Reversible and Irreversible Elongation of Ischemic, Infarcted, and Healed Myocardium in Response to Increases in Preload and Afterload

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Background. Left ventricular aneurysm formation after myocardial infarction (MI) has been associated with elongation of infarcted tissue in response to wall stress. Such elongation most commonly occurs in acutely infarcted or partially healed regions during the early post-MI period; however, recent reports have indicated that mature (15-week-old) healed infarct regions also undergo elongation after stress.

Methods and Results. To assess factors contributing to post-MI left ventricular aneurysm formation, we subjected isolated strips (n=50) of rabbit myocardial tissue from acutely ischemic (noninfarcted left ventricular), acutely infarcted (24 hours after MI), and healed infarct (3 and 15 weeks after MI) regions to a range of loading conditions and measured the reversible and irreversible length changes that occurred. The isolated strips were repetitively stretched for 1 hour at 4 Hz to impose cyclical physiological peak and resting stresses of 2.0 and 0.2 g/mm². During a second hour, either peak stress (“afterload”) or resting stress (“preload”) was tripled, and the increase in strip length (strain) was measured. During a third hour, peak and resting stresses were returned to the initial values to assess the reversibility of length changes occurring during increased load. Elongation was expressed as the increase in natural strain from the first hour. Increasing afterload caused similar irreversible length increases of 4–5%/hr in acutely infarcted and 3- and 15-week-old healed infarct strips; acutely ischemic tissue length increased by 7.4%/hr (p<0.05 versus acutely infarcted tissue and scars). Increasing preload in acutely ischemic and acutely infarcted tissue caused a reversible length increase of less than 1%/hr. (Scar strips were not tested for the effect of preload.)

Conclusions. Since an irreversible length increase may represent an early event in aneurysm formation, our results suggest that 1) afterload increases are more likely to lead to aneurysm development than preload increases, 2) acutely ischemic tissue is the most vulnerable to increased afterload, and 3) for a given wall stress level, healing scar tissue is as susceptible to irreversible length changes as is acutely infarcted tissue. The observation that even mature post-MI scar elongated in response to increases in afterload implies that long-term pharmacological management of afterload in post-MI patients may be beneficial in preventing tissue elongation and aneurysm formation and that factors that increase wall stress (e.g., hypertension and exercise stress) have the potential to promote aneurysm formation in healed infarct scars. (Circulation 1991;84:387–399)

Left ventricular (LV) aneurysm formation after myocardial infarction (MI) is associated with a high rate of mortality and morbidity.1,2 Factors contributing to post-MI aneurysm formation have recently been reviewed.3 The initial step in aneurysm formation is the elongation of infarcted tissue in response to the load of wall stress, and such elongation most commonly occurs in acutely in-
farcted or partially healed regions during the early post-MI period. However, Jugdutt et al has recently reported that mature (15-week-old) healed infarct regions can also undergo expansion when patients with large anterior infarcts undergo exercise conditioning. No previous study has separated the influence of preload and afterload on aneurysm formation.

To clarify the influence of post-MI healing and loading conditions on aneurysm formation, we assessed the ability of strips of acutely ischemic (noninfarcted) myocardium, 24-hour-old infarct tissue, and 3- and 15-week-old scar tissue to withstand an imposed load (stress) without undergoing an irreversible increase in length (strain), because such a strain increase would represent the initial step in aneurysm formation. Isolated tissue strips were repetitively stretched between physiological peak and resting stresses at a frequency of 4 Hz.

We tested the effect of a threefold increase in peak stress (modeling an increased afterload) for 1 hour in all four tissue types and a threefold increase in resting stress (modeling an increased preload) for 1 hour in acutely ischemic (noninfarcted) myocardium and 24-hour-old infarct tissue. We assessed the length changes that occurred with each change in loading condition. The length (strain) was measured before and after the increased stresses were imposed and was compared with the length of tissue strips exposed to normal physiological resting and peak stresses. The length (strain) change was also measured after release of the increased stresses to determine the reversibility of the elongation response. Thus, the independent contributions of increases in "preload" and "afterload" on potential for aneurysm formation at different stages during post-MI healing were determined.

**Methods**

Thirty-five New Zealand White male rabbits (1.5–2.0 kg) were studied. The studies conformed to the guidelines established by the American Physiological Society for the care and use of experimental animals. The rabbits were anesthetized with sodium thiopental (2.5 mg/kg) and respired with a mechanical ventilator after tracheostomy. A left thoracotomy was performed at the level of the apical impulse, and the heart was suspended in a pericardial cradle. The large marginal branch of the circumflex coronary artery was identified and permanently occluded halfway between apex and base. Our previous work has shown that midventricle coronary occlusion results in a relatively consistent infarct size of 25±3% of the LV by weight at 24 hours after MI with a surgical mortality of approximately 20%. Eighteen rabbits were studied at 1 day after MI; 11 rabbits, at 3–4 weeks after MI; and six rabbits, at 15–16 weeks after MI. Previously, we have shown that 3-week-old scar tissue from permanent occlusion results in healed infarct tissue that is 75% fibrotic and 25% residual nonviable myocytes, with the scar tissue encompassing the entire midwall region and the dead myofibrillar elements located along the subendocardial and subepicardial scar edges.

