Passive Stiffness of Isolated Cardiac and Skeletal Myocytes in the Hamster

Daniel Fish, Jan Orenstein, and Sherman Bloom
From the Department of Pathology, George Washington University Medical Center, Washington, D.C.

SUMMARY. Single cardiac myocytes and skeletal myocyte fragments, devoid of interstitial collagen but with intact glycocalyx, were prepared by mechanical disaggregation of hamster ventricular myocardium and caudal gracilis muscle, respectively. Passive stiffness was studied by examining the sarcomere length-tension relationship over the approximate Eulerian stress range of 0-20 mN/mm² for cardiac myocytes and 0-120 mN/mm² for skeletal myocytes. Creep and stress-relaxation became apparent only when cells were stretched to sarcomere lengths close to, or exceeding, 2.2 μm for the cardiac myocytes, and 2.7 μm for the skeletal myocytes. Stress-relaxation and creep occurred simultaneously, suggesting that the sarcomere is at least one of the structural components responsible for viscoelasticity. The differential strain stiffness constant was calculated from the regression of natural stress [Ln(mN/mm²)] against differential strain [(L-Lo)/Lo] and found to be 7.48 ± 1.73 for the ventricular myocytes and 5.77 ± 0.87 for the skeletal myocyte fragments. The natural strain stiffness constant was obtained from the regression of natural stress against natural strain [Ln(L/Lo)]. The natural strain stiffness constant was 30-50% higher than the differential strain constant. The high correlation coefficients obtained for both regressions indicate that the length-tension relationships for these isolated cardiac and skeletal myocytes can be very closely fitted to the single exponential function, \( a = C \cdot \exp[K't] \). The length-tension curves obtained for the skeletal myocyte fragments are qualitatively and quantitatively similar to those obtained with intact skeletal muscle. The cardiac myocyte length-tension curves are qualitatively, but not quantitatively, similar to those obtained with cardiac muscle. Isolated ventricular myocytes are stiffer than similarly isolated skeletal myocytes. These findings suggest that cellular structures contribute to myocardial stiffness in the hamster. (Circ Res 54: 267-276, 1984)

PASSIVE stiffness at long sarcomere lengths is much greater in cardiac muscle than in skeletal muscle, and may function to limit excessive diastolic stretching (Braunwald et al., 1976). This restriction of diastolic stretching is a critical function, inasmuch as, without it, pathological ventricular dilatation would occur. On the other hand, excessive stiffness is undesirable because it reduces contraction velocity (Pollack, 1970; Mirsky et al., 1981; Natarajan et al., 1979). The sites of the stiffness-inducing structures in cardiac muscle are unknown. Many intracellular anatomic sites have been suggested as candidates, including the sarcolemma, residual actomyosin crossbridges, actin-dependent "intracellular structures," actin links across the H zone (S filaments), myosin-Z-line connections (C filaments), and Z-line-to-Z-line connections. These studies have been reviewed by Sonnenblick (1974).

Recent studies have reported the isolation and characterization of an intracellular elastic protein, connectin, thought to be the source of the parallel elastic element (PE) which is responsible for cardiac stiffness at long sarcomere lengths (Maruyama et al., 1977a, 1977b; Fabiato and Fabiato, 1978). Fabiato and Fabiato showed that the passive mechanical properties of "skinned" cardiac myocytes (sarcolemma removed) are highly variable, depending on the particular species of animal under study. Their results suggest an intracellular site for the high resting stiffness of intact rat cardiac muscle, whereas frog and dog cardiac muscle stiffness depends more on elements in either the cell surface complex or extracellular connective tissue.

Interstitial collagen is attractive as a source of diastolic stiffness (Borg et al., 1981a, 1981b), especially in view of the physical properties of collagen and its widespread function in loadbearing (Fung, 1981). However, the interstitium does not show an anatomic arrangement that suggests a specific role in generating diastolic stiffness. That is, there is no clear longitudinal component to the system of interstitial collagen fibers. If collagen were the PE, its role would be mechanically complex, and depend on longitudinal stress vectors of nonlongitudinal fibers.

