th>
<th>% Change Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV/EDV</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>External circumference</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Midwall circumference</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Internal circumference</td>
<td>26</td>
<td>29</td>
</tr>
</tbody>
</table>

arteries (fig. 10). Glutaraldehyde is an excellent fixative for this purpose, since it fixes quite rapidly with minimal distortion. Although fixation times in isolated fibers is of the order of 50 to 100 msec, it is undoubtedly longer in the intact heart. Nevertheless, fixation has been confirmed by changes in the ventricular topography and volume and subsequently supported by electron microscopic studies.

In figure 10 are shown the silastic casts of the left ventricle of two such hearts, derived from ventricles of similar weight. The cast on the left is taken from a heart fixed in diastole, while the cast on the right is taken from a ventricle fixed in systole. With systole, the decrease in intraventricular volume accompanied by an increased imprint of the papillary muscles and endocardial infolding is evident.

In table 1 certain features of topography are shown as taken from a recently published study. A 58% ejection fraction was found. This was accompanied by a 28% increase in ventricular wall thickness. External circumference shortened 8%, midwall circumference by about 15%, and internal circumference, by 26%. As noted in figure 11, such an increase in wall thickness is to be anticipated. These differences in shortening in the outer and inner circumferences are also readily explained by spherical thick-walled models. In figure 12 changes in circumference have been related to intraventricular volume for such a model. The vertical difference between the dashed lines represents ventricular ejection fraction of 50%. Of note, circumference shortenings of 5, 12, and 20% are found in the three layers of the heart, the largest amount of

Figure 11
The relation of changes in left ventricular wall thickness to ejection fraction (SV/EDV).

Figure 12
The relation of intraventricular volume to changes in circumference in the inner (i), middle (m), and outer (o) layers of the ventricular wall. The dashed horizontal lines demark the volume at 50 and 25 cc, that is, a 50% ejection fraction. Such an ejection fraction requires a 50% change in the outer circumference, a 12% change in the midwall, and a 20% change in the inner circumference.
shortening occurring in the inner layers of the ventricle. In table 1, then, the experimentally determined and predicted changes in dimension have been compared. The ventricular ejection fractions have been made identical, and it is apparent that the changes in wall thickness and the circumferences are well predicted from thick wall equations. 

In order to follow the course of sarcomere shortening in the wall of the intact heart, sarcomere lengths from the midwall of the ventricle have then been analyzed in these hearts as previously described, and the results are summarized in table 2. The weights of the two groups of ventricles are similar, as are LVED's prior to fixation. Under these conditions sarcomere length in diastole in the midwall of the ventricle averaged 2.07 μ, and decreased to 1.81 μ during systole, representing a 13% change. Theoretically this 13% change

\[ \text{SARCOMERE LENGTH-TENSION CURVE} \]

Average sarcomere lengths determined in the intact heart in diastole and systole have been portrayed on the sarcomere length-tension curve.

*In this light, it is significant that at any given ventricular filling pressure, average sarcomere length has been found to be longer in the endocardium and shorter in epicardium when compared to the midwall. This supports the view that the highest tension in the ventricular wall during diastole is at the endocardial surface. Teleologically, it is useful that those sarcomeres which must bear the greatest tension and shorten the farthest in systole are indeed relatively the longest and thus the strongest.

![Figure 13](image)

![Figure 14](image)

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Wt (g)</th>
<th>Vol (cc)</th>
<th>LVED (mm Hg)</th>
<th>Sarcomeres (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diast.</td>
<td>95.5</td>
<td>51.6</td>
<td>8.8 ± 1.7</td>
<td>2.07 ± 0.04</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syst.</td>
<td>102.4</td>
<td>21.4</td>
<td>6.1 ± 1.2</td>
<td>1.81 ± 0.03</td>
</tr>
<tr>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
<td>(1.73 - 1.87)</td>
</tr>
<tr>
<td>%Δ</td>
<td>58</td>
<td></td>
<td></td>
<td>13%*</td>
</tr>
</tbody>
</table>

(SV/EDV)

*13% Δ Sarcomere length → SV/EDV of 55%.

The relation between left ventricular volume of the dog and midwall sarcomere length. Taken from the intact heart fixed in diastole (○), systole (○), diastole following acute dilatation (△), and following postextrasystolic potentiation (●). The dashed line shows the calculated change in sarcomere length necessary to explain these volume changes. (Modified from Sonnenblick, E. H.: Amer J Physiol 207: 1330, 1964, by permission of the publisher.)

