Load Regulation of the Structure, Composition, and Function of Mammalian Myocardium

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SUMMARY. It is established that the transition from normal to hypertrophied myocardium occurs in the adult mammal through a process of myocyte enlargement in response to increased mechanical stress. Abnormal myocardial structure, composition, and function have been characterized extensively in this situation. The present study employs a model of reduced mechanical loading to examine the opposite end of this spectrum of myocardial response to stress: the hypothesis tested is that normal myocardial structure, composition, and function depend critically on the maintenance of normal, as opposed to reduced, myocyte loading conditions. Sham-operated and experimental cats had right ventriculotomies performed under venous inflow occlusion. