"Pressure-Volume" Relations in Isolated Cat Trabecula

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SUMMARY We studied isolated cat trabecula under conditions closely resembling those present for muscle fibers in the left ventricular wall. The purpose of the study was to see if muscle contraction under those circumstances could be described by a time-varying compliance as reported for intact canine left ventricle. We found the time of the end of systole to depend on the history of contraction. This time varied between 100 and 180 msec as measured from the onset of contraction. Similar dependency, although less percentage-wise, was found by reanalysis for intact canine left ventricles. We concluded that the behavior of the canine left ventricle as a time-varying compliance may be related to the complex organization of the cardiac muscle fibers in the wall of the heart rather than to muscle properties. Circ Res 49: 388-394, 1981

THE viscoelastic model of contracting muscle represents the muscle as a system of elastic (springs) and dissipating elements (dashpots). At the beginning of this century, such models received considerable interest in an attempt to describe the fundamental processes of skeletal muscle contraction. Over the last 10 years, comparable electrical analogs, time-varying compliances (springs) with or without resistors (dashpots), have been used to describe the behavior of the cardiac pump.
be due to differences in the type of muscle or related to the complex organization of the cardiac muscle fibers in the wall of the heart (Streeter et al., 1969).

This study questions the validity of the time-varying compliance model to describe the mechanical behavior of isolated cardiac muscle, studied under conditions chosen to resemble the intact heart situation as closely as possible. Such a preparation circumvents the effects of the complexities of myocardial geometry.

**Methods**

**Muscle Preparation**

Male adult cats (2250-2900 g) were anesthetized with thiopental, 45 mg/kg, ip. A midsternal thoracotomy was performed while the animals were ventilated. A ligature was placed around the aorta, and heparin (5000 IU) was given intravenously. The aorta was then cut and a cannula was introduced into its proximal end to provide retrograde coronary perfusion at a pressure of 80 mm Hg with oxygenated Tyrode's solution (Elzinga, 1972). When the coronary venous fluid, dripping from the heart, was completely clear, the heart was transferred to a preparation bath. Retrograde coronary perfusion then was ended.

The right ventricle was opened carefully and a thin, cylindrically shaped trabecular muscle (a in Fig. 1) was selected (see Table 1 for cross-sectional areas). The muscle was dissected free from the heart and one end was tied with a thin (10-0) monofil suture to a small platinum hook made of platinum wire 0.1 mm in diameter (b). The other end of the muscle remained attached to a relatively large piece of myocardium. The muscle was mounted between the force transducer (b) and the puller arm (g). The lump of tissue at the puller end was gripped by four stainless steel hooks at the origin of the muscle. The muscle bath was perfused with temperature-controlled oxygenated Tyrode's solution at 38°C. Two platinum stimulating electrodes were placed along the sides of the muscle.

**Force and Length Control**

The muscle puller (i in Fig. 1), used to control either the length or the force of the muscle, was constructed from a loudspeaker (Philips AD125HP4). Moving parts were made as light as possible and movement was restricted to one axis only by stiff suspensions. The maximum stroke of the puller was 3 mm: 2 mm forward and 1 mm backward.

Changes in muscle length were measured by a position transducer (n) made of two light-emitting diodes (HP 5082-4420) (r) and two silicon photovoltaic readout segmented cells (Sensor Technology, STN-EC-200-100-600S) (q). The output of the two light-sensitive elements was summed and the sum was fed back and kept constant by adjusting the output of the light emitting diodes. The ampli-

**TABLE 1**

<table>
<thead>
<tr>
<th>Muscle Size, Preload, and Developed Stress</th>
<th>23-10</th>
<th>25-10a</th>
<th>25-10b</th>
<th>26-10</th>
<th>29-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>0.060</td>
<td>0.072</td>
<td>0.034</td>
<td>0.037</td>
<td>0.058</td>
</tr>
<tr>
<td>Working muscle length (mm)</td>
<td>3.2</td>
<td>3.4</td>
<td>3.0</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Preload (N·mm⁻²)</td>
<td>0.0016</td>
<td>0.0019</td>
<td>0.0031</td>
<td>0.0027</td>
<td>0.0016</td>
</tr>
<tr>
<td>Developed isometric stress at working length (N·mm⁻²)</td>
<td>0.046</td>
<td>0.033</td>
<td>0.036</td>
<td>0.057</td>
<td>0.042</td>
</tr>
</tbody>
</table>