The LVs were excised, cut along the posteroseptal border, and immersed in oxygenated Krebs-Henseleit buffer. Tissue strips were cut along the long axis between the papillary muscles in the anteropapillary region of the LV in the infarcted and healing hearts for subsequent testing (see below).

Noninfarcted myocardial strips (n=15) were also studied. Longitudinal strips of myocardium were taken from the lower posterior wall in these noninfarcted rabbit hearts because the LV geometry in this region produced strips similar in shape to the infarct and scar strips. These strips were considered to be acutely ischemic but noninfarcted, since they were not perfused after dissection from the LV wall and since their cross-sectional area was too great to permit adequate oxygenation by superfusion. Hereafter in the text, these myocardial strips will be referred to as noninfarcted myocardium.

A computer-controlled test apparatus comprised of a low-inertia servomotor, strain-gauge force transducer, and a capacitive position length transducer was used to test the materials properties of the tissue (Figure 1). The computer program could continuously measure and alter the force and length of the strip. The infarct, scar, or noninfarcted myocardial strips were mounted vertically between two stainless steel clips with spring-loaded, serrated hinged clamps and submerged in a Krebs-Henseleit bath bubbled with 95% O₂–5% CO₂. The initial resting dimensions of the clamped strip, at zero load, were measured with a dissecting microscope and entered in the computer program. The force and length of the strips were continuously monitored and displayed by the computer system with the data expressed as Eulerian stress (i.e., force per instantaneous cross-sectional area) and as natural strain [a percentage; ln (L/Lo)×100, where L is instantaneous length and Lo is initial length at zero resting stress]; the strip dimensions were incorporated into these calculations.

The experimental design consisted of three protocols (Table 1); each consisted of three 60-minute phases. During each phase, a 4-Hz periodic sawtooth waveform (equivalent to a rabbit heart rate of 240 beats/min) of constant velocity (16 mm/sec) stretches and releases was applied to the strip. Using Laplace's law, we estimated that a stress of 2.0 g/mm² was comparable to a peak LV systolic pressure of 80 mm Hg, which is physiological for the rabbit, and that a stress of 0.2 g/mm² was comparable to an LV diastolic pressure of 8 mm Hg (see "Appendix"). Thus, the hour 1 of protocols I–III consisted of a baseline equilibrium phase with stretch–release cycles between normal physiological resting stress of 0.2 g/mm² and peak stress of 2.0 g/mm².

During hour 2, the baseline physiological resting and peak stresses were maintained in protocol I; in protocol II (increased peak stress), the peak stress was tripled to 6.0 g/mm² (comparable to an LV peak systolic pressure
of 240 mm Hg) while the resting stress remained at 0.2 g/mm². In protocol III (increased resting stress), the resting stress was tripled to 0.6 g/mm² (comparable to an LV diastolic pressure of 24 mm Hg) while the peak stress remained at 2.0 g/mm². During hour 3, the physiological stress levels of the first baseline period were applied to all strips (Table 1).

The myocardial tissues tested in protocol I included the noninfarcted myocardium, 24-hour-old infarct, and 3-week-old scar; in protocol II the tissue tested included the noninfarcted myocardium, 24-hour-old infarct, 3-week-old scar, and 15-week-old scar. The latter group was included in protocol II to determine whether additional healing altered behavior of the tissue with increased afterload. In protocol III, only the noninfarcted myocardium and 24-hour-old infarct tissue were tested. Since only small reversible changes occurred in these tissue groups with increased preload, additional tests were not carried out in healed scar.

Stress–strain curves during the ascending portion of the stretch–release cycle were stored in the computer memory. Measurements were made of strain versus stress at 2, 5, 10, 15, 30, 45, and 60 minutes during each hour. Curves were also stored every 30 minutes after the first 3 hours as noninfarcted myocardium was cycled for 6 continuous hours in protocol I and for 4 hours after release of either high peak or resting stress in protocols II and III.

Any strip elongation during the period of increased loading in hour 2 of protocols II and III was compared with the strip length during hour 3 to determine whether the elongation was reversible with strip unloading. Tissue strips subjected to increases in peak stress (protocol II) or resting stress (protocol III) were compared with the strips in protocol I, where physiological resting and peak stresses were maintained throughout the 3-hour period.

To evaluate the time-dependent nature of tissue elongation after ischemia,14,15 tissue strain at a stress of 1.8 g/mm² was plotted versus time, comparing the strips in protocol I (normal physiological stresses) with those in protocol II (increased peak stress). A stiffness coefficient for all four tissue types was determined after the first hour of cycling by measuring the slope of the stress–strain curve divided by the stress and was compared among the groups at a common stress level of 1.8 g/mm².