Circulation, Volume XXXVIII, July 1968
a rather close correlation between theory and the observed changes.

In figure 13, the average sarcomere lengths determined in the intact heart in diastole and systole have been plotted on the length-tension curve, which has been derived from the isolated muscle. It is apparent that the limits to sarcomere shortening which were previously suggested (fig. 5) have been closely adhered to. Thus, the normal intact ventricle functions on the upper half of the ascending portion of the sarcomere length-tension curve, with diastolic sarcomere length being only about 5% shorter than the apex of the curve. This, however, still provides adequate reserve for the Starling phenomenon to function such that increased ventricular volume would generate increased fiber shortening and, hence, increased stroke volume. Further, the passage of thin filaments into the center of the sarcomere occurs under physiological conditions, since this would occur when sarcomere length falls below 2.0 μ.

In figure 14, midwall sarcomere length has been related to intraventricular volume for left ventricles fixed in diastole (●), systole (○), following acute dilatation (∆), and during potentiated systole resulting from an extrasystolic contraction (■).18,19 Using the diastolic heart as the focal point, a line of prediction for changes in volume as a function of midwall sarcomere length has been constructed (dashed line). It is apparent that changes in sarcomere length follow this line of prediction quite well, not only during normal systole, but following either marked potentiation of contractility, or acute dilatation. Of some note, sarcomeres which are markedly potentiated can produce an 80% ventricular ejection fraction, but then only by maximal shortening.

**Chronic Dilatation**

With Roger Taylor and John Ross, Jr., we have also explored the effects of chronic dilatation on sarcomere changes in the wall of the heart.20 This has been done by creating an arteriovenous fistula between the aorta and vena cava in dogs. Subsequently, profound peripheral congestion ensues with an enlarged heart, high cardiac output, and elevated left ventricular filling pressure. These hearts have then been fixed in situ at their ambient filling pressures, and midwall sarcomere lengths in the left ventricles have been determined. It has been found that sarcomere lengths were shorter than predicted from the volumes observed. Further, despite the marked ventricular dilatation which occurred, sarcomeres longer than 2.2 μ were not the general rule, and one must suggest either that new sarcomeres had been synthesized in series with those initially present, or that slippage of cells had occurred in chronic dilatation. Either phenomenon would result in the large dilated heart with sarcomere length being shorter than volume changes would predict. Support for the latter phenomenon has been gained in isolated muscle strips, and it would appear that both sarcomere stretch and “fiber slippage” have occurred. Thus, with sarcomere length at 2.2 μ, little if any reserve for the Starling mechanism would remain for the dilated heart and this is generally observed clinically.

**Dispersion of Sarcomere Lengths**

Another problem of current interest to us has been the dispersion of sarcomere lengths which occur within the wall of the heart.19 A significant variation in sarcomere length has

![Figure 15](image-url)
been found within a given layer of the ventricular wall. Indeed, there is significant variation of sarcomere length within a given fiber; average sarcomere length with its changes between diastole and systole reflects the change in this distribution. This dispersion of sarcomere length represents at least two problems: first, the error implicit in measurement, which is necessitated by the large number of measurements for the mean, and second, the possibility that force is not uniformly distributed across the cells of the myocardium. Thus, average sarcomere length is a statistical entity. During the course of contraction, the dispersion of sarcomere lengths would be decreased as the longer, and hence, stronger sarcomeres reach the length of the shorter ones and sarcomere lengths become synchronized. However, the situation is more complex in the dilated heart. When the histogram of sarcomere length is studied in the presence of massive ventricular dilatation, one finds that some sarcomeres remain at less than 2.2 μ in length, which is the apex of the sarcomere length-tension curve, while others are stretched beyond the apex of the curve (fig. 15). Thus, weak sarcomeres on the descending limb of the sarcomere length-tension curve are placed in series with stronger sarcomeres at the apex and along the ascending portions

**Figure 16**

Electron micrographs. Panel A shows left ventricular septal tissue taken at the time of surgery from a patient with idiopathic hypertrophic subaortic stenosis. Marked variation in sarcomere length is apparent. Indeed, thin filaments have been drawn entirely out of three sarcomeres (arrows). In the lower left hand corner is seen the frequent finding of markedly shortened sarcomeres (star).