**FIGURE 1** Isolated heart muscle set-up. The muscle (a) is attached on one side (b) to the force transducer and gripped at the other side by four bent stainless steel pins (c). The muscle bath (d) is perfused with Tyrode's solution and the overflowing fluid (e) is pumped back to a central reservoir (not shown). The muscle bath is surrounded by a water jacket (f) to minimize temperature loss. The bent stainless steel pins (c) are attached to an arm (g) and the force transducer to a micrometer (h). The arm (g) connects the muscle with the muscle puller (i) via a cone (k) and a coil (l). The coil is suspended in a magnetic field (m). The suspensions, which are not shown in the figure, allow movement of the coil, cone, arm, pins, and muscle assembly along one axis only. The position of this assembly is registered by a position transducer (n). The working principle of the position transducer is given in the right upper inset of the Figure. It consists of a vane (p), attached to the cone (k), which moves between two light-emitting elements (r) and two light-sensing elements (a). The sum of the outputs of the light-sensing elements is used to stabilize the position transducer; the difference can be used to record muscle length changes. The left upper inset shows tracings of muscle length and force of a contraction controlled in such a way as to imitate the ventricular contraction phases in the intact heart.
tude frequency response of the length measurement thus obtained was flat to over 10 kHz, its noise level was equal to 5 µm, and its linearity was such that the voltages obtained were within ±0.5% over the range of positions used in this study. The closed loop position feedback amplification resulted in a −3 dB point of the amplitude frequency response at 1.1 kHz.

The force transducer (Aksjeselskapet Mikro-Elektronikk AE 801) was made watertight and insensitive to light. The compliance of the force-measuring device was 1 µm/mN. Its frequency response in the bath with a muscle attached was approximately 5 kHz with no detectable creep. The noise level of the force measuring system was 1.5 mg. The amplification in the closed loop force control system was optimized in each experiment, since the gain in the force control loop is different for different muscles. Because the phase of maximum stiffness determines the optimal setting of the feedback amplifier, force control at rest and during relaxation is not optimal. To improve on this, the closed loop force feedback amplification was modulated during contraction with a factor equal to f(F + Δl), where F is force, Δl the change in length, and f an adjustable factor.

Experimental Conditions

The experimental conditions were chosen such that the contraction of the isolated trabeculum resembled closely that of the myocardial fibers in the wall of the isolated cat heart used in our earlier studies (Elzinga and Westerhof, 1973, 1978; Elzinga et al., 1980). Therefore, the experimental animals were taken from the same stock of male adult cats and experiments were performed at about 37.5°C. Temperature differences between experiments were less than 1°C. The oxygenated perfusion fluid used for the muscle experiments was the same as used for our cardiac studies; PO₂, Pco₂, and pH for the bathing solution were 492 ± 8 (SD) mm Hg, 35.1 ± 1.8 (SD) mm Hg and 7.31 ± 0.03 (SD), respectively. A stimulation frequency of 2 Hz was used which corresponds to most of our studies on the isolated cat heart.

We have assumed that the muscles should at least be able to generate isometrically 2.5 times the stress present in the left ventricular wall under normal ejecting conditions [100-200 g/cm²] in circumferential direction (Burns et al., 1971; Huysman et al., 1980). All muscles used in this study could fulfill this criterion (see Table 1). The resulting length at which our muscles were studied (working length) in relation to lmax is shown by example in Figure 2A.

The contraction mode of the isolated heart muscle was made comparable to that of the intact heart as shown in the inset of Figure 1 and described by Brutsaert and Paulus (1977), Paulus et al. (1979), Sulman et al. (1974), Westerhof and Elzinga (1978), and Wiegner and Bing (1977, 1978).