Table 1. Stress Levels of Tissue Strips in Protocols I, II, and III

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Stress (g/mm²)</th>
<th>Types of tissue tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hour 1 (baseline)</td>
<td>Hour 2 (experimental)</td>
</tr>
<tr>
<td>I</td>
<td>Peak</td>
<td>Resting</td>
</tr>
<tr>
<td>II</td>
<td>2.0</td>
<td>0.2</td>
</tr>
<tr>
<td>III</td>
<td>2.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Noninfarcted, infarcted, and scar tissue are defined in "Methods."
TABLE 2. Dimensions of Infarct and Scar Strips

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Thickness (mm)</th>
<th>Width (mm)</th>
<th>CSA (mm²)</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfarcted</td>
<td>15</td>
<td>4.65±0.3*</td>
<td>1.53±0.2</td>
<td>7.12±0.9†</td>
<td>5.02±0.2*</td>
</tr>
<tr>
<td>Infarcted</td>
<td>18</td>
<td>1.51±0.1</td>
<td>2.97±0.1</td>
<td>4.44±0.3</td>
<td>3.73±0.1</td>
</tr>
<tr>
<td>Scar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-week-old</td>
<td>11</td>
<td>1.63±0.1</td>
<td>3.46±0.1</td>
<td>5.69±0.5</td>
<td>3.41±0.1</td>
</tr>
<tr>
<td>15-week-old</td>
<td>6</td>
<td>1.55±0.1</td>
<td>3.53±0.4</td>
<td>5.48±0.8</td>
<td>4.00±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CSA, cross-sectional area (thickness x width).
Noninfarcted, infarcted, and scar tissue are defined in "Methods."
*p<0.05 vs. infarcted and 3- and 15-week-old scar tissue.
†p<0.05 vs. infarcted tissue.

Statistical comparisons for more than two groups were made by analysis of variance (ANOVA) followed by the Newman-Keuls test when the ANOVA indicated significant intergroup differences. A repeated measures ANOVA was used for the serial measurements of strain changes over time. A multiple linear regression analysis was used to identify the influence of strip dimensions on strain changes. All values represent mean±SEM.

Results

Table 2 shows the transmural thickness, width, cross-sectional area, and length of the tissue strips. We anticipated transmural thinning of infarct or scar in the occluded region, relative to noninfarcted myocardium. Therefore, strips of noninfarcted myocardium were intentionally cut with a narrower width to compensate for their greater transmural thickness. Noninfarcted myocardial strips were oriented in the clamps so that the strip width separated the clamps, whereas the infarct and scar strips were oriented in the clamps with the thickness separating the clamps. This orientation resulted in a similar amount of tissue between the clamps in all groups, because the width of the myocardial strips (1.53±0.2 mm) was not significantly different from the thickness of the infarct (1.51±0.1 mm), 3-week-old scar (1.63±0.1 mm), and 15-week-old scar (1.55±0.1 mm) strips (p=NS, Table 2).

However, the thickness of the noninfarcted strips was greater than the width of the infarct or scar strips (p<0.001, Table 2). Thus, cross-sectional area for the noninfarcted tissue was greater than that for infarct strips. Noninfarcted strips were also slightly longer than infarct or scar strips.

Stress–Strain Curves

Figure 2 shows an example of the stress–strain curves for two representative 24-hour-old infarct strips. The lower end of each curve represents an end-diastolic stress of 0.2 g/mm² (equivalent to 8 mm Hg). The upper end of the curve represents end-systolic stress, either 2.0 g/mm² corresponding to 80 mm Hg (normal systolic pressure for the rabbit) or 6.0 g/mm² corresponding to 240 mm Hg (curve 2a, increased afterload). To plot Figure 2, zero strain was considered to be resting strain at the

Figure 2. Stress–strain curves for two 24-hour-old infarct strips. Lower end of each curve represents resting stress (0.2 g/mm²=8 mm Hg), and upper end represents peak stress (either 2.0 g/mm² [80 mm Hg] or 6.0 g/mm² [240 mm Hg]). A zero reference point (strain 0.0) was chosen as strain at resting stress level of 0.2 g/mm² at the beginning of the first hour of strip conditioning, when strips were cycled between 0.2 and 2.0 g/mm² (physiological resting and peak stress). Curves 1–3 (protocol I, see text) were recorded at the end of each hour of strip cycling thereafter at these physiological stresses. Curves 1a–3a were from second strip (protocol II, see text). Curve 1a was recorded after strip was cycled for 1 hour at physiological stresses; curve 2a was recorded after second hour of cycling with peak stress increased to 6.0 g/mm², and curve 3a was recorded 1 hour after return to initial physiological peak stress. Rightward shift from curve 1a to curve 2a indicates strip elongation due to the hour of increased peak stress; shift from curve 1a to curve 3a represents the irreversible elongation that persisted despite strip unloading during third hour of protocol.
TABLE 3. Initial Strain and Strain Increase During the First “Conditioning” Hour

<table>
<thead>
<tr>
<th>Strain</th>
<th>Noninfarcted</th>
<th>Infarcted</th>
<th>3-week-old</th>
<th>15-week-old</th>
</tr>
</thead>
<tbody>
<tr>
<td>protocol I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hour 0</td>
<td>18.0±2.9</td>
<td>18.0±1.5</td>
<td>20.3±2.3</td>
<td>...</td>
</tr>
<tr>
<td>Hour 1</td>
<td>23.5±3.0</td>
<td>21.0±1.7</td>
<td>25.7±2.6</td>
<td>...</td>
</tr>
<tr>
<td>Δ Strain</td>
<td>5.4±0.4</td>
<td>3.0±0.4*</td>
<td>5.4±0.8</td>
<td>...</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>...</td>
</tr>
<tr>
<td>protocol II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hour 0</td>
<td>14.8±1.7</td>
<td>18.9±1.8</td>
<td>19.7±1.3</td>
<td>17.7±2.1</td>
</tr>
<tr>
<td>Hour 1</td>
<td>18.5±2.0</td>
<td>21.8±2.1</td>
<td>24.2±1.3</td>
<td>21.4±2.7</td>
</tr>
<tr>
<td>Δ Strain</td>
<td>3.7±0.5</td>
<td>3.0±0.3</td>
<td>4.6±0.4</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>protocol III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hour 0</td>
<td>18.2±1.3</td>
<td>19.4±1.7</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Hour 1</td>
<td>21.9±1.7</td>
<td>21.9±2.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Δ Strain</td>
<td>3.7±0.6</td>
<td>2.5±0.4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Average Δ strain</td>
<td>4.3±0.3</td>
<td>2.8±0.2*</td>
<td>5.0±0.4</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>18</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Strain, ln(L/Lo)×100, where L is instantaneous length and Lo is initial length of unloaded strips; Δ Strain, strain increase (measured at 1.8 g/mm² peak stress); n, number of tissue strips examined for a given protocol; N, total number of tissue strips examined for all three protocols. Noninfarcted, infarcted, and scar tissue are defined in “Methods.”