Data concerning the correlation between age, collagen content, and various indices of intrinsic myocardial stiffness tend also to be species dependent (Mirsky et al., 1976; Kane et al., 1976; Weisfeldt et al., 1971). The observed increase in ventricular distensibility with increasing age in the rat (Mirsky et al., 1981) is interesting, in light of the fact that an elevated level of connective tissue in the heart of the old male rat has been well established (Gay and...
stiffness of hamster myocardial tissue is therefore reflected in isolated cardiac myocytes. An intracellular appearance to be qualitatively but not quantitatively different from the glycocalyx, but devoid of interstitial collagen, poses a question about the cell-surface complex that could be a source of passive stiffness is the system of unbanded fibrils, or "cell-surface cables," that are present on the surface of heart muscle cells (Orenstein et al., 1980). These cables are partially buried in the glycocalyx, and, in contrast to interstitial collagen, are largely parallel to the long axis of the cell. The orientation of these cables suggests that they could contribute to diastolic stiffness.

The mechanical disaggregation procedure, by which cells were prepared in this study, has been shown to yield cells with an intact glycocalyx (Orenstein et al., 1980). Cell surface cables, roughly parallel to the long axis, and partially embedded in the glycocalyx, are abundant. Interstitial collagen, however, is absent, except for rare random strands. Although the glycocalyx appears intact, the cells behave as though their membranes are leaky, in that they present no permeability barrier to calcium or many other substances, ionic and non-ionic (Bloomstein et al., 1980; Tsokos et al., 1977). This leakiness may be due to occasional physical defects in the cell surface, or to biochemical factors. The important point is that, although the cells are leaky, the cell surface complex is morphologically intact. For this reason, cells prepared by mechanical disaggregation might be suitable for testing the hypothesis that the PE resides within hamster cardiac myocytes or their immediate surface coat, rather than the interstitium. An intact glycocalyx is also present on skeletal myocytes obtained by this method (Caceci et al., 1981). Therefore, comparison of the mechanical properties of these two myocyte preparations is justified.

In an attempt to further narrow the range of possible choices for the anatomic locus of the PE, we have studied the passive mechanical properties of single cardiac myocytes and skeletal myocyte fragments in the hamster. The aim of this work was to determine whether single myocytes, with intact glycocalyx, but devoid of interstitial collagen, possess passive mechanical properties similar to those of intact cardiac muscle in the hamster. The passive mechanical properties displayed by these isolated myocytes that are of interest in intact muscle include the nonlinear length-tension relationship, a linear relationship between natural stress and natural or unstressed shape. It is a dimensionless quantity. Both Lagrangian and Eulerian strain are used in muscle mechanics. Lagrangian strain assumes (among other things) a constant cross-sectional area for materials subjected to variable amounts of stress. Eulerian strain assumes that for large deformations, defined as greater than 0.2%, the physical dimensions of stressed materials undergo significant alteration. Therefore, Eulerian stress-strain relationships are evaluated on the basis of instantaneous cross-sectional area. Eulerian theory is particularly applicable in studies such as this, since muscle undergoes large deformation over the range of load applied. Values of stress calculated using either Lagrangian or Eulerian strain are referred to as Lagrangian or Eulerian stress, respectively.

Two different mathematical expressions for strain are used in this study and the results are compared. 

\[(L-Lo)/Lo: \text{"differential strain" (DS)}\]

\[Ln(L/Lo): \text{"natural strain" (NS)}\]

Either of these expressions of strain are applicable to the study of both Eulerian and Lagrangian stress-strain relationships.

Methods

Heart muscle cells were prepared from Syrian golden hamsters, as previously described (Orenstein et al., 1980). The same method was applied to skeletal muscle cells obtained from the caudal portion of the gracilis muscle. The hamsters were male, random bred, 100–130 g, and 2–3 months old. In brief, about 500 mg of left ventricular myocardium were homogenized in a VirTis-45 homogenizer in 25 ml of ice-cold homogenization medium (HM) containing, in mmol/liter: KCl, 135; Tris buffer at pH 7.1, 50; MgCl₂, 2; ATP, 2; Na⁺, 1. The homogenate was passed through a 100-mesh stainless steel screen in a 2.5-cm Swinnex filter holder (Millipore Filter Corp.) to remove large fragments and sedimented once at 150 g for 30 seconds to remove subcellular debris. The cell pellet was resuspended in 30 ml of HM and kept at 0°C. A drop of this homogenate, containing many cells, was transferred to a plexiglass flow cell. A single cell was selected, impaled at each end with glass needles, and superfused at a constant flow rate of 0.33–0.66 ml/min with HM containing 2 mm EGTA (HM-EGTA) at 25°C. The calculated [Ca²⁺] of HM-EGTA was 10⁻⁶ M, assuring complete relaxation. Cells were selected for impalement on the basis of regularity and clarity of the sarcomere pattern and overall appearance. The glass needles used for impalement were prepared from capillary tubing, using an electrode puller. The needle tips were curved into hooks with a de Fonbrune microforge.
One glass needle, mounted on a motorized micromanipulator (Eric Sobotka Co.), served as a mechanical anchor. The other needle was attached to a high-sensitivity small displacement force transducer (Ade Corp.) mounted on a micromanipulator fashioned from a microscope base. The transducer was calibrated with wisps of thread of known mass. Transducer compliance did not influence the measurements reported here, since direct measurement of sarcomere length was used. For the same reason, high compliance at the sites of impalement would not influence the results. Tension was recorded on a strip-chart recorder. The entire experimental apparatus rested on a Micro-g vibration isolation table (Technical Manufacturing Corp.). This, in turn, was surrounded by a wind-screen, and was located in a room with minimal forced-air ventilation.