When one wants to simulate muscle contraction of the myocardial fibers in the wall of the ventricle, one has to account for the shape of the cavity and the direction of the muscle cells. We have chosen a thin-walled cylindrical model with fibers running in the circumferential direction, i.e., the 2D pump described by Gabe (1974). The relationships between force and pressure and between length and volume in this model are given by:

\[ V = k_1(l - \Delta l)^2 = k_2 l^2 \]  
\[ P = k_3 F/l \]

where V is volume, l is muscle length, k₁ is resting muscle length, Δl is the change in muscle length, P is pressure, F is force, and k₁ and k₂ are constants. These relationships were part of the muscle control unit such that P and V could be controlled instead of length and force.

A comparison between heart and muscle in this way may lead to a confusing terminology because now pressures and flows may be measured in cardiac as well as in muscle experiments. To avoid confusion we have placed words like pressure, flow, isovolumic, etc., in quotes when data were obtained from muscle experiments through the 2D model.

Systemic arterial input impedance was modeled by a direct electric analog R-C-R network (Fig. 2B). The hydraulic version of the same network has been used as the load for our isolated heart studies (Westerhof et al., 1971). An electrical current proportional to the positive part of the first derivative of the "volume" change ("aortic bloodflow") was taken as input for this network. The resulting volt-
age was used as the "aortic pressure" loading the "ventricle," i.e., the 2D pump. The control values of the resistances and the compliance in the network were chosen to be comparable with our isolated cat heart studies. Control "pressure" in the 2D pump during ejection should be about 40% of peak "isovolumic pressure." This value was obtained by setting the resistance $R_p$, which represents the peripheral resistance. Total arterial compliance was chosen in such a way that an $R_pC$ time of 1 second was obtained (Westerhof et al., 1971) and resistance $R_c$ then was set to a value of 10% of that found for the resistance $R_p$. We could also introduce a constant "pressure" level in the "arterial system" as a load for the pump. It was possible to switch from one load (R-C-R network) to the other (constant "pressure" level) on a beat-to-beat basis.

Experimental Protocol, Recording, and Analysis

After the muscles had been mounted and had contracted isometrically for about 20 minutes at a stimulation frequency of 2 Hz at approximately the correct peak stress level, the contraction mode was changed such that a normal cardiac contraction was imitated. This situation is referred to as the control situation. Using the control situation as a steady state, one out of every ten contractions was given a different fixed value for the "arterial pressure" (for an example, see Fig. 3). This series of beat-to-beat load changes started and ended with an isometric contraction and contained about 10 different fixed "pressure" levels. At the end of the experiment the length-tension relationship was determined on a steady state basis. Finally, muscle width in the middle of the preparation was measured with the microscope at 40x in two perpendicular directions. Cross-sectional areas were calculated assuming an ellipsoid.

"Aortic pressure," "ventricular pressure," "aortic flow," "ventricular volume," muscle force, muscle length, stimulus, and trigger pulses were recorded on an Elema Schönander ink recorder (EMT 81) and an SE analog tape recorder (SE 7000). For display of superimposed tracings, use was made of a storage oscilloscope (Tektronix R5103N). For the measurements of peak values, values at a given time, and average values, use was made of an analog device especially designed for such a purpose (Puls and Elzinga, 1978).

Results

In five stable experiments, difference in peak isometric "pressure" at the start and end of each experiment was less than 3.5%, and "aortic pressure" was changed for one cycle every tenth beat to a preset value while the muscle was still working under the control conditions. A recording of "aortic pressure," "ventricular pressure," and "aortic flow" is shown in Figure 3. Table I gives sizes and isometric peak forces of these preparations.