*p<0.05 vs. noninfarcted and 3-week-old scar tissue (by analysis of variance, F=6.22, p<0.01).

High Peak Stress

Figure 3 plots the average shift of the stress–strain curves after 1 hour of increased loading, that is, between the end of hour 1 and the end of hour 2 for the different strip types cycled at either normal peak stress (protocol I) or increased peak stress (protocol II). To quantify strip elongation in response to an increase in loading, we defined baseline systolic strip length as the length at a physiological systolic stress of 1.8 g/mm² after 1 hour of the conditioning cycling. This zero reference point was chosen to reflect the strip length at a peak physiological systolic stress common to all tissue strips after 1 hour of cycling at physiological resting and peak stresses and to negate differences in strip elongation that occurred during the 1 hour of conditioning cycling before strip loading. Therefore, the results in Figures 3–5 are expressed as the percent change in strain from this baseline systolic strain value.

Figure 3 (upper panel) shows that strips of noninfarcted myocardium, 24-hour-old infarct, and 3- and 15-week-old scars exposed to a peak stress of 6.0 g/mm² (protocol II) had shifted significantly to the right (by ANOVA, F=13.53, p<0.001) compared with the strips cycled at a physiological peak stress (protocol I). Furthermore, in response to increased peak stress, the strips of noninfarcted myocardium shifted significantly more to the right relative to the strips of 24-hour-old infarct or 3- or 15-week-old scar (p<0.05, Newman-Keuls test), indicating greater strip elongation in response to 1 hour of increased afterload. The 24-hour-old infarct and scar strips did
not differ from each other with regard to strip elongation in protocol II.

Figure 3 (lower panel) reports stress–strain curve position at the end of hour 3, when all strips were cycled at the normal peak stress of 2.0 g/mm². Despite return of peak stress to physiological values, the curves from protocol II remained shifted significantly to the right of the curves from protocol I, which had been cycled at physiological peak stress levels for 3 hours.

Strain Versus Time

Figure 4 emphasizes the irreversible nature of the strain changes seen in the strips with increased peak stress. This figure shows the average change in strain measured at a stress level of 1.8 g/mm² during the entire experimental time period for strips with physiological peak and resting stress (protocol I) and strips with increased peak stress (protocol II).

The strips with physiological stress levels increased in length continuously (ranging from 0.2±0.03% in 24-hour-old infarct tissue to 1.2±0.1% in noninfarcted myocardium) throughout the duration of the experiment. The strips subjected to increased peak stress showed tissue strain increasing or decreasing most rapidly immediately after the increase (hour 2) or decrease (hour 3) of peak stress. The resulting increases in strip length with increased peak stress during hour 2 ranged from 3.9±0.9% in 24-hour-old infarct tissue to 4.8±0.6% in the scar tissues and to
7.4±1.3% in the noninfarcted myocardium. After the hour of increased load (end of hour 2), the strips subjected to the increased load were continuing to elongate in response to the increase in peak stress (i.e., the slope was not flat, Figure 4), suggesting that a longer duration of load increase would have resulted in greater strip elongation.

One hour after removal of the high peak stress, the 24-hour-old infarct and scar strips from protocol II remained significantly elongated, ranging from 3.2±0.9% in infarct tissue to 4.1–4.3±0.6% in the scar tissues, compared with the same tissue types in protocol I, ranging from 0.3±0.1% (infarct) to 0.9±0.2% (scar). Four hours after release of the high peak stress, the noninfarcted myocardial strips were still significantly elongated (6.4±1.2%) compared with the noninfarcted strips in protocol I (1.2±0.1%).

**High Resting Stress**

Figure 5 plots the stress–strain curves at the end of the second (upper panel) and third (lower panel) hours of protocols I and III for strips of noninfarcted myocardium and 24-hour-old infarct tissue. Zero strain was defined as in Figure 3. In contrast to the curves from protocol II (high peak stress), the curves from protocol III (high resting stress) show only a slight shift to the right relative to the curves from protocol I. No significant difference in curve position was seen between noninfarcted myocardium and 24-hour-old infarct tissue at this time in protocol III (Figure 5, upper panel).