The flow cell was located on the stage of a Leitz Diavert inverted microscope equipped for 35-mm photography. A pneumatic shutter release and automatic film advance, selected for minimum vibration, were used. After impalement, cells were photographed to determine the sarcomere length at zero tension. Then the impaling microneedles were moved further apart, to produce a length and tension increment. Another photograph was taken and the tension again raised. This was continued until a set of photographs was obtained, one for each of a series of tensions. The length of the preparation was generally adjusted by hand-controlled movements of the transducer micromanipulator. Sarcomere length was determined by projecting 35-mm negative images of cells onto a surface where measurements could be accurately made. These were compared to a projected scale obtained from the photographic image of a stage micrometer. For cardiac and skeletal myocytes, the average sarcomere length at each tension increment was obtained by measuring groups of 3-40 consecutive sarcomeres and dividing by n (n = 20-150). For some cells, more than one set of sarcomeres was counted. Hence, the average number of sarcomeres counted per cell exceeds the maximum number present in any one consecutive series. Sarcomeres close to attachment sites were not included in the stress-strain analysis, due to optical interference from the microneedles, and the higher probability that these areas had sustained internal structural damage during the impalement process.

The stiffness constant, K, was taken from the equation:
\[
\ln(s) = K(e) + \ln(C)
\]
where: 
- \( s \) = stress in mN tension/mm² of x-sectional area 
- \( C \) = either natural strain \([L/(L-Lo)]\) or differential strain \([L/(L-Lo)]\) 
- \( L \) = mean sarcomere length at any given tension 
- \( Lo \) = mean sarcomere length at zero tension 
- \( K \) = a constant

K was calculated as the slope of the regression of Ln(stress) against either Ln(L/Lo) or (L-Lo)/Lo. We use the notation K(NS) or K(DS) to indicate K calculated by natural or differential strain, respectively.

The stress-strain relationships described here are not valid when stress is zero. That is, Ln(stress) does not equal \( K \) or \( C \) when Ln(stress) is zero. However, this expression is otherwise in conformity with the fundamentals of elasticity theory. Values for instantaneous stress at 2.2 and 2.6 \( \mu \)m were obtained for each cell (Tables 1 and 2) by substituting the appropriate sarcomere lengths into the natural or differential expressions for strain:
\[
\sigma = C \cdot \exp(K \cdot \ln(L/Lo))
\]

and solving for \( \sigma \). Each of these equations permits calculation of Eulerian stress, since, in both instances, instantaneous values of cross-sectional area were employed at each level of load. The cross-sectional area of the cell was calculated from the measured cell diameter, assuming the cell was circular in cross-section. Results are stated as mean ± SD. Student’s t-test was used for the comparison of mean values. The concentration of ionized calcium in the medium was calculated by the Newton-spontaneous overrelaxation method (Ortega and Rheinboldt, 1970).

After completion of the length-tension recording, the cell was superfused with HM-EGTA containing 2.5% glutaraldehyde for 45 minutes and rinsed with HM-EGTA for 5 minutes. Cells then were carefully extricated from the needles and transferred with a 10-μl micropipet to a 6 × 6 mm coverslip pretreated by a modification of the method of Sanders et al. (1975). We applied a thin film of poly-L-lysine to an acid-alcohol washed coverslip, and allowed this to dry in room air. This provided a surface to which the cells would adhere tightly for processing. The suspended cells were allowed to settle onto the coverslips overnight, at 4°C, prior to processing for scanning electron microscopy (SEM).

Cells were prepared for SEM as previously described (Orenstein et al., 1980). In brief, the coverslips were passed through increasing concentrations of ethanol up to dessicated 100%, and critical-point dried with a Samdri critical-point drier (Tousimis Research Corporation). After critical-point drying, the coverslips were mounted on aluminum stubs with cyanacrylic cement and silver paint. The stubs were then coated with 150–200 Å of 60:40 gold:pallidium, using a Denton evaporation unit and tilting mini stage (Denton Vacuum Incorporated), and scanned in a JEOL JSM-35 SEM equipped with a tungsten filament. Images were recorded, at magnifications of 20X–30,000X, on Polaroid P/N 55 film.

**Results**

The sarcomere length of ventricular myocytes suspended in HM-EGTA and photographed immediately after impalement (Lo) was 1.74 ± 0.09 μm (Table 1). This measurement was made with no tension load on the cells. In fact, care was taken to provide a small amount of slack. This sarcomere length was much greater than was noted in cells suspended in HM and not impaled for mechanical studies (Orenstein et al., 1980). This is due to the low [Ca++] of HM-EGTA, calculated to be about 10⁻¹⁰ M. In comparison, the [Ca++] of HM is about 10⁻⁷ M (Bloom et al., 1974). The long, load-free sarcomere length of impaled cells [Lo (Tables 1 and 2)] indicates that the contractile apparatus was quiescent, as would be expected at this low ionized calcium level (Fabio et al., 1976). In several early experiments, the medium was briefly changed to HM, to verify that the cardiac myocytes would show spontaneous contractile activity. This was, in fact, observed for every cell thus tested.

Cardiac myocytes selected for impalement had a mean diameter of 29.2 ± 6.3 μm. Whereas we do not have prior data on the diameter of isolated hamster ventricular myocytes, this value is not sig-
significantly different from that reported for rat cells. These have been found to be 12–40 μm in diameter (Bishop and Drummond, 1979; Carlson et al., 1978; Moses and Kasten, 1979; Powell et al., 1978). The appearance of impaled cells, by both light microscopy and SEM (Figs. 1 and 2) was that of mechanically disaggregated heart muscle cells, as previously described (Orenstein et al., 1980). Transmission electron microscopy of cells embedded in plastic directly from the coverslip, where they had been attached for SEM, revealed that selected cells were single cells. Skeletal myocyte fragments selected for impalement had a mean diameter of 62 ± 14 μm.

Cells recovered after impalement showed scant evidence, at the SEM level, of injury by the glass microtools. For cardiac myocytes, the only evidence of prior impalement was a small, barely recognizable hole in the cell surface at the impalement site (Fig. 2, c and d). Although it is obvious that impalement must produce local injury, and probably a region of high compliance, the absence of a conspicuous rent in the cell, or distortion of the sarcomere pattern, implies that the injury was not as severe as anticipated. At one impalement site, however, there was denudement of the cell surface complex distal to the site of impalement (Fig. 2d), revealing the underlying myofilaments. This change was not observed at any other impalement site examined. It appeared that this membrane was avulsed at the time the cell was removed from the glass microneedles.

A typical tension recording is shown in Figure 3, which is a photocopy of an actual experiment (cell no. 1, Table 1). The sarcomere length, obtained from photographs taken at the indicated times, is also given, together with the simultaneous tension, in mN/mm². It is apparent, from this figure, that there is a substantial amount of stress-relaxation and creep observable at sarcomere lengths approaching 2.2 μm, and above. Since our view of the cells under study is limited to one focal plane or optical section, our observations are, clearly, incomplete. In addition to sarcomere length variation along the length of the cell, variation in the transverse plane must also be considered. That is, there may be some radial variation in strain, since the angular relationship among sarcomeres did show some variation across the cell width (Fig. 1). However, this could not be estimated with sufficient reliability to justify an attempt at quantification. We would anticipate that radial variation in strain is randomly distributed, and is not a significant source of error.

The sarcomere length-tension curves obtained for isolated hamster ventricular myocytes (Figs. 4a and 5) are similar to those obtained previously with cardiac muscle and whole heart preparations (Spiro and Sonnenblick, 1964; Spotnitz et al., 1966; Winegrad, 1974). The skeletal myocyte length-tension curves (Figs. 4a and 5) are similar to those obtained previously with intact skeletal muscle preparations (Spiro and Sonnenblick, 1964; Lannergren and Noth, 1973; Moss and Halpern, 1977). These data are also similar to those obtained with intact heart and skeletal muscle preparations, in that they demonstrate a linear relationship between natural stress [Ln(mg/mm²)] and both NS and DS (Fig. 4b). This technique for linearization of length-tension data has been discussed in detail (Mirsky and Parmley, 1973; Natarajan et al., 1979).
Figure 2. Part a: cardiac myocyte held with two microneedles for tension recording. Sarcomere length is 2.16 ± 0.14 μm for 51 sarcomeres. The cell was fixed at this level of tension and processed for SEM (bar = 10 μm). Part b: scanning electron micrograph of the cell shown in part a. Surface undulations can be seen, but surface cables are not visible at this magnification. Part c: high magnification of the impalement site indicated on the left, in part b. Four mitochondria are seen at the edge of the elongated defect. Undulations and surface cables (arrow) are apparent. Part d: high magnification of the impalement site indicated on the right in part b. This defect is smaller. The surface complex has been removed distal to the defect, exposing myofilaments. Magnifications: part a, 540×, bar = 10 μm; part b: 585×, 30° tilt; part c: 10,000×, 30° tilt, bar = 1 μm; part d: 10,000×, 30° tilt.
3.

FIGURE 3. Recording of a length-tension curve obtained with an isolated hamster cardiac myocyte (cell no. 1, Table 1). Sarcomere length, in μm, is indicated along with the simultaneous tension, in mN/mm². Arrows indicate points along the tension recording where photographs were taken for sarcomere length determination. At the end of the experiment, a repeat baseline recording was made. This has been moved to the left to conserve space.

TABLE 1
Stress-Strain Parameters of Cardiac Myocytes

<table>
<thead>
<tr>
<th>Cell no</th>
<th>Lo (μm)</th>
<th>Cell width (μm)</th>
<th>K(NS)</th>
<th>r</th>
<th>Stress (mN/mm²)</th>
<th>K(DS)</th>
<th>r</th>
<th>Stress (mN/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Calculated using Ln(L/Lo)</td>
<td>2.2 μm</td>
<td>2.6 μm</td>
<td>A. Calculated using Ln(L/Lo)</td>
<td>2.2 μm</td>
<td>2.6 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.45</td>
<td>35.9</td>
<td>8.48</td>
<td>0.971</td>
<td>0.62</td>
<td>2.57</td>
<td>5.28</td>
<td>0.961</td>
</tr>
<tr>
<td>2</td>
<td>1.62</td>
<td>30.2</td>
<td>7.98</td>
<td>0.988</td>
<td>0.72</td>
<td>2.71</td>
<td>5.84</td>
<td>0.989</td>
</tr>
<tr>
<td>3</td>
<td>1.80</td>
<td>29.3</td>
<td>12.09</td>
<td>0.961</td>
<td>0.17</td>
<td>1.30</td>
<td>8.71</td>
<td>0.950</td>
</tr>
<tr>
<td>4</td>
<td>1.85</td>
<td>33.0</td>
<td>10.78</td>
<td>0.982</td>
<td>0.82</td>
<td>4.94</td>
<td>8.85</td>
<td>0.986</td>
</tr>
<tr>
<td>5</td>
<td>1.77</td>
<td>37.7</td>
<td>7.66</td>
<td>0.986</td>
<td>1.02</td>
<td>3.67</td>
<td>6.44</td>
<td>0.989</td>
</tr>
<tr>
<td>6</td>
<td>1.80</td>
<td>23.0</td>
<td>13.86</td>
<td>0.980</td>
<td>0.38</td>
<td>3.83</td>
<td>10.40</td>
<td>0.987</td>
</tr>
<tr>
<td>7</td>
<td>1.66</td>
<td>24.3</td>
<td>9.58</td>
<td>0.991</td>
<td>1.44</td>
<td>7.15</td>
<td>6.78</td>
<td>0.994</td>
</tr>
<tr>
<td>8</td>
<td>1.74</td>
<td>20.4</td>
<td>10.14</td>
<td>0.994</td>
<td>1.83</td>
<td>9.86</td>
<td>7.58</td>
<td>0.995</td>
</tr>
<tr>
<td>Mean</td>
<td>1.74</td>
<td>29.2</td>
<td>10.07</td>
<td>0.982</td>
<td>0.88</td>
<td>4.50</td>
<td>7.48</td>
<td>0.981</td>
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<tr>
<td>SD</td>
<td>0.09</td>
<td>6.3</td>
<td>2.14</td>
<td>0.011</td>
<td>0.55</td>
<td>2.78</td>
<td>1.73</td>
<td>0.017</td>
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</table>

K, r, stress at 2.2 μm, and stress at 2.6 μm were calculated from the regression of natural stress [Ln(mN/mm²)] against either natural (NS) or differential strain (DS).

TABLE 2
Stress-Strain Parameters of Skeletal Myocytes

<table>
<thead>
<tr>
<th>Cell no</th>
<th>Lo (μm)</th>
<th>Cell width (μm)</th>
<th>K(NS)</th>
<th>r</th>
<th>Stress (mN/mm²)</th>
<th>K(DS)</th>
<th>r</th>
<th>Stress (mN/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Calculated using Ln(L/Lo)</td>
<td>2.2 μm</td>
<td>2.6 μm</td>
<td>A. Calculated using Ln(L/Lo)</td>
<td>2.2 μm</td>
<td>2.6 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.34</td>
<td>42.5</td>
<td>8.81</td>
<td>0.997</td>
<td>0.28</td>
<td>1.20</td>
<td>5.73</td>
<td>0.992</td>
</tr>
<tr>
<td>2</td>
<td>2.63</td>
<td>78.3</td>
<td>7.06</td>
<td>0.981</td>
<td>0.33</td>
<td>1.07</td>
<td>5.22</td>
<td>0.980</td>
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<tr>
<td>3</td>
<td>2.16</td>
<td>71.7</td>
<td>10.04</td>
<td>0.998</td>
<td>0.10</td>
<td>0.52</td>
<td>7.00</td>
<td>0.995</td>
</tr>
<tr>
<td>4</td>
<td>1.98</td>
<td>57.6</td>
<td>8.23</td>
<td>0.982</td>
<td>0.27</td>
<td>1.06</td>
<td>5.12</td>
<td>0.988</td>
</tr>
<tr>
<td>5</td>
<td>2.15</td>
<td>60.4</td>
<td>8.67</td>
<td>0.997</td>
<td>0.19</td>
<td>0.79</td>
<td>5.81</td>
<td>0.998</td>
</tr>
<tr>
<td>Mean</td>
<td>2.25</td>
<td>62.1</td>
<td>8.56</td>
<td>0.991</td>
<td>0.25</td>
<td>1.02</td>
<td>5.77</td>
<td>0.991</td>
</tr>
<tr>
<td>SD</td>
<td>0.25</td>
<td>13.8</td>
<td>1.08</td>
<td>0.007</td>
<td>0.06</td>
<td>0.15</td>
<td>0.87</td>
<td>0.007</td>
</tr>
</tbody>
</table>

K, r, stress at 2.2 μm, and stress at 2.6 μm were calculated from the regression of natural stress [Ln(mN/mm²)] against either natural (NS) or differential strain (DS).
Table 3 summarizes some of the information given in Tables 1 and 2. Here, we can see an explicit comparison of the parameters of passive mechanical properties calculated from experimental data, using either natural or differential strain. The use of natural or differential strain significantly affects the calculated K, but has less of an effect on the calculated stress. For the data shown in Table 3, the use of NS vs. DS had no effect on calculated values of stress, except for skeletal myocytes at a sarcomere length of 2.2 μm. The data in Table 3 can also be used to compare cardiac and skeletal myocytes in regard to these same parameters. Since skeletal muscle is known to be more compliant than cardiac muscle, a one-tailed t-test was used for calculation of the P values. Comparison of stiffness constants and calculated stress at sarcomere lengths 2.2 and 2.6 μm shows that cardiac myocytes are more stiff than skeletal myocytes. The difference between values of K(NS) is not significant, but it is highly significant for K(DS).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Cardiac myocytes</th>
<th>Skeletal myocytes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(NS)</td>
<td>10.07 ± 2.14</td>
<td>8.56 ± 1.08</td>
<td>&lt;0.086</td>
</tr>
<tr>
<td>K(DS)</td>
<td>7.48 ± 1.73</td>
<td>5.77 ± 0.87</td>
<td>&lt;0.031</td>
</tr>
<tr>
<td>Stress at</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2.2 μm NS</td>
<td>0.88 ± 0.55</td>
<td>0.25 ± 0.06</td>
<td>&lt;0.014</td>
</tr>
<tr>
<td>2.6 μm DS</td>
<td>0.88 ± 0.48</td>
<td>0.44 ± 0.16</td>
<td>&lt;0.037</td>
</tr>
<tr>
<td>Stress at</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.6 μm NS</td>
<td>4.50 ± 2.78</td>
<td>1.02 ± 0.15</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>2.6 μm DS</td>
<td>4.91 ± 2.58</td>
<td>1.20 ± 0.36</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
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</table>

Abbreviations: NS, natural strain; DS, differential strain. Units of stress: mN/mm².