When the contractions obtained by varying "aortic pressure" on a beat-to-beat basis during the control situation are superimposed, a picture like the one shown in Figure 4A is obtained. Because fixed "pressure" levels have been used during "ejection," the end of systole—i.e., the point where "pressure" starts to fall—can be seen easily. This point is not reached for all contractions at the same time. This variation in occurrence of the end of systole is presented for all five experiments in Figure 4B. The different symbols used in this plot represent the different experiments. It is clear that the pattern is very consistent in all muscles. A small amount of shortening—i.e., a high level of end-systolic "pressure"—lengthens the duration of systole considerably. Shortenings at "pressures" lower than 75% of "isovolumic" have the opposite effect.

The data presented in Figure 4 show that isolated heart muscle in a 2D model of the ventricle does not behave as a time-varying compliance according to the formula (Suga et al., 1973):

$$E(t) = \frac{P(t)}{V(t) - V_d}$$ (3)

where $E$ is the stiffness, $P$ pressure, $V$ volume, and $V_d$ volume.

![Figure 3](https://example.com/figure3.png)

**Figure 3** "Aortic pressure" was changed once every 10 beats to a fixed level of variable magnitude. Two such contractions are shown. The "pressure" level of the second one is higher and "flow" correspondingly lower. "Pressures" and "flow" are given as percentages of their maximum value.
FIGURE 4 A: Superimposed tracings of the effects of a series of different "arterial pressures" on "ventricular pressure" and "ventricular volume." The various "pressure" levels were obtained on a beat-to-beat basis (see Fig. 3). B: The moment of the end of systole, corresponding in Figure 4A to the point at which "pressure" starts to fall, related to the end-systolic "pressure" level. The five different symbols correspond to the five muscles studied. "Pressure" and "volume" are given as percentages of their maximum values. The end of systole corresponds to the moment of occurrence of "Em.*" i.e. the maximum value of the "pressure-volume" ratio during the cycle.

Vd the intercept of the pressure-volume relationship on the volume axis. In Figure 5, the "pressure-volume" relationship is given at different times during the contraction. The data presented were obtained from one of the experiments but are fully representative of what was found in the other four muscles (see Fig. 4B). The graph is constructed by relating the "pressures" with the corresponding "volumes" obtained from a series of beats "ejecting" at different "pressure" levels such as shown in figure 4A. In the example of Figure 5, peak "isovolumic pressure" is reached at 120 msec. It is clear that the 120-msec "pressure-volume" relationship differs from the other maxima of the "pressure-volume" ratio (unfilled circles). The times given in the graph for the unfilled symbols correspond to those presented in Figure 4B. Figure 5 shows that the shape of the instantaneous relationship between "pressure" and "volume" changes with time and that no single unique "Em.*" line exists, independent of the loading conditions. Moreover, it seems to be very unlikely that the family of lines does have a common intercept on the "volume" axis.

Discussion

We have used for this study muscles with a diameter less than 325 μm, and we found no indication that the performance of such muscles was inhibited by limitations in diffusion of oxygen or substrates. If metabolic depression did occur due to inadequate supply by diffusion, it would be likely to take place during the first 2 minutes after the onset of stimulation when isometric force increases considerably. However, an increase followed by a decrease in force during this time is found only when muscles with a diameter larger than approximately 500 μm are used. Peak stresses generated isometrically by the isolated trabecula (Table 1) were as high as those predicted for intact left ventricles in the circumferential direction (Huisman et al., 1980). This is another sign of the absence of anoxic regions. Furthermore, on the basis of the diffusion equation given by Hill (1965) for a cylindrical muscle, we calculated the critical diameter which is still compatible with an adequate supply of oxygen. If one assumes an oxygen consumption of 10 ml/min per 100 g, a critical value of 450 μm is obtained. The fact that myoglobin probably facili-
tates oxygen transport in myocardial tissue is neglected in the calculation.

Over the last 10 years, several studies have appeared (Suga et al., 1973; Suga and Sagawa, 1974; Sagawa, 1978; Westerhof and Elzinga, 1978; Suga et al., 1979) showing evidence suggesting that the left ventricle can be regarded during systole as a simple time-varying compliance of the type given in Equation 3. However, the present study shows that such a behavior of the left ventricle cannot result from basic myocardial contractile properties alone.