However, the infarct strips from protocol III were significantly elongated at a stress level of 1.8 g/mm² (rightward curve shift) compared with the infarct strip strain in protocol I (0.74±0.12% versus 0.23±0.03%, p<0.05, protocol III versus protocol I, respectively) (Figure 5, upper panel). A borderline difference in strip elongation (p<0.06) was seen between noninfarcted myocardial strips from protocol III (0.74±0.12%) and those from protocol I (0.48±0.04%). Thus, a threefold increase in resting stress levels elongated the 24-hour-old infarct tissue and noninfarcted myocardial tissue, but the observed elongation was minimal compared with that which occurred with a tripling of peak stress.

Figure 5 (lower panel) reports the average stress–strain curve position at the end of hour 3, when all strips were cycled at the physiological resting stress level. This figure shows that there was no longer a significant difference between the strips from protocols I and III, indicating that the tissue strip elongation seen during high resting stress (Figure 5, upper panel) was reversible when the increased resting stress was removed.

**Stiffness**

Figure 6 shows the stiffness coefficients from the stress–strain curves for individual tissue strips measured at a common stress of 1.8 g/mm² after the first hour of cycling at physiological resting and peak stress values. The stiffness values were directly related to the degree of post-MI healing, since the stiffness coefficients of 3- and 15-week-old scar tissue were similar and significantly greater than noninfarcted myocardium and 24-hour-old infarct tissue values (by ANOVA, F=8.27, p<0.05). The stiffness coefficients of noninfarcted myocardium and 24-hour-old infarct tissue were similar (see Figure 6).

**Strip Dimensions, Stiffness, and Strain**

Because there were unanticipated differences in tissue strip geometry among the groups (Table 2), we wished to determine if our measurements of stiffness and elongation (strain) were influenced by strip geom-
These effects did function of differences in sectional area, $r=-0.30$, $p=0.007$. When adjusted for length and area, the stiffness coefficients for the 3- and 15-week-old scar groups were still significantly greater than those for the noninfarcted and acutely infarcted groups.

Cross-sectional area, but not initial length, influenced the dimensionless change in strain ($d\text{Strain}$) during hour 1; the relation is expressed as $d\text{Strain}=1.75+0.35A$ ($r=0.58$, $p<0.0001$). Overall, the initial elongation (strain) during hour 1 was least for the 24-hour-old infarct tissue and greatest for the 3-week-old scar tissue. Elongation (strain) during hours 2 and 3 was not influenced significantly by length or area.

Effect of Initial Strain

The initial elongation during hour 1, however, was a predictor of the elongation (strain) in response to

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**Figure 5.** Tissue strip elongation during increased preload (hour 2) and subsequent load removal (hour 3). These panels show shifts in stress-strain curves from a common reference point (strain $b$) after 1 hour of increased preload (upper panel) and after a subsequent hour of unloading (lower panel). For protocol I, control strips were cycled at physiological stresses throughout the 3-hour protocol. For protocol III, strips were subjected to an hour of increased resting stress (0.6 g/mm²) during hour 2 and subsequently returned to physiological resting stress (0.2 g/mm²) during hour 3. Curves in upper panel represent relative increase in tissue elongation at end of hour 2 in strips cycled with increased resting stress (protocol III) or strips cycled with physiological resting stress (protocol I); curves in lower panel represent relative increase in tissue elongation 1 hour subsequently (at the end of hour 3), either after removal of the increased resting stress (protocol III) or after a third hour of physiological resting stress (protocol I). The x and y axes and format for this figure are the same as Figure 3. Relative $\Delta$ strain is expressed as a percentage $[\ln(L/L_0)\times 100$, where $L$ is instantaneous length and $L_0$ is initial length] and measures the change in strain relative to strain $b$ (as defined in Figure 3). The dashed line indicates acutely ischemic, noninfarcted myocardium (Myo.). The solid line indicates myocardial infarct tissue at 24 hours after myocardial infarction. Each plotted curve represents the average of five to seven strips per group. The SEM bars are given at a stress level of 1.8 g/mm². *$p<0.05$ vs. strain in 24-hour-old infarct tissue in protocol I measured at a stress level of 1.8 g/mm².
increased resting or peak stress during hour 2 and the continued elongation during hour 3. Figure 7 shows the change in strain (on a log scale) from hour 1 to 2 (upper panel) and from hour 1 to 3 (lower panel) as functions of the initial change in strain during hour 1. For each of the three protocols, the log of elongation during hour 2 was linearly related to the initial elongation during hour 1. Analysis of covariance indicates that there were no significant differences in the slopes of these relations.

As shown in Figure 7 (upper panel), when normal physiological stresses were maintained in hour 2 (protocol I), elongation of the 24-hour-old infarct tissue was slightly less \((p<0.05)\) in relation to initial elongation than that for noninfarcted myocardial tissue or 3-week-old scar tissue, which both fell on a common line. During increased peak stress (protocol II), 24-hour-old infarct tissue and 3- and 15-week-old scar tissue fell on a common line that was shifted upward by 1.08 log units, or 12-fold \((p<0.0001)\), compared with the relation obtained during maintained physiological stress levels (protocol I). The values for noninfarcted myocardium fell along a line that was shifted further by 0.24 log units, 1.7-fold \((p<0.01)\), compared with the infarct or scar tissue types subjected to increased peak stress. During increased resting stress (protocol III), noninfarcted myocardium and 24-hour-old infarct tissue fell along a common line that was shifted upward by 0.36 log units \((p<0.002)\) compared with the relation obtained during maintained physiological stresses (protocol I). This corresponds to a 2.3-fold increase in strain.

Figure 6. Bar graph showing stiffness coefficients of tissue strips from acutely ischemic, noninfarcted myocardium, 24-hour-old infarct tissue, and 3- and 15-week-old scar tissue measured at a stress level of 1.8 g/mm² after the first hour of conditioning. Values shown represent the average slope of the stress–strain curves divided by the stress reported at a common stress level of 1.8 g/mm² for all strips at the end of hour 1. Values from tissue strips from protocols one, two, and three were combined as all strips were cycled between physiological peak and resting stress at this time. Data represent mean±SEM; number of strips is indicated in parentheses.

*p<0.05 vs. strips of noninfarcted myocardium and 24-hour-old infarct tissue (Newman-Keuls test after analysis of variance, \(F=8.27, p<0.001\)).

Figure 7. Tissue elongation during loading (hour 2) and unloading (hour 3) as a function of elongation during conditioning (hour 1). The increase of strain during conditioning (hour 1) is plotted on the x axis in both the upper and lower panels. The y axis indicates the increase in strain, on a log scale that occurred from the end of hour 1 to the end of hour 2 (upper panel) and from the end of hour 1 to the end of hour 3 (lower panel). During hour 2 (upper panel) strips in protocol I were maintained at physiological stresses, strips in protocol II were subjected to increased peak stress, strips in protocol III were subjected to increases in resting stress. During hour 3 (lower panel) all strips were maintained or returned to physiological stresses. Symbols indicate mean x and y values for each group \((\circ, \text{acutely ischemic, noninfarcted myocardium}; \bullet, \text{24-hour-old infarct tissue}; \triangle, \text{3-week-old scar tissue}; \blacktriangle, \text{15-week-old scar tissue})\); means and error bars on y axis are geometric mean±SEM. Lines indicate linear regression relations \(\log y=mx+b\) for different protocols. Lengths of lines indicate range of x values from which lines were computed. Differences among linear relations were determined by analysis of covariance; see text for statistical comparisons. Elongation is expressed as increase of natural strain \(d\text{Strain}_t\), \(\ln(Lt/L0)\times100\), where \(L\) is instantaneous length and \(L0\) is initial length, measured at a stress of 1.8 g/mm².
After hour 3 (Figure 7, lower panel), during which all strips were returned to baseline peak and resting stresses, all tissue types from protocols I and III fell along the same line, indicating again the reversibility of the excess elongation that had occurred during increased resting stress. The strips subjected to increased peak stress (protocol II) during hour 2 remained relatively elongated in hour 3 compared with the other protocols, and noninfarcted myocardium remained relatively elongated compared with 24-hour-old infarct tissue and 3- and 15-week-old scar strips subjected to increased peak stress.

Discussion

LV aneurysm develops after MI in 10–15% of patients surviving an MI and is associated with a dismal prognosis with a 50% mortality at 5 years.1,2,17 After acute MI, regional LV wall stress can attain values that are two to five times normal.18 Moreover, post-MI topographical changes can result from a combination of factors including hemodynamic stresses2,4,8,10,11,19 and infarct tissue composition.20 Parmley et al20 examined the length–tension characteristics of dissected human aneurysms of varying composition (muscular, mixed muscular–fibrous, and fibrous); in vivo wall stresses of these aneurysms were calculated to be between 68 and 162 g/cm² (end diastolic) and between 268 and 817 g/cm² (end systolic). In the current study, we imposed similar stresses on isolated tissue strips from acutely ischemic, infarcted, and scarred myocardium to determine the extent and reversibility of strip elongation when exposed to these stress levels.

Previous studies21–23 have generally focused on diastolic pressure–length and compliance relations in post-MI ventricles. However, the irreversible tissue elongation that results in aneurysm formation is probably related to the viscous and/or “creep” properties of post-MI tissue. Glover et al24 studied the relation between diastolic creep and systolic function with 15 minutes of coronary occlusion with reperfusion in dogs and noted a 16% increase in diastolic segment length. These authors suggested that it would be beneficial to separate the diastolic length changes from the systolic functional effects. In our study, we were able to isolate the relative contribution of preload (resting stress) and afterload (peak stress) on the extent of creep.

The purpose of our study was to determine whether an increase in peak stress (modeling increased afterload) or in resting stress (modeling increased preload) during different stages of infarct healing would cause an irreversible elongation of noncontractile tissue. Such a length increase would suggest the propensity of acutely ischemic, recently infarcted, or healing scar tissue to develop LV aneurysm. By oscillating strips of tissue at normal physiological peak and resting stresses and then selectively tripling either the peak or resting stress, we were able to assess independently the effects of an increase in resting or peak stress on tissue strain (elongation). A strip strain increase was deemed irreversible if a significant increase in strip length persisted after the return to physiological stress levels, compared with the strips subjected only to physiological peak and resting stress levels for the same time period. Thus, the ability of the tissue to resist elongation despite an increased stress was measured, and on return to normal physiological conditions, the reversibility of any elongation was ascertained.

Four types of myocardial tissue were studied. Nonperfused strips of noninfarcted myocardium were used to model acutely ischemic tissue. Infarcted tissue at 24 hours after MI was used to model acutely infarcted dyskinetic tissue preceding healing and collagen deposition.12,25,26 Three- and 15-week-old myocardial scar tissue was used to evaluate any changes after scar formation. Scar composition and percent fibrosis are known to influence its mechanical properties.20,27 Our previous quantitative histological assessment of 3-week-old scar tissue formed after permanent occlusion showed such scar tissue to be composed of 75% fibrous tissue and 25% residual nonviable myocytes. The residual myofibrillar elements were found only at the subendocardial and subepicardial edges of the scar when the entire midwall region was stained for collagen.13 Thus, by 3 weeks after MI the majority of the infarcted tissue had been replaced by scar tissue in our experimental model.

Effect of an Increase in Resting Stress (Preload)

Noninfarcted myocardium and 24-hour-old infarct strips elongated slightly in response to an increased resting stress compared with noninfarcted myocardium and infarcted tissue maintained at physiological resting stress. However, this length change was reversible with strip unloading in contrast to the irreversible changes seen with increased peak stress. Although the length changes observed with increased resting stress in the noninfarcted myocardium and 24-hour-old infarct tissue were minimal (<1%/hr) and reversible, a more prolonged increase in preload might potentially result in an irreversible elongation of the tissue. Also, in the intact ventricle, significant increases in preload usually cause an associated increase in afterload because of the preload-induced increases in chamber radius.28 Thus, large increases in preload could influence aneurysm formation by such a secondary effect on afterload.

Effect of an Early Increase in Peak Stress (Afterload)

We found that the greatest change in tissue elongation in response to an increase in peak stress (afterload) occurred in acutely ischemic (noninfarcted) myocardial tissue rather than in 24-hour-old or healed infarct strips. This finding is in agreement with a number of studies21–23,27,29 that have shown bulging of acutely ischemic tissue after coronary occlusion.

Some studies10,30 suggest that ventricular unloading decreases infarct expansion; others have shown complete recovery of creep in passive rabbit papillary
muscle after 100 seconds of a 2–3% length increase under isotonic conditions. However, we have shown that beyond certain limits of either time (60 minutes) or degree of increased afterload (threelfold increase) there is an irreversible elongation of acutely ischemic, infarcted, and scar tissue.

Hammerman et al increased systolic pressure for 4 hours in dogs during acute coronary occlusion and found infarct expansion and wall thinning at 1 week after occlusion. Other studies have shown or suggested a “point of no return” for tissue length changes. Little and Wead tested rabbit papillary muscle and reported that an irreversible length change occurred when the muscle was stretched to more than 15% of its initial length for 5 minutes. In our study, irreversible length changes occurred when an imposed stretch of 4–7% persisted for 1 hour. Longer periods of increased afterload would likely result in continued tissue elongation as shown in Figure 4. The irreversible elongation in ischemic dyskinetic tissue may be due to overstretched sarcomeres that have been found to develop in ischemic tissue. Glower et al found widened I bands with transient ischemia in dogs; others have shown that creep correlates with increased sarcomere length and myofilament disarray.

Early post-MI expansion has been associated with an irreversible change in infarct topography and later aneurysm formation. Animal studies suggest that irreversible expansion of an infarct region provides a template for later scar formation, and a clinical study has shown infarct expansion within 46–72 hours after MI without any further change in infarct topography for the next 19 days. Thus, these studies support the concept that LV aneurysm is the result of an early elongation of the acutely infarcted tissue.

Our current study is in agreement with these previous studies since a threefold increase in afterload imposed on both acutely ischemic (noninfarcted) myocardial tissue and infarct tissue at 24 hours after MI resulted in a significant increase in strip elongation that was irreversible despite strip unloading. Furthermore, the acutely ischemic (noninfarcted) myocardium was found to be more vulnerable to changes in peak stress, with a significantly greater degree of elongation for the same increase in stress than 24-hour-old infarct tissue.

The 24-hour-old infarct tissue had the least amount of elongation in all three protocols (Table 3 and Figure 7). This finding raises the possibility that the 24-hour-old infarct tissue manifested substantial elongation that had occurred in vivo, before being excised and studied at 24 hours after MI. We calculated the predicted in vivo elongation of the ischemic region during the first 24 hours after MI in the following way: Using the elongation response of the noninfarcted myocardium (1.24%, Figure 4) in protocol I (physiological peak and resting stress) at 6 hours (5 hours after the initial conditioning hour) and assuming the same strain rate for the next 24 hours, we calculated in vivo elongation of approximately 5.95% for the first 24 hours with MI at physiological stress levels. Combining this value with the elongation of the 24-hour-old infarct tissue subjected to increased peak stress in protocol II (3.9±0.9%) results in a total elongation for the calculated in vivo elongation plus the strip elongation on our test apparatus of approximately 9.8%, a value slightly greater than the strain response of noninfarcted myocardium after increased peak stress in protocol II (7.4±1.3%). Thus, the 24-hour-old infarct tissue may have already been elongated by approximately 5.95% in vivo before being subjected to the increase in peak stress in our test apparatus.

Effects of Increased Peak Stress (Afterload) Late in Healing

Although the initial phase of ventricular “remodeling” after myocardial infarction usually occurs before development of fibrous scar, our results suggest that healed infarct scars may also be subject to expansion after mature fibrous tissue has formed. Some experimental studies have shown a progressive dilatation of the normal and infarcted region of the LV over time although the relative contributions of the normal and scar regions to this chamber dilatation have not been delineated.