* Indicates not significant, or P > 0.05.
suggestive. This difference in \( K \) would probably be significant if the range of stress for the skeletal myocyte fragments (0–120 mN/mm\(^2\)) was limited to the same range obtained for the cardiac myocytes (0–20 mN/mm\(^2\)). In general, comparisons of muscle stiffness (\( K \)) must be made over the same range of stress. In spite of this discrepancy, the skeletal myocyte \( K(DS) \) is significantly less than the cardiac myocyte \( K(DS) \). The values of stress at sarcomere lengths 2.2 and 2.6 \( \mu \)m are clearly higher for the cardiac than for the skeletal myocytes.

The correlation coefficients (\( r \)) indicate a tight clustering of data points around the calculated regression lines (Table 1). The values of \( K(NS) \) and \( K(DS) \) were calculated using only “peak” tension values obtained from stress-strain curves of isolated cells. To evaluate the effect of the observed viscoelasticity, a worst-case calculation was performed in which only “plateau” or "steady state" length and tension values were used for the natural strain regression analysis of the cardiac myocytes. This calculation yielded a mean \( K(NS) \) for the eight cells of 9.23 ± 0.71 (mean ± SEM). This was significantly different from \( K(NS) \) calculated using the peak values (\( P < 0.05 \)).

### Discussion

The data shown here are similar to those obtained by others, in that the cardiac and skeletal muscle length-tension data can also be interpreted as being bilinear or biexponential, resulting from different stiffness elements dominating the high and low stress ranges of the curve. The same data can be, and usually are, interpreted as being unexponential. The high \( r \) values for the regression of Eulerian stress against either NS or DS for all cells studied (Tables 1 and 2) indicate that unexponential linearization is appropriate. This suggests that a single stiffness element is operative throughout the full range of both the cardiac and skeletal myocyte length-tension curves.

The high \( r \) values also suggest that both expressions of strain (NS and DS) are equally valid for linearization of the sarcomere length-tension curves. However, as shown by Mirsky and Parmley, (1973), values of \( K \) are dependent on the particular mathematical expression of strain employed. This indicates that care must be exercised when comparing indices of stiffness obtained from length-tension or pressure-volume studies. Our results show (Tables 1 and 2; Fig. 4b) that NS increases more slowly with increases in sarcomere length than DS, resulting in a 30–50% higher value of \( K(NS) \). \( K(NS) \) obtained for the cardiac myocytes (10.07 ± 2.14) is similar to the \( K \) obtained indirectly from pressure-volume data (10.93 ± 0.51; mean of nine age groups, from 1 to 24 months ± SEM) by Kane et al. (1976) with intact hamster ventricles. However, DS was the definition of strain used by Kane et al., who obtained \( K \) by plotting the slope of the length-tension curve at a given value of strain, as a function of strain at that point on the curve. \( K(DS) \) is therefore more comparable to the \( K \) obtained by Kane et al., and can more reliably be compared to length-tension data obtained by others who have also used DS. It should be noted that, as \( L \) approaches \( L_0 \), NS approaches DS. Although the Y-intercepts for the NS and DS methods are slightly different in Figure 4b, they are theoretically identical, and observed to be not significantly different.

Comparison of the cardiac myocyte \( K(DS) \) (7.48 ± 1.73) with the \( K \) obtained by Kane et al. indicates that the isolated ventricular myocytes are not as stiff as the wall of the intact left ventricle. Furthermore, \( K(DS) \) is comparable to a \( K \) (6.12) similarly obtained for isolated, intact frog atrial myocytes (Tarr et al., 1979). The atrial cells studied by Tarr et al., were found to be more compliant than intact frog atrial trabeculae studied by Winegard (1974). Using DS, we linearized the data of Winegrad [(1974) Fig. 5] and obtained a \( K \) of 11.7 with a stress at 2.2 \( \mu \)m of 5.1 mN/mm\(^2\). This stress is 5–10 times higher than our mean value of stress at 2.2 \( \mu \)m (0.88 ± 0.55 mN/mm\(^2\)) for the ventricular myocytes. However, it should be noted that trabecular preparations have not been proven to be entirely representative of ventricular myocardium in general (Sonnenblick and Skelton, 1974).