Panel B shows tissue from the left ventricular apex of another patient with idiopathic hypertrophic subaortic stenosis. The stars indicate contracture formation. Such areas of contracture are frequent in this condition.
of the curve. During the course of contraction, the already elongated sarcomeres remain at a mechanical disadvantage. Theoretically the end-result would be the production of further sarcomere dispersion. Obviously more information to this point is required.

Speculative Thoughts on IHSS

In at least one specific pathological situation, sarcomere dispersion may play an important role and may, indeed, create a vicious cycle for the deterioration of cardiac function. Shown in figure 16 are two electron micrographs of ventricular muscle taken from a patient with idiopathic hypertrophic sub-aortic stenosis (IHSS),\textsuperscript{20} where unexplained, inappropriate ventricular and septal hypertrophy has occurred. This tissue is typical of that removed from the ventricular septum (fig. 16A) of 10 patients with IHSS by Dr. Andrew Morrow at the time of surgery to relieve the muscular obstruction to ventricular ejection which this tissue created.\textsuperscript{21} In three instances, cardiac tissue from the apex of the ventricle (fig. 16B) was also studied. Notably, in addition to an increase in nuclear size and irregularity, and an increase in the number of mitochondria, great variation in sarcomere length is found within a given cell in IHSS; that is, very long sarcomeres are seen in series with very short ones. Indeed, in figure 16A sarcomeres are seen in which one half shows marked over-stretching with the thin filaments pulled out from the A band with the production of a huge I band (arrows), while the other half of the sarcomere is shortened to the point that the I zone is no longer present. Furthermore, large groups of sarcomeres have gone into contracture with sarcomeres less than 1.5 μ (see stars on figure). Clearly, one has sarcomeres which are weakened by being placed at both lower ends of the sarcomere length-tension curve. The end point of such sarcomere dispersion would be an inability to develop force. Theoretically, this could result in localized areas within the cell which are unable to generate tension, and could lead to abnormal loading of the remainder of the fiber. It is then conceivable that such alterations in force generation could result in increased tissue growth. Such a viewpoint however must remain within the realm of speculation for the present.

The underlying mechanism of the sarcomere length dispersion in IHSS is unknown but could involve asynchronous activation of the cell. Were this to occur, activated sarcomeres would shorten at the expense of non-activated ones which could be stretched. These activated sarcomeres might then proceed to contracture, since shortening is not adequately resisted. Indeed, the state of the excitation system remains to be studied in this as well as other forms of hypertrophy.

Of course, an element of caution must also be expressed relative to such interpretations. The tissue studied has been obtained with a rongeur and tissue orientation for fixation has been difficult. The possibility of fixation artifact remains although mitochondrial structure is well preserved and similar observations have not been noted in hypertrophied muscle removed from patients with tetralogy of Fallot where the fixation procedure was the same. Obviously, further work to this point is required.

Recapitulation

In summary, the ultrastructural basis of cardiac contraction has been reviewed. It is clear how the properties of the sarcomere can serve to explain the basic length-tension relation of heart muscle and in turn, Starling's law of the heart. The geometry of contraction during diastole and systole has been examined, and the limits to sarcomere performance have been shown to determine such generalities as the ejection fraction and its identity in a broad range of animal species. The effects of ventricular dilatation have been studied, and here, too, the function of the sarcomere is central.

These findings are only a beginning, and several related problems are presently being explored. The significance of sarcomere dispersion within the myocardium, both within a given cell and in various layers of the heart in terms of force distribution, is being studied.
This is being related to fiber orientation across the myocardial wall. Similar approaches are being used in hypertrophy and ischemia.

A more difficult problem receiving increasing interest is the ultrastructural basis of the force generator and the bridges between myofilaments.

These studies have strengthened our belief that quantitative ultrastructural studies in concert with the controlled physiological experiments offer an opportunity to obtain broad insights into normal and pathological organ function. Hopefully, time and further effort will fill in the innumerable gaps in our knowledge.

References


Correlation of Myocardial Ultrastructure and Function
EDMUND H. SONNENBLICK

Circulation. 1968;38:29-44
doi: 10.1161/01.CIR.38.1.29

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/38/1/29

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/