When the instantaneous "pressure-volume" relationship is determined at various times (Fig. 5), its time dependence is dissimilar to that reported for the intact left ventricle (Suga et al., 1973; Suga and Sagawa, 1974) or that implied by Equation 3. For the muscle, we found neither a linear relationship nor an intercept on the "volume" axis which could be regarded as the center of rotation (Vd in Equation 3). The findings for the muscle resemble somewhat more those obtained in the right heart where no center of rotation on the volume axis was found (Maugham et al., 1979). This suggests either that right ventricular myocardium differs from that of the left or that left ventricular geometry may be of major importance for the pressure-volume relationship obtained for that cavity. If the latter is true the 2D pump seems to be a model with very limited possibilities.

From Figure 4A it should be clear that plotting the "pressure-volume" relationship for times later than peak "isovolumic pressure" has no use. Compare for instance the "isovolumic pressure" with the "pressure" of the contraction with the smallest amount of shortening at the moment when relaxation starts in the latter. At this moment, "pressure" of the contraction where shortening was measured is even slightly higher than the "pressure" level found in the "isovolumic" beat. This is in contrast to what happens in a time-varying compliance.

In the present experiments, a definite load dependency was found for the time to "E_{max}" (Fig. 4B). The point in time at which "E_{max}" was reached is undoubtedly better defined in the present experiments than in the intact left ventricle because it shows up as a clearly defined corner in the pressure record. This may be of some relevance for the explanation of this discrepancy, but it is unlikely that this can fully solve the problem.

The finding that the moment at which "E_{max}" is found (Fig. 4B) depends on the history of contraction could be related to the use of the 2D model for ventricular geometry in this study. The influence of models of ventricular geometry can be eliminated by using the force-length relationship instead of a "pressure-volume" relationship. An example of such an experiment has been shown by Elzinga and Westerhof (1980b). From this experiment, which is fully representative of other similar experiments, it must be concluded that the absence of the 2D model does not take away the dependency of the time at which "E_{max}" occurs on the history of contraction.

Instead of making use of the 2D model and isolated trabecula, one can study intact left ventricles to determine the time at which E_{max} is attained. We measured the duration between the onset of contraction and the end of systole in five isolated cat hearts (experiments described by Elzinga et al., 1980). The method of measurement employed is shown in Figure 6A. The onset of

![Figure 6](http://circres.ahajournals.org/)

**Figure 6**: A: In intact left ventricles (Elzinga et al., 1980) the time between the onset of contraction and the start of relaxation was measured. The method of measurement is shown. B: The times found, using the method shown in A, are plotted as a function of stroke volume (open symbols) expressed as a percentage. The dashed line is obtained by reanalysis of one of the experiments using pressure (stroke) volume loops to determine the end of systole instead of the method shown in A. The relationships found are compared to those determined for the isolated trabecula (filled symbols).
contraction was found by defining the moment when left ventricular pressure started to rise. The end of systole was taken as the time at which the aortic blood flow signal crossed the baseline line. In the pressure-volume loop, the latter moment in time corresponds to the highest pressure value at the end-systolic volume. The results of this analysis are shown in Figure 6B. For one of the experiments (dashed line through black dots), we measured the end of systole also from pressure-stroke volume loops, but the end of systole is less well defined by that method. A comparison is made with the data obtained from the isolated trabecula which also were presented in Figure 4B, although in a different manner. Now times are not related to a fixed pressure level but plotted as a function of "stroke volume" because aortic pressure was not kept precisely constant in the cardiac studies. The way in which the time of "E_max" changes with alterations in output is similar in intact ventricle and isolated muscle, although, on average, the duration is longer in the intact hearts. It must be concluded that viscoelastic models are untenable for heart muscle as was shown long ago for skeletal muscle. Therefore, the findings for intact hearts which show that the left ventricle can be regarded as a time-varying system rather than to basic myocardial properties.

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