Values of stress at 2.2 \( \mu \)m for hamster intact myocardial preparations are not available for comparison. However, studies involving mammalian intact cardiac preparations have reported a stress at 2.2 \( \mu \)m which is considerably higher than values listed for frog trabecular preparations. These include 14.0 mN/mm\(^2\) for cat papillary muscle (Julian and Sollsins, 1975), 15.7 mN/mm\(^2\) for the 11–12-month-old rat (Weisfeldt et al., 1971), up to 58.0 mN/mm\(^2\) for dog papillary muscle (Kitabatake and Suga, 1978), with a mean of about 30.0 mN/mm\(^2\) for all reported values (Spiro and Sonnenblick, 1964; Spann et al., 1967; Natarajan et al., 1979; Krueger and Pollack, 1967). We have conducted preliminary length-tension studies on trabecular muscle preparations isolated from the hamster right ventricle (data not shown). These studies indicate that stress at 2.2 \( \mu \)m for hamster trabeculae is approximately 3.0–8.0 mN/mm\(^2\) compared with 15.7 mN/mm\(^2\) in the rat (Weisfeldt et al., 1971). This appears to be reasonable, since rat myocardium, at all ages, is considerably more stiff than hamster myocardium (Borg and Caulfield, 1981a).

For the skeletal myocyte fragments, the DS regression gave a stress at 2.3 \( \mu \)m of 0.56 ± 0.19 mN/mm\(^2\). This value is comparable to a range of 0.46–0.77 mN/mm\(^2\) at 2.3 \( \mu \)m for frog isolated twitch muscle fibers (Lannergren and Noth, 1973) and 0.29 ± 0.02 mN/mm\(^2\) at 2.25 \( \mu \)m for whole semitendinosus muscle (Moss and Halpern, 1977). Compared with the skeletal myocyte fragments, the stress at 2.30 \( \mu \)m for the cardiac myocytes (1.34 ± 0.73 mN/mm\(^2\)) is higher. Furthermore, the stress at 2.35 \( \mu \)m
for the cardiac myocytes (1.67 ± 0.90 mN/mm²) is higher than a Lagrangian stress at 2.35 µm (0.60 mN/mm²) obtained by Tarr et al. (1979) with single intact frog atrial cells. The cells studied by Tarr et al. were enzymatically isolated, so that the glycocalyx was probably absent (Bishop and Drummond, 1979; Carlson et al., 1978; Moses and Kasten, 1979). At any rate, the length-tension curve obtained by Tarr et al. is shifted considerably to the right, compared with those obtained with frog atrial trabeculae (Winegrad, 1974). In contrast, the composite length-tension curves obtained here for cardiac myocytes and skeletal myocyte fragments (Figs. 4a and 5) are qualitatively similar to curves obtained by others with isolated muscle and whole heart preparations (Braunwald et al., 1976). Our cardiac myocyte length-tension curves are also qualitatively comparable to curves obtained with isolated, "skinned" ventricular myocytes of the rat (Fabiato and Fabiato, 1978). Stress at 2.2 µm is not obtainable for skinned rat cells, since the length-tension curve obtained by Fabiato and Fabiato employed percent tension rather than force/area plotted against sarcomere length.

One possible source of error in a study such as this would be mistaking two cells for one. A range for hamster cardiac cell width has not been established in the literature. Values reported for rat ventricular cells range anywhere from 12 to 40 µm (Bishop and Drummond, 1979; Carlson et al., 1978; Moses and Kasten, 1979; Powell et al., 1978). Our cell diameters were within this range (Table 1). If some of our cardiac myocytes had been 2 cells thick, there might have been an increase in stiffness due to the presence of interstitial matrix. However, transverse sections of two myocytes, examined by transmission electron microscopy (data not shown) showed them to be actually one cell thick. Furthermore, the three lowest calculated values of K(DS) for the ventricular myocytes (5.28, 5.84, and 6.44) were obtained with the three widest cells (35.9, 30.2, and 37.7 µm, respectively). There was no significant correlation between cell diameter and the stiffness constant, K. It is apparent that wide cells were not more stiff than thin cells.

The simplest representation of diastolic stiffness is the sarcomere length-tension curve. The curves obtained in this study suggest that the resting stiffness of hamster cardiac tissue, like that in the rat, is due at least in part to intracellular structural elements. Since it has not been shown conclusively that trabecular preparations accurately reflect ventricle wall stiffness, data from future length-tension studies of isolated ventricle wall should be compared with the ventricular myocyte data. This comparison would more reliably reflect the interstitial contribution to wall stiffness. The model used here seems appropriate for further studies of this type. Future applications might also include studies of hypertrophic changes at the cellular level during cardiac hypertrophy. Likewise, studies dealing with the impact of exercise, nutrition, and aging on cardiac muscle function at the cellular level can be envisioned.

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Address for reprints: Department of Pathology, George Washington University, Medical Center, 2300 Eye Street, Washington, D.C. 20037. Received January 21, 1983; accepted for publication January 11, 1984.

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D Fish, J Orenstein and S Bloom

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