Load reduction with captopril, given both early (2 days) and late (21 days) after MI, reduced the tendency for post-MI LV cavity dilatation to occur, suggesting that hemodynamic changes both early and late in healing can affect post-MI LV geometry. Although we increased rather than decreased peak stress in our study, our results both support and expand the findings of Pfeffer et al by addressing specifically the effect of increased afterload on elongation in isolated strips of infarct and scar tissue. In our study, irreversible length changes occurred in both infarcted and healing scar tissue with an increase in peak stress, modeling an increase in afterload (Figures 3 and 7). Thus, our study suggests that LV aneurysm may develop late in the course of post-MI healing in response to an increase in afterload, after scar tissue has already formed.

Our results are also consonant with the finding of infarct expansion occurring 15 weeks after MI, when patients with large anterior wall infarcts underwent exercise conditioning. In this study, serial two-dimensional echocardiography demonstrated the expansion of the healed infarct region, as well as the noninfarcted LV cavity, in response to the stress of exercise. Our study of isolated 15-week-old scar strips shows that such scars have the potential to elongate when stress levels are increased. In contrast, Theroux et al found that end-diastolic segment length decreased during 3 weeks of post-MI healing in dogs; however, their experiments did not include a hemodynamic challenge that increased wall stress, as in the study of Jugdutt et al and in our study. We found that both 3-week-old scar and 15-week-old scar elongated to the same extent as...
24-hour-old post-MI infarct tissue after imposition of a threefold increase in peak stress (Figures 3 and 4) despite the difference in stiffness coefficient of these tissue types (Figure 6). Therefore, increased stiffness of healing scars did not make them more resistant to elongation (i.e., creep) after a sustained increase in peak stress.

Consistent with our results and those of Jugdutt et al.,13 the contractile state of the noninfarcted region has been shown to correlate with post-MI aneurysm formation. Arvan and Badillo34 have reported that post-MI aneurysm development is associated with greater fractional shortening in the noninfarcted LV region. Although the time course of aneurysm development in these patients was not studied serially, this finding suggests that increased contractile function of the noninfarcted ventricle could contribute to stretching of the infarct or the scar region.

The similarity in the elongation response to increased peak stress (afterload) between 24-hour-old infarct tissue and 3- and 15-week-old scar tissue suggests a finite limit on resistance to elongation, consistent with Little and Wead,31 who found a finite limit on the extent of the viscous element in cardiac muscle. However, the physiological mechanisms responsible for this resistance in infarct and scar tissue differ. Previous work25 from our laboratory has shown that post-MI collagen synthesis is not significantly increased by 24 hours after MI, suggesting that the resistance to elongation at this time may be due to edema and phagocytic infiltration of the necrotic tissue and preexisting collagen, while collagen deposition and the healing process contribute to the resistance to elongation in the scar tissue.

The noninfarcted myocardium in our experiments did not have time to undergo any physiological response to injury in vivo; thus, the strain response was in direct proportion to the applied stress. Creep after acute myocardial ischemia has been shown to be load dependent in other animal models.15 In our experiments, the average elongation in noninfarcted myocardial tissue during the high resting stress of 0.6 g/mm² was approximately one tenth as large as the average elongation during the high peak stress (6.0 g/mm²) (Figure 7), suggesting that acutely ischemic, noncontractile myocardial tissue is vulnerable to tissue elongation in direct proportion to an increase in wall stress.

In summary, our studies of isolated strips of acutely ischemic, infarcted, and healing post-MI tissue are generally consistent with post-MI observations made in intact ventricles. Using isolated strips allowed us to independently impose increases in peak stress (afterload) or resting stress (preload). Increasing preload for 1 hour produced very small degrees of elongation that were reversible with strip unloading. Increasing afterload for 1 hour resulted in irreversible tissue elongation in 15-week-old scars as well as in acutely ischemic tissue. These observations support the concept that late long-term alterations of post-MI wall stress can have a significant effect on LV geometry and remodeling.

Appendix

**LV Wall Stress in Relation to LV Pressure in the Rabbit**

Laplace’s law states that wall stress=(pressure×radius)/(2×thickness). The volume of 1.76±0.04 ml has been determined to be equivalent to 10 mm Hg LV end-diastolic pressure (unpublished data, C.S. Apstein and W.N. Grice, Cardiac Muscle Research Laboratory, Boston University School of Medicine). The radius (r) was determined as follows:

\[ \text{LV wall stress} = \frac{0.01363^* \times \text{pressure} \times \text{radius}}{2 \times \text{thickness}} \]

Thus, at 10 mm Hg,

\[ \text{LV wall stress} = \frac{0.01363 \times 10 \text{ mm Hg} \times 0.749 \text{ cm}}{2 \times 0.197 \text{ cm}} = 0.26 \text{ g/mm}^2 \]

Likewise, at 8 mm Hg (physiological LV end-diastolic pressure),

\[ \text{LV wall stress} = 0.21 \text{ g/mm}^2 \]

At 80 mm Hg (physiological LV systolic pressure),

\[ \text{LV wall stress} = 2.1 \text{ g/mm}^2 \]

At 24 mm Hg (high LV end-diastolic pressure),

\[ \text{LV wall stress} = 0.63 \text{ g/mm}^2 \]

At 240 mm Hg (high LV systolic pressure),

\[ \text{LV wall stress} = 6.3 \text{ g/mm}^2 \]

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**References**


*0.01363 is a conversion factor to convert millimeters of mercury to grams per millimeters squared.*13

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