Papillary Muscle Shortening in the Intact Dog

A CINERADIOGRAPHIC STUDY OF TRANQUILIZED DOGS IN THE UPRIGHT POSITION

By Arthur F. Grimm, Bessie L. Lendrum, and Hun-Lin Lin

ABSTRACT

Shortening of the anterior papillary muscle of the left ventricle was demonstrated in six intact, tranquilized dogs. Two small metal markers that had been surgically implanted 3–50 months earlier were cineradiographically photographed during approximately ten sequential cardiac cycles in each of two orthogonal positions. Distances between markers were plotted for successive frames. The resulting curves were used to obtain maximum velocities of papillary muscle shortening and lengthening: 1.08 ± 0.29 muscle lengths/sec and 1.39 ± 0.48 muscle lengths/sec, respectively. From the two orthogonal planes, the average maximum spatial distance and the average minimum spatial distance between the markers were calculated. The mean percent shortening of 22.8 ± 6.5% was surprisingly large: it approximated the distance from the foot to the peak of the ascending limb of the myocardial length-tension curve derived from isolated muscle studies. Mechanical studies on isolated papillary muscle have consistently shown reduced shortening with increasing loads. Since the in vivo dog papillary muscle has been reported to be under considerable tension during systole, there appears to be some contradiction between the degree of shortening found in the present study and the shortening observed in isolated papillary muscle studies.

KEY WORDS

- myocardial shortening
- length-tension curves
- papillary muscle
- electrocardiogram
- cardiac metal markers
- myocardial velocities

Papillary muscle has been investigated for two relatively independent reasons: (1) it is an experimentally convenient sample of ventricular myocardium, and (2) it is a clinically important portion of the functioning heart. Papillary muscle is easy to isolate and to remove from the ventricle. Its small diameter in some species and its participation during cardiac growth also contribute to its utility as an experimental material. Most papillary muscle studies have employed the isolated muscle (1–10), although more recently mechanical properties have been studied in the in situ muscle (11–14). The essentially linear fiber arrangement within the muscle greatly simplifies the interpretation of data from both mechanical and microscopic studies (15).

Papillary muscle function in the intact heart has, most often, been deduced indirectly from isolated muscle studies, clinical observations, autopsy reports, and theoretical considerations. Burch et al. (16) and Burch and Depasquale (17) have postulated that during ventricular systole the papillary muscle, by creating tension, acts as a stay to restrict mitral valve motion. When normal tension development does not occur, excessive mitral valve motion results in mitral regurgitation (the papillary muscle dysfunction syndrome).

An absence of papillary muscle shortening during ventricular systole has been reported by Karas and Elkins (18) who studied the normal intact dog with a cineradiographic technique using metal markers, one on the free edge of the mitral valve leaflet or the chordae tendineae and the other at the left ventricular apex. Their surprising conclusion is quite different from the findings described in the present paper.

In the present study using cineradiographic techniques, the papillary muscle shortening of the left ventricle in intact,
morphinized dogs was shown to be substantial and relatively consistent. The extent of shortening approximated the entire ascending limb of the length-tension curve derived from isolated muscle studies (8).

Methods

Mongrel dogs were anesthetized with sodium thiopental and ventilated via an endotracheal tube using a Harvard pump; a left thoracotomy was performed to expose the heart. The left anterior papillary muscle was located by inserting a finger through the mitral valve via the left atrial appendage. Two small barbed metal markers (Fig. 1) less than 2 mm in length and 0.5 mm in width were placed (by penetration from the epicardial surface using a fine forceps) in the papillary muscle, one near its attachment to the chordae tendineae and the other approximately 1 cm away along the long axis of the papillary muscle. The surgical incisions in the atrial appendage, the pericardium, and the chest wall were closed, and the dog was allowed to recover. Verification of marker location in the papillary muscle was obtained post-mortem by X-raying the excised papillary muscle. Migration of these markers was never observed. The heart was arrested with potassium and fixed with formalin in four anesthetized closed-chest dogs at the time of death. After the metal markers in the excised papillary muscle had been removed with forceps under fluoroscopy, the muscle was sectioned for microscopic examination.

A variety of metal markers were tested in the present study. The different types of markers resulted in identical experimental findings. The most convenient, stable marker over long periods of time and the one least likely to produce surgical and postsurgical complications proved to be a segment of a barbed broach, an instrument used in dental pulp removals (Cresent Broach). The only complication encountered was a small sterile abscess found in one of the dogs when another type of metal marker was used in a preliminary study (the data from this dog were not included in the present paper).

The dogs were studied 3-50 months after surgery by X-ray cineradiography using a General Electric Fluoricon. The X-ray tube and the image intensifier-camera unit were rigidly mounted on opposite sides of a moveable C-arm (Fig. 2) which could be rotated about an axis perpendicular to the plane of the C-arm. This axis, parallel to the long axis of the dog, passed through the heart at the approximate location of the markers. Rotation of the C-arm was motor controlled, and the angle between the central X-ray beam and the horizontal beam could be read accurately to within a degree on a large circular "protractor" permanently mounted coaxially with the C-arm. The dog was placed in a specially constructed sling from 0.5-1 hour after administration of either morphine alone or a combination of morphine, sparine, and phenergan. The dog's long axis was perpendicular to the X-ray path, and the heart markers were in the center of the field (Fig. 3). Due to the lack of suitable biplane equipment, the images of the markers were obtained sequentially in two orthogonal planes, 45° superior and 45° inferior to the horizontal plane. Cineradiograms were obtained for ten or more cardiac cycles in each view to ensure recording over one or more respiratory cycles. An electronic flash triggered by the QRS complex of the electrocardiogram (ECG) was simultaneously recorded on the film while the entire ECG was recorded on paper with a Beckman Dynograph recorder. The flash mark on a given cine frame was matched precisely with the appropriate QRS complex of the ECG by moving the ECG base line to "clip off" the QRS wave and thus to bring the triggering potential of the flash unit below threshold level for activation of the electronic flash. Thus, the last QRS complex before the base line was moved was matched to the last
A cineradiographic image of the intact dog heart showing papillary muscle markers. The single small white arrow points to the QRS light marker (white dot). The two white arrowheads point to the barbed broaches. The larger radio-opaque spots are assumed to be birdshot pellets located subcutaneously.

Flash signal on the cine frame. Confirmation of the match was obtained by repeating the maneuver. Moreover, longer cycles on the recorded ECG (due to sinus arrhythmia) corresponded to an appropriately increased number of frames between the matching flash-marked frames.

The cineradiographic filming rate was 30 frames/sec. In three dogs an additional filming sequence at 60 frames/sec was also obtained. Frames were numbered sequentially starting with 1 for each filming sequence.

The metal markers may be thought of as forming the ends of a spatial vector that varies in length and orientation throughout the cardiac cycle. The spatial vector projections along the x- and y-axes were determined from the superior plane and those along the x- and z-axes were determined from the inferior plane. Measurements were made from the cine films taken in the two orthogonal planes in the following manner (Fig. 2). An optical enlarger projected the image of a single cine frame (Fig. 3) onto graph paper with fixed distances from film to lens and lens to paper. The center of each metal marker image was plotted with the edge of the image parallel to the graph-paper grid. Care was taken to maintain constant optical magnification of all cine frames obtained in both orthogonal views during each experiment. The length of the line joining the two plotted points on the paper as well as the projections of this line on the horizontal and vertical axes were measured in graph-paper units. B is the distance between the points on the superior plane and A is the distance between the points on the inferior plane. The projections of A and B on the x-, y-, and z-axes were x, y, and z. There were two sets of z values, one from each projection; this procedure was repeated for each frame.

Calculation of spatial distances between markers from orthogonal projections (obtained sequentially rather than simultaneously) was possible only for the averaged maximum and minimum intermarker distances. Graphs were made of the A and B distances plotted against the frame number. Successive frames represented time intervals of 33.3 msec. The time course of distance between the markers in one projection is presented in Figure 4A. For each view, the maximum distances (diastolic) for a given cardiac cycle were calculated from the average value of the two or three frames at the peak of the curve to minimize the effect of errors in individual measurements. The mean ± SD of these averaged maximum A distances of all cardiac cycles during a cine sequence was then calculated; the mean ± SD was calculated in a similar manner for the averaged minimum A (systolic) distances. Similar calculations were made for the B values (Table 1). The corresponding mean x values (x max and x min), mean y values (y max and y min), and mean z values (z max and z min) were also similarly calculated for the same frames.

Since both image planes were parallel to the long axis of the dog, the x measurements along the abscissa in each plane represented the projection of the spatial vector along an axis parallel to the dog’s long axis and should therefore be identical in both projections. However, unless the markers were in the center of the X-ray beam and located on the axis of rotation about which the fixed X-ray source and input phosphore of the image intensifier rotated, different magnification of the intermarker distance was obtained in the two orthogonal images. Accordingly, the measured differences in x values were assumed to be due to differences in the marker locations relative to the X-ray source in the two projections. These magnification differences were quite small (less...
Distances between Papillary Muscle Markers Projected on Orthogonal Planes

<table>
<thead>
<tr>
<th>Muscle length (arbitrary units)</th>
<th>Maximal diastolic length</th>
<th>Minimal systolic length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Dog no.</td>
<td>Body weight (kg)</td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>101</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>78</td>
</tr>
</tbody>
</table>

N = Number of determinations included in the average.

Conversion of graph-paper units to equivalent centimeter units was not necessary for the calculation of percent shortening or velocity of muscle shortening or lengthening, because by normalizing muscle lengths the actual units were removed.

Results

Microscopic examination of the papillary muscle revealed minimum tissue disturbance around the barbed broach metal markers. After removal, the barbed broaches were found to be covered with a thin layer of fibrous scar tissue. Microscopic sectioning and standard staining procedures showed that the void in the muscle due to removal of a broach was 1 x 1.1 mm. There was no microscopic evidence of scarring or inflammation beyond the void. The barbed broaches were apparently stabilized by the...
covering layer of collagen and were essentially nonirritating as reflected by the normal-appearing myocardium.

Figure 4A is a typical plot of the distances between the papillary muscle metal markers obtained by single-plane cineradiography. The individual data points are 33.3 msec apart (filming rate of 30 frames/sec). Except at greatly elevated heart rates, which usually resulted from the administration of cardioacceleratory agents, the greater filming speed of 60 frames/sec had no obvious advantage; however, it did greatly increase the time and the effort needed for the frame-by-frame analysis. Similar conclusions have been reached by Bove et al. (19) from their studies of the beating canine heart. From the simultaneously recorded ECG and the locations of the appropriate QRS complex on both the ECG trace and the film, P and T waves were located. Under the experimental conditions, the distance between markers slowly but progressively increased during much of diastole. Atrial contraction, which was determined from the location of the P wave, apparently caused a slight further increase in the distance between markers. After ventricular excitation, the extent of shortening of the papillary muscle was substantial and reproducibly consistent (Table 1). Shortening appeared to be in progress within 1 or 2 frames of the QRS complex (30–70 msec) and reached its maximum within 2–4 frames after the peak of the T wave (60–130 msec). Lengthening of the muscle was always a more rapid process than muscle shortening.

The means of the maximum velocities of shortening and lengthening shown in Table 2 were 1.08 ± 0.29 muscle lengths/sec and 1.39 ± 0.48 muscle lengths/sec, respectively. The velocity of muscle lengthening was approximately 30% faster than the velocity of shortening. In Figure 4A, where the entire cardiac cycle was approximately 1,000 msec long (heart rate of 61 beats/min), the entire shortening-lengthening cycle of the papillary muscle (from the initiation of shortening to the end of rapid lengthening) was approximately 400–460 msec.

The extent of shortening per heart beat in the six dogs is presented in Table 2. Two of the dogs (nos. 2 and 5) were examined on different days with quite comparable results. The average papillary muscle shortening per heart beat for these six dogs was 22.8 ± 6.5% of the maximum diastolic length.

Beat-to-beat variability was estimated from the standard deviations of both maximum and minimum lengths (Table 1). The

**TABLE 2**

Calculated Spatial Differences between Papillary Muscle Markers

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>( D_{\text{max}} )</th>
<th>( D_{\text{min}} )</th>
<th>( % ) shortening</th>
<th>Contraction Mean ± SD N</th>
<th>Relaxation Mean ± SD N</th>
<th>(Contraction/relaxation) ( \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.61</td>
<td>2.21</td>
<td>15.3</td>
<td>0.73 ± 0.08 18</td>
<td>1.03 ± 0.24 16</td>
<td>141</td>
</tr>
<tr>
<td>2 trial 1</td>
<td>2.18</td>
<td>1.63</td>
<td>25.3</td>
<td>1.23 ± 0.21 21</td>
<td>1.70 ± 0.34 21</td>
<td>138</td>
</tr>
<tr>
<td>2 trial 2</td>
<td>2.17</td>
<td>1.58</td>
<td>27.2</td>
<td>1.65 ± 0.19 17</td>
<td>1.89 ± 0.33 17</td>
<td>115</td>
</tr>
<tr>
<td>average</td>
<td></td>
<td></td>
<td></td>
<td>26.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.13</td>
<td>0.79</td>
<td>30.0</td>
<td>1.11 ± 0.12 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.94</td>
<td>1.38</td>
<td>28.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 trial 1</td>
<td>1.87</td>
<td>1.54</td>
<td>17.7</td>
<td>1.18 ± 0.25 16</td>
<td>1.69 ± 0.39 16</td>
<td>143</td>
</tr>
<tr>
<td>5 trial 2</td>
<td>1.91</td>
<td>1.47</td>
<td>23.0</td>
<td>1.36 ± 0.19 20</td>
<td>1.90 ± 0.25 21</td>
<td>140</td>
</tr>
<tr>
<td>average</td>
<td></td>
<td></td>
<td></td>
<td>20.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.71</td>
<td>2.28</td>
<td>16.0</td>
<td>0.87 ± 0.11 20</td>
<td>0.91 ± 0.18 18</td>
<td>105</td>
</tr>
<tr>
<td>mean ± SD</td>
<td>22.8 ± 6.5</td>
<td>1.08 ± 0.29 5</td>
<td>1.39 ± 0.48 4</td>
<td>129 ± 17.0*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The velocity of shortening and lengthening was derived from the method illustrated in Figure 4B. The mean percent shortening is based on the average of the six dogs. N = number of determinations included in the average.

*\( N = 4 \).
values in Table 1 are the means ± SD from the two orthogonal views (A and B values). Variability in the end-diastolic measurements as expressed by the standard deviations was quite small (0.4-6.0%). The somewhat greater variability between the endsystolic (minimum) lengths (1-9%) was not unexpected. Some of the increased variability was almost certainly due to the fact that a much higher filming speed would be necessary to record more accurately the very rapid length changes at the extremes of shortening. Since the shortest distance may not have been filmed and since the calculated minimum lengths were average values, the actual extent of shortening of the papillary muscle was probably slightly underestimated.

Discussion

The metal markers, particularly the segments of the barbed broaches, provided stable, long-term reference points within the myocardium. Microscopic examination revealed minimum tissue disturbance about the markers. The markers were stabilized by a covering layer of collagen and were essentially nonirritating as reflected by the normal-appearing myocardium which surrounded them. Indeed, the markers were not extruded during the present study which lasted for several years. This stability and lack of tissue reaction ensured that the radiographically observed movement accurately reflected the changes in papillary muscle lengths during the cardiac cycle. Since the fibers of the papillary muscle are essentially linearly arranged along the muscle’s long axis and since the markers were placed in this axis, the marker movement also described the muscle fiber and sarcomere length changes.

The radiographic records were obtained in intact, tranquilized dogs supported in an upright position. The dogs were sedated with morphine and probably remained in a very low inotropic state. There was no real evidence that the dogs were excited or disturbed by their surroundings after the first few filming sessions; the results of these initial sessions were discarded. The low heart rates support this conclusion (Table 1). Thus, we feel confident that the technique used in the present study yielded a good index of papillary muscle fiber and sarcomere shortening during the cardiac cycle in a relatively normal dog in a basal state.

The greatest limitation of our technique was due to the lack of appropriate biplane equipment. Simultaneous orthogonal films are necessary to calculate instantaneous spatial distances. However, in our study an average maximum distance was obtained for each filming sequence. The mean maximum spatial distance was calculated from the average obtained in the orthogonal views. The average mean spatial distance was calculated in a similar fashion. Moreover, since the maximum and minimum distances for each cardiac cycle were determined from the average of several frames, these distances were almost certainly underestimated. An attempt was made to evaluate the magnitude of this underestimation. The most probable curve of the dimensional changes during the cycle was drawn visually based on the measured points. A comparison with the actual values used for calculation suggested that our technique, although reproducible and consistent, probably underestimated the actual papillary muscle shortening by several percent points.

To our knowledge, the present study is the first direct demonstration of shortening of the left ventricular papillary muscle in the intact dog heart. There was a surprisingly large amount of shortening (22.8%) during cardiac function. If end-diastolic muscle fiber-sarcomere lengths closely approach the optimum length for force production (4, 8, 20, 21), then the end-systolic lengths must be essentially at the foot of the length-tension curve. The functioning papillary muscle apparently can at times use the entire ascending limb of the myocardial length-tension curve.

If the papillary muscle is considered to be a chord of the circle representing the longitudinal cross section of the ventricle, it can readily be shown that the percent shortening in the chord should be the same as that in the circumference of the circle. It must be remembered that the distance from apex to valve equals the sum of the papillary muscle length and the chordae tendineae length. When these dimensional relationships were examined in a few hearts that were arrested with potassium and fixed with formalin in situ in closed-chest dogs, the papillary mus-
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load. In a study of open-chest animals, Salisbury et al. (34) directly recorded force along one of the chordae tendineae that connects to the aortic leaflet of the mitral valve. Their recorded forces were a summation of the forces that arose from the papillary muscle and the ventricular wall as well as the forces that were borne by the mitral valve itself. During the ejection phase of the cardiac cycle, the recorded forces were substantial (50–250 g) and either fell, remained relatively constant, or even rose during ejection, depending on conditions. Nevertheless, even though their results are not simply interpreted in relation to the forces produced by the papillary muscle, it is clear that there was a substantial force on the papillary muscle during the ejection phase. Unfortunately, because of the open-chest condition of the preparations and other complications, it is hard to extrapolate information directly to the present study. However, more recently Cronin et al. (13) have directly recorded substantial systolic forces produced by the in situ papillary muscle in the intact left ventricle of open-chest dogs. From their study it seems reasonable to assume that the in vivo papillary muscle does support a substantial load during ejection, and at the same time, according to the results of the present study, a “maximum” amount of shortening is occurring. The present results are rather surprising in view of the mechanical results derived from isolated muscle studies of isotonic shortening (2, 5). Isolated muscles stimulated under isometric conditions, when released with various afterloads, approach the point on the length-tension curve appropriate to that tension (4, 35). The less the afterload, the greater the extent of shortening. However, to get the percent shortening obtained in the present study, the isolated papillary muscles must shorten almost under conditions of zero afterload (as in a quick-release preparation). Yet experimental data on isolated cardiac muscle (2) as well as skeletal muscle (36) suggest that maximum rapid shortening is incompatible with large tension development, possibly because the so-called active state may be reduced by rapid length changes (quick stretch or quick release [37]). Recently, however, data obtained by Edman and Nilsson (6) using damped releases, which may more nearly duplicate in vivo conditions, suggest that

cle length was approximately 70% (65–73%) and the chordae tendineae length was approximately 30% of the total apex-valve length. Therefore, the 22.8% papillary muscle shortening resulted in only a 16% shortening of the total length. It is interesting to compare these results with reported left ventricular dimensional changes during systole in intact closed-chest dogs. Sonnenblick et al. (20) estimated that a 16–20% change in the circumference of a sphere was necessary to expel a normal stroke volume (43–50% of end-diastolic volume). Furthermore, this value is virtually identical to those determined in cineradiographic studies on intact, unanesthetized human subjects who had silver-tantalum clips sutured to the external surface of their right and left ventricles (22–24). Both left (Fig. 9 and 10 in ref. 24) and right (Fig. 2 in ref. 22) ventricular length changes closely approximate our 16% estimate. Since these measurements were made from a single plane, the values must be considered as relative values. The change in base-to-apex length (major axis) recorded in the biplane cineradiographic studies of Wiedenthall and Mitchell (25) and Leshin et al. (26) also closely approximates our calculated values. Quite similar values were also found in the biplane cineradiographic study of Bove and Lynch (27) on intact dogs receiving radioopaque contrast injections. They observed a 14.5% decrease in left ventricular length.

Length changes occur not only because of shortening during left ventricular ejection but also because of changes in the physiological state. For example, end-diastolic volume, which is maximum in the completely relaxed, recumbent normal dog, becomes smaller under other conditions, even with the simple lifting of the dog's head in response to an unexpected noise (28). The role of papillary muscle shortening under tension in the functioning heart may be to adjust to these length changes during systole so that the remainder of the mitral valve apparatus (chordae tendineae plus mitral valve) does not become slack and thereby permit mitral regurgitation. This theory is supported by experimental evidence in dogs (29, 30) as well as by data obtained in certain pathological states in humans (31–33).

It should be emphasized that the observed large percent of shortening (22.8%) must be occurring with the muscle under significant
both large tension development and considerable shortening can occur simultaneously. The reasons for the discordant results obtained between the papillary muscle shortening relationships in the isolated papillary muscle preparation and those relationships in the intact animal are obscure. These differences, which need to be confirmed, are interesting and merit further investigation.

Addendum

Preliminary studies on three dogs using newly acquired biplane cineradiographic equipment to obtain simultaneous orthogonal views appear to confirm the validity of the results described in the present paper.

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References

17. BURCH GE, DEPAQUALE NP: Time course of tension in papillary muscles of the heart. JAMA 192:117-120, 1965
20. SONNENBLICK EH, SPIRO D, SPOTNITZ HM: Ultrastructural basis of Starling’s law of the heart: Role of the sarcomere in determining ventricular size and stroke volume. Am Heart J 68:335-346, 1964

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LEFT VENTRICULAR PAPILLARY MUSCLE SHORTENING

35. KILEN SM: Characteristics of the mechanical response and "active state" of rat cardiac muscles. Ph.D. Thesis, University of Illinois at the Medical Center, Chicago, 1971
37. KAUFMANN RL, BAYER RM, HARNASCH C: Autoregulation of contractility in the myocardial cell. Pfluegers Arch 332:96-116, 1972
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