Mechanical and Structural Correlates of Canine Pericardium

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With Scanning Electron Microscopy by

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SUMMARY We have assessed viscoelastic properties of pericardium within the physiological range of stresses and related mechanical behavior to fiber direction as defined by scanning electron microscopy. Stiffness, stress relaxation, and creep were measured in samples taken from the anterior surface of 14 canine pericardia. Stress-strain relations generally were not exponential; stiffness at a stress of 1 g/mm² ranged from 12.9 to 239 g/mm² during stretch and varied both from pericardium to pericardium and with the orientation of the strip within the sample (anisotropy). The strips exhibited hysteretic behavior which was not proportional to rate of strain. Following a rapid increase in stress, creep averaged less than 1% and stress relaxation, 34%, in a 30-minute test period. The orientation of the strip with the greatest stiffness was consistent from pericardium to pericardium, and correlated with a layer of collagen fibers oriented along the major axis of the strip. Circ Res 49: 807-814, 1981

SOME previous studies of the properties of pericardial strips (Rabkin et al., 1974, 1975) have applied large, non-physiological loads to the tissue in order to define such material properties as tensile strength (stress of failure). Others (Vito, 1979) have not taken into account orientation of the strips because of claimed isotropy of the pericardium (Hildebrandt et al., 1969). Finally, no studies have combined detailed analysis of mechanical properties with microscopic examination of the structures responsible for those properties. In the present study, we investigated the elastic and viscoelastic properties of the pericardium, particularly at low stresses, while avoiding high, non-physiological stresses. To relate pericardial structure to physiology, mechanical properties were related to the orientation of the collagen fibers within the strips, visualized by scanning electron microscopy.

Methods

A circular sample of parietal pericardium approximately 3 cm in diameter was removed from the anterior surface of the heart of 14 dogs and maintained in oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solution at 37°C. Fatty tissue overlying the pericardium was dissected away if necessary. From one to four strips, approximately 2 x 13 mm, were cut from each section, for a total of 42 strips. Adjacent parallel strips were used to test the reproducibility of analysis of mechanical properties, and strips oriented to each other at 45° or 90° were tested for isotropy of the pericardium.

Spring clips were attached to the ends of the pericardial strips which then were studied in an apparatus usually used for isolated papillary muscle studies (Sulman et al., 1974; Wiegner and Bing, 1977) with a force range of 0-10 g. Quantization error was less than 20 mg for force and 5 μm for length measurements. Length and width of the strips were measured with a Gaertner cathetometer and telescope. Length and force servosystems facilitated computer control of strip force and length. Under program control, constant velocity stretches and releases were performed at rates from 0.01 to 5.0 mm/sec. Maximum force applied during a stretch was approximately 5 g. A “run” will be defined as a series of 10 stretch/release cycles used to pre-condition the strip, followed by three more stretch/release cycles which were recorded and presented on a cathode ray tube display. We performed as many runs as were necessary to obtain a consistent response in the displayed cycles. A total of 3-6 suitable cycles from each strip were stored in a digital computer (Nova 2) for later analysis.

Force and length data were expressed as Eulerian stress (σ) and natural strain (ε):

\[ \sigma = \frac{Force}{Area} \]
\[ \epsilon = \ln \left( \frac{L}{L_0} \right) \]
where \( A_i \) = instantaneous cross-sectional area, \( \ell_i \) = instantaneous length, and \( \ell_0 \) = zero-stress length. Because the strips are very compliant at low stresses, it is difficult to determine exactly their zero-stress length. The amplitude of the stretch/release cycle was adjusted so that the strip was fully released between cycles, extended only by the weight of the bottom spring clip (20 mg). At this time, of course, the force transducer was momentarily unloaded and we could take note of, and correct for, any offset. In this way, error in measuring force could be held to a maximum of approximately 20 mg during each stretch/release cycle. Given the compliance of the pericardium at this level of stress, we estimate that zero-stress length could be measured with an error of no more than 1 or 2%.

A number of equations have been proposed to describe the stress-strain behavior of elastic animal tissue (Fung, 1967; Blatz et al., 1969; Mirsky and Parmley, 1973). If stress-strain curves can be fit by an exponential curve, for example,

\[
\sigma = B(\lambda^A - 1) = B(\lambda^A - 1)
\]  

\[
\text{(1)}
\]

where \( \lambda = \ell / \ell_0 \) and \( A \) and \( B \) are constants, then the stiffness-stress curve is linear: \( d\sigma / d\ell = A(\sigma + B) \). However, stress-strain curves were not uniformly exponential in the present study, nor could the curves be consistently well fitted with the power law (Blatz et al., 1969):

\[
\sigma = 2(G/A)(\lambda^{\lambda - 1} - \lambda^{-\lambda^{(\lambda - 1)}})
\]  

\[
\text{(2)}
\]

where \( A \) and \( G \) are constants. For this reason, a four-parameter version of the Blatz model was fitted to some of the data:

\[
\sigma = \sum_{i=1}^{2} 2(G_i / \lambda^A_i)(\lambda^{\lambda - 1} - \lambda^{-\lambda^{(\lambda - 1)}}).
\]  

\[
\text{(3)}
\]

In addition, in order to obtain stiffness-stress data independent of any particular model, stiffness was calculated for a number of levels of stress by a moving ensemble modified least squares technique and plotted as shown in Figure 1. For example, to calculate stiffness at a stress of 0.1 g/mm\(^2\), a window of 20 points (approximately 100 \( \mu \)m) was chosen from the stress-strain curve such that 10 lay on each side of the point where \( \sigma = 0.1 \) g/mm\(^2\). A least squares technique then fit a straight line through these 20 points, the slope of which is the desired stiffness. A modification was made to the standard least squares technique such that, instead of minimizing squared differences between calculated and actual values of the dependent variable (here, stress), the computer program iteratively minimized the squared distance of each point from the least-squares line. We found this iterative technique to be considerably more accurate than the standard method when random errors (noise) are found in both the dependent and independent variables. Values of \( d\sigma / d\ell \) at \( \sigma = 0.3 \) and 1.0 g/mm\(^2\) were tabulated, as were values of \( d\sigma / d\ell \) and \( \epsilon \) for values of \( \sigma \) = 0.5, 1.0, 3.0, and 5.0 g/mm\(^2\). All stiffness data are expressed as means ± SE.

Creep is the time-dependent elongation of a material held at a constant stress. Creep was studied in nine strips by raising force rapidly from near zero to 10 ± 2 g/mm\(^2\) and maintaining that force for 30 minutes while sampling, at intervals, the length of the strip.

Stress relaxation is the time-dependent diminution of stress of a material held at constant strain. Seven strips were adjusted rapidly from a length at which stress was near zero to a length at which stress was 15 ± 1.7 g/mm\(^2\) and maintained at that length for 30 minutes while force was repeatedly sampled.

Following completion of the previously described protocol, eight strips were prepared for scanning electron microscopy (SEM). Pericardial strips maintained at a stress of approximately 5 g/mm\(^2\) were immersed in 3% 0.1 M sodium cacodylate buffered glutaraldehyde (pH 7.4) for 3 hours. Strips then were rinsed in sodium cacodylate, cut flush with the clips, blotted, and weighed. Cross-sectional area of the strips was calculated from the strip length and weight assuming a density of 1 g/cm\(^3\).
strip thickness was calculated from measured width and calculated area. Strips then were placed in 2% 0.1 M sodium cacodylate buffered OsO₄ for 2 hours, dehydrated in a graded series of acetones, critical point dried, mounted on specimen stubs, coated with gold to enhance conductivity, and examined by SEM.

Results

Average physical dimensions of 42 strips were (mean ± SD): length, 9.0 ± 1.9 mm; width, 2.2 ± 0.6 mm; and thickness, 0.17 ± 0.06 mm. Strips ranged from 5.9 to 15.1 mm in length, 1.0 to 2.9 mm in width, and calculated thicknesses, 0.09 to 0.29 mm. There was no correlation between any of the strip dimensions and normalized stiffness.

After the first two experiments, it was evident that velocity of stretch/release had no effect on measured stiffness, at least over the range of velocities used. Therefore, all subsequent experiments were conducted with a velocity of 2 mm/sec.

Most strips displayed a curvilinear stiffness-stress curve for low-to-moderate stresses (<2.0 g/mm²), as shown in Figure 1. For this reason no "elastic stiffness constant" (Mirsky and Parmley, 1973) was defined for these strips; rather, stiffness was calculated directly, as previously described, at various levels of stress. In 42 strips, stiffness during stretch at a stress of 0.3 g/mm² ranged from 3.9 to 60.2 g/mm² with a mean of 14.4 ± 2.0 g/mm². At a stress of 1.0 g/mm², the range was 12.9 to 239 g/mm², with a mean of 61 ± 8.5 g/mm². These stiffness values during stretch are plotted in Figure 2 to illustrate the spread of values within the range. Stiffness data were available for stresses up to 5 g/mm² in 18 of the strips; these values are given in Table 1, and illustrate the concave upward nature of the stiffness-stress relation during stretch and release. The properties of this subset did not differ significantly from those of the remaining strips.

Table 1

<table>
<thead>
<tr>
<th>Stress (g/mm²)</th>
<th>Stiffness (g/mm²) during Stretch</th>
<th>Stiffness (g/mm²) during Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>14.5 ± 3.2</td>
<td>16.4 ± 3.8*</td>
</tr>
<tr>
<td>1.0</td>
<td>53.8 ± 12.9</td>
<td>62.2 ± 15.9*</td>
</tr>
<tr>
<td>3.0</td>
<td>210 ± 31</td>
<td>246 ± 40*</td>
</tr>
<tr>
<td>5.0</td>
<td>383 ± 41</td>
<td>475 ± 53*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n = 18).

* P < 0.001 vs stretch (paired t test).

Figure 3 shows the result of fitting Equations 1 and 2 to stress-stretch data from a relatively stiff and a compliant strip. Only one fit is shown to each curve, as there was no difference in the fits obtained with Equations 1 and 2. This is not surprising, as the two equations are equivalent for A > 1.
Neither model describes the shape of the more compliant stress-strain curve very well, although the stiffer curve is well fitted. The more compliant strip is typical of many strips which tended to show a gradual increase in stress with increasing strain, followed by an "elbow" and rapidly increasing stress thereafter. However, no general pattern relating goodness of fit to strip stiffness or orientation was noted. It is probable that the complex structure of collagenous sheets is responsible for the inconsistent curve fits obtained with two-parameter models such as Equations 1 and 2.

Table 2 lists the parameters obtained by fitting Equation 3 to the data from pericardia 8-14. In general, a better fit was obtained using Equation 3 than Equation 2. However, in a number of cases, shown with asterisks in Table 2, the fit could not be improved by the addition of two more parameters, and the results represent Equation 2.

Mean stiffness values during release exceeded those during stretch at all levels of stress, as shown in Table 1. This hysteresis was found despite the lack of sensitivity of stiffness to rate of stretch or release. In strip no. 2, which was 9.6 mm long and had a cross-sectional area of 0.25 mm², the area of the hysteresis "loop" was calculated at strain rates of 0.14, 0.54, 2.0, 3.7, and 5.0 mm/sec; the areas, ergs, respectively. Since the area within the hysteresis "loop" was calculated at strain rates during stretch at all levels of stress, as shown from 0.098 ± 0.051 to 0.166 ± 0.085 during stretch, and from 0.105 ± 0.051 to 0.168 ± 0.089 during release. The average strain is larger at any given stress during release than during stretch. Although large standard deviations reflect the variability of

![Graph showing stress-strain relationship](image-url)

**FIGURE 4** Strain of pericardial strips during stretch and release. Mean of 18 strips; strain rate was 2 mm/sec. At each level of stress, strain during release exceeded strain during stretch (paired t-test, t < 0.001).

**Table 2 Parameters Obtained by Fitting Equation (3) to Stress-Strain Data**

<table>
<thead>
<tr>
<th>Strip no.</th>
<th>Orientation</th>
<th>G₁</th>
<th>A₁</th>
<th>G₂</th>
<th>A₂</th>
<th>G₁</th>
<th>A₁</th>
<th>G₂</th>
<th>A₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>8A</td>
<td>0°</td>
<td>0.56</td>
<td>24</td>
<td>0.41 x 10⁻⁶</td>
<td>118</td>
<td>0.45</td>
<td>25</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>8B</td>
<td>90°</td>
<td>0.22</td>
<td>15</td>
<td>*</td>
<td>*</td>
<td>0.16</td>
<td>16</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>8C</td>
<td>45°</td>
<td>0.20</td>
<td>22</td>
<td>0.20 x 10⁻⁵</td>
<td>118</td>
<td>0.17</td>
<td>22</td>
<td>0.38 x 10⁻⁹</td>
<td>141</td>
</tr>
<tr>
<td>8D</td>
<td>45°</td>
<td>0.28</td>
<td>22</td>
<td>0.59 x 10⁻⁵</td>
<td>106</td>
<td>0.21</td>
<td>23</td>
<td>0.15 x 10⁻⁷</td>
<td>127</td>
</tr>
<tr>
<td>9A</td>
<td>45°</td>
<td>0.19</td>
<td>21</td>
<td>0.24 x 10⁻⁵</td>
<td>83</td>
<td>0.13</td>
<td>23</td>
<td>0.75 x 10⁻⁹</td>
<td>100</td>
</tr>
<tr>
<td>9B</td>
<td>135°</td>
<td>0.16</td>
<td>22</td>
<td>0.19 x 10⁻⁵</td>
<td>52</td>
<td>0.19</td>
<td>25</td>
<td>0.29 x 10⁻⁹</td>
<td>113</td>
</tr>
<tr>
<td>9C</td>
<td>0°</td>
<td>0.23</td>
<td>14</td>
<td>0.71 x 10⁻⁷</td>
<td>68</td>
<td>0.13</td>
<td>17</td>
<td>0.12 x 10⁻⁹</td>
<td>198</td>
</tr>
<tr>
<td>9D</td>
<td>0°</td>
<td>0.19</td>
<td>14</td>
<td>0.18 x 10⁻⁶</td>
<td>86</td>
<td>0.16</td>
<td>15</td>
<td>0.50 x 10⁻⁹</td>
<td>124</td>
</tr>
<tr>
<td>10A</td>
<td>90°</td>
<td>0.40</td>
<td>18</td>
<td>0.22 x 10⁻⁵</td>
<td>131</td>
<td>0.23</td>
<td>23</td>
<td>0.14 x 10⁻¹⁰</td>
<td>171</td>
</tr>
<tr>
<td>10B</td>
<td>0°</td>
<td>0.34</td>
<td>54</td>
<td>0.45 x 10⁻⁷</td>
<td>134</td>
<td>0.16</td>
<td>56</td>
<td>0.52 x 10⁻⁷</td>
<td>157</td>
</tr>
<tr>
<td>10C</td>
<td>0°</td>
<td>0.067</td>
<td>64</td>
<td>*</td>
<td>*</td>
<td>0.024</td>
<td>73</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>10D</td>
<td>135°</td>
<td>0.73</td>
<td>12</td>
<td>0.17 x 10⁻³</td>
<td>54</td>
<td>0.51</td>
<td>14</td>
<td>0.54 x 10⁻⁶</td>
<td>62</td>
</tr>
<tr>
<td>11A</td>
<td>45°</td>
<td>0.021</td>
<td>185</td>
<td>*</td>
<td>*</td>
<td>0.003</td>
<td>223</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>11B</td>
<td>0°</td>
<td>0.90</td>
<td>133</td>
<td>*</td>
<td>*</td>
<td>0.095</td>
<td>178</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>11C</td>
<td>90°</td>
<td>1.49</td>
<td>86</td>
<td>*</td>
<td>*</td>
<td>0.34</td>
<td>107</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>11D</td>
<td>45°</td>
<td>0.22</td>
<td>132</td>
<td>*</td>
<td>*</td>
<td>0.011</td>
<td>189</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>12A</td>
<td>90°</td>
<td>0.11</td>
<td>38</td>
<td>*</td>
<td>*</td>
<td>0.068</td>
<td>40</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>12B</td>
<td>45°</td>
<td>0.18</td>
<td>34</td>
<td>0.64 x 10⁻⁴</td>
<td>116</td>
<td>0.10</td>
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<td>0.17 x 10⁻⁶</td>
<td>155</td>
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<tr>
<td>12C</td>
<td>90°</td>
<td>1.27</td>
<td>28</td>
<td>0.31 x 10⁻⁴</td>
<td>113</td>
<td>0.92</td>
<td>33</td>
<td>0.17 x 10⁻⁷</td>
<td>163</td>
</tr>
<tr>
<td>12D</td>
<td>135°</td>
<td>0.96</td>
<td>20</td>
<td>0.98 x 10⁻⁶</td>
<td>83</td>
<td>0.54</td>
<td>29</td>
<td>0.30 x 10⁻⁹</td>
<td>164</td>
</tr>
<tr>
<td>13A</td>
<td>0°</td>
<td>0.72</td>
<td>31</td>
<td>0.25 x 10⁻⁷</td>
<td>82</td>
<td>0.64</td>
<td>29</td>
<td>0.44 x 10⁻³</td>
<td>96</td>
</tr>
<tr>
<td>13B</td>
<td>135°</td>
<td>0.24</td>
<td>16</td>
<td>0.98 x 10⁻⁷</td>
<td>78</td>
<td>0.17</td>
<td>17</td>
<td>0.24 x 10⁻⁴</td>
<td>115</td>
</tr>
<tr>
<td>13C</td>
<td>90°</td>
<td>0.46</td>
<td>17</td>
<td>0.39 x 10⁻⁴</td>
<td>129</td>
<td>0.25</td>
<td>21</td>
<td>0.11 x 10⁻³</td>
<td>164</td>
</tr>
<tr>
<td>14A</td>
<td>90°</td>
<td>0.57</td>
<td>21</td>
<td>0.91 x 10⁻⁷</td>
<td>163</td>
<td>0.52</td>
<td>20</td>
<td>0.71 x 10⁻⁷</td>
<td>118</td>
</tr>
<tr>
<td>14B</td>
<td>0°</td>
<td>0.38</td>
<td>38</td>
<td>0.64 x 10⁻⁵</td>
<td>85</td>
<td>0.18</td>
<td>44</td>
<td>0.30 x 10⁻³</td>
<td>110</td>
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<td>14C</td>
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<td>0.94</td>
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<td>0.18 x 10⁻⁵</td>
<td>70</td>
<td>0.74</td>
<td>17</td>
<td>0.19 x 10⁻⁴</td>
<td>146</td>
</tr>
</tbody>
</table>

* Unable to improve fit with second pair of parameters.
stiffness among strips, on a pairwise basis, all differences are significant \((P < 0.001)\). The area of this average loop was estimated (by Simpson's Rule) to be 17 ergs/mm².

Two further tests of viscoelastic behavior were performed. Creep averaged 0.80% of strip length in nine strips 30 minutes after stress was increased from near zero to 10 ± 2 g/mm². Creep records from three of these strips, from one pericardium, are shown on a linear time scale (Fig. 5A) and on a logarithmic time scale (Fig. 5B). There was no evidence of asymptotic behavior of creep during this time.

Stress relaxation was measured in seven strips after stress was rapidly increased from near zero to 15 ± 1.7 g/mm² by means of a step change in length. Stress declined in an exponential manner, such that after 30 minutes, strip stress was 66 ± 3% of the stress recorded immediately after the increase in length. Figure 6 demonstrates the exponential nature of stress relaxation and creep in a strip in which these two tests of viscoelastic behavior were performed sequentially. Overall, there was no correlation between the stiffness of the strips and either creep \((r = -0.05)\) or stress relaxation \((r = -0.29)\), expressed as percent of initial value, after 30 minutes.

Figure 7 represents the topography of the strips cut from six pericardia and indicates the stiffness,
during stretch, at a stress of 1.0 g/mm². Strip direction is defined in terms of degrees rotation clockwise from a cranial-caudal orientation. These data demonstrate both a wide range of average stiffness from heart to heart and variability of stiffness with direction within each pericardial sample (anisotropy). It is evident that parallel strips are of similar stiffness, whereas perpendicular strips often differ markedly. In addition, there is a consistent pattern of anisotropy in the six groups of strips. For example, dog no. 8 had a compliant pericardium, whereas that of dog 11 was quite stiff. Yet in each, the 0° strip was about twice as stiff as the 90° strip. This pattern is also seen in dogs 10, 13, and 14. The pattern of stiffness has been summarized in the lower portion of Figure 7, obtained by expressing the stiffness in the 0°, 45°, and 135° directions as a percentage of the stiffness in the 90° direction, which is defined as 100%. For the particular region of the pericardium used in this study, the greatest stiffness consistently lies in the 45° direction.

Scanning electron micrographs suggest the existence of a multilayer network of collagen fibers which are at times parallel and at other times interwoven. In Figure 8, fibers within the top layer, oriented along the direction of 5 g/mm² stress during fixation, appear straight. Middle fibers, oriented across the direction of primary stress, remain wavy, as do those within the deep layer. This strip was oriented at 45°, the direction of maximal stiffness for this particular pericardial sample. Other micrographs, from strips not in the 45° orientation, tended not to show a dominant layer of nearly straightened fibers.

Discussion

Intrapericardial pressures corresponding to the experimentally applied stresses (g/mm²) or tensions (g/mm) can be calculated by considering the pericardium to be a thin walled sphere (for the moment, isotropic) and applying Laplace's Law:

\[ T = \frac{P r}{2} \]

where \( T \) is tension, \( P \) is intrapericardial pressure, and \( r \) is the radius. If we assume \( r = 4 \) cm, and a pressure of 1 mm Hg (= 1.35 g/cm² or 1.32 \( \times \) 10³ dynes/cm²), then \( T = 2.7 \) g/cm (2.6 \( \times \) 10² dynes/cm), or, given a mean strip thickness of 0.17 mm, \( \sigma = 1.6 \) g/mm² (1.6 \( \times \) 10⁵ dynes/cm²). Since a pressure of 1 mm Hg yields a stress of 1.6 g/mm², the data presented in Table 1 cover inflation pressures up to 3-4 mm Hg. In this study, care was taken not to apply stresses in excess of 30 g/mm² (20 mm Hg) to the strips, in order to ensure that large, non-physiological loads did not permanently deform the strips. This is in contrast to most previous mechanical studies of the pericardium, which generally imposed larger loads on the pericardial strips (Rabin, 1974; Vito, 1979).

It was not possible to define a modulus of elasticity for the pericardial strips used in this study.
because no linear range of the stress-strain curve was seen within the range of stresses examined. Indeed, the linear region seen by Rabkin (1974) occurred at forces in excess of 500 g/mm². The slope of the stiffness-stress curve in our study was generally concave upwards. This may be consistent with the gradual alignment and straightening of collagen fibers during stretch, although it does not clearly separate two possible models of flat collag-
enous tissue structure (Lanir, 1979): (1) collagen undulations induced by prestressed elastin fibers attached at numerous points to each collagen fiber, which has been reported in the skin (Finlay, 1969; Crisp, 1972); (2) inherently undulating collagen fi-
bers with few interconnections to an independent network of straight elastin fibers, as has been re-
ported in the mesentery of the cat (Chu et al., 1972). Our scanning electron micrographs were more suggestive of model 2 than model 1.

That the stiffness-stress relation remains concave upward, at least to a stress of 5 g/mm², suggests that either the collagen fibers are not completely straight at this point, or straight collagen fibers obey a uniaxial stress-strain power law more steep than exponential.

We have indicated that two-parameter models such as Equations 1 and 2 are inadequate to de-
scribe the shape of the stress-strain data for low stresses, largely because of an “elbow” which is often found at a stress between 0.5 and 1.0 g/mm² (Fig. 1). Clearly, models which fit more constants to similar data \[ \sigma = A + B \exp(C_1 e) + B_2 \exp(C_2 e) \] (Wiegner, 1978) will be more accurate, at the cost of greater complexity. Such complexity may be necessary to model the straightening, then stretch, of wavy col-
lagenous fibers (Lanir, 1979). Thus, use of the four-
parameter model, Equation 3, can be justified here on histological grounds: one pair of constants may correspond to elastin fibers, or perhaps unstraight-
ened collagen fibers, whereas the second pair may represent straightened collagen fibers. The four-
parameter model provides certain advantages: the fit is very good, and breaking up the curve into a compliant and stiff portion tends to reduce the variability seen in the stiffness constants A; (Table 2) compared to the variability in stiffness seen at 0.3 g/mm² (Fig. 2). On the other hand, certain characteristics of the data, such as the fact that the stiffness of 0° strips at a stress of 1 g/mm² is consistently double that of 90° strips, is not apparent from the stress-stretch parameters in Table 2. This 2-to-1 stiffness relation may well not continue at much higher stresses, where all collagen fibers would be straightened and physiological orientation of collagen layers might be distorted.

Our data reveal that, despite the considerable stiffness of the pericardium at large, non-physiologi-
ical stresses, there is significant extension of the pericardium beyond its zero-stress length before stiffness becomes large. In particular, Figure 4 in-
dicates that an average strip extends by 18% (\( \epsilon = 0.166 \)) under a load of 5 g/mm². Retraction of the intact pericardium observed immediately following incision indicates that the normal pericardium is not unstressed; on the other hand, pressure across the pericardial membrane is normally negligible (Holt, 1970), suggesting that the intact pericardium is lightly loaded, with significant capacity to expand before reaching a point where extensibility is lim-
ited.

Given the technical difficulty of defining a zero-
stress length for the pericardium, the above figures should be taken as approximations. Nevertheless, our ability to measure small forces and extensions greatly exceeds that of previous studies of pericar-
dial strips: force resolution was 20 mg, and force offset was carefully eliminated during the analysis of each stretch/release. Difficulty in recognizing low forces is evident in Rabkin’s (1974) figure of 41% extension at first rise in tension: Hildebrandt (1969) found 33% extension at a stress of 5 g/mm², and Vito (1979), only 2%.

Although the creep of the pericardial strips is quite small, whereas stress relaxation is relatively large, these two measures of the same viscoelastic property are quite consistent. At the rather large stresses at which creep was measured, the length-
tension curve rises so steeply that shortening of the strip by 0.8% will decrease the stress to two-thirds of its previous value. Thus during a 30-minute pe-
riod, such a strip would either creep by 0.8% at constant stress or undergo 34% stress relaxation at constant strain. In the light of the slow rate of creep, one can see why, in the absence of other linear viscoelasticity, the strain rate has no discern-
able effect on measured stiffness, a result also re-
ported by others (Rabkin, 1974; Vito, 1979). The strain rate used in this study ranged from 6% to 3000% of strip length per minute, whereas the creep rate of the strips was less than 2% per hour. Only if the strain rate were slow enough to be comparable to the creep rate would the viscoelasticity exemplified by creep have an effect on measured stiffness.

The slow rate of creep demonstrates that, despite the considerable extensibility of the pericardium at low stresses, creep is not able to significantly modify intrapericardial pressure-volume relationships at large stresses, as during the development of acute tamponade.

Stiffer strips were generally found to have a preponderance of collagen fibers parallel to the axis of the strip, which were straight, rather than wavy, having been under tension at the time of fixation (Fig. 8). More compliant strips tended to have more wavy fibers, running obliquely or perpendicular to, the axis of the strip; these fibers were not straightened by tension applied during fixation. Thus, we have found a correlation between in-
creased stiffness and fiber orientation along the axis of the strip. This is consistent with previous obser-
vations on the directional stiffness of collagen
sheets (Gibson et al., 1969; Lanir and Fung, 1974) and suggests that, despite the diversity of orientation of fibers within a given region, local differences in collagen fiber orientation result in anisotropy of pericardial stiffness.

We consistently found that pericardial strips oriented at 45° were stiffer than for other orientations; a cross-section of such a strip (Fig. 8) demonstrates a major layer of collagen fibers aligned with the strip axis. Wallraff (1937) originally described the course of the superficial, middle, and deep layers of collagen fibers in the parietal pericardium of the dog. In particular, within the region which was examined in the present study, Wallraff found the superficial collagen layer to be oriented in what we have found to be the direction of maximum stiffness.

References
Mechanical and structural correlates of canine pericardium.
A W Wiegner, O H Bing, T K Borg and J B Caulfield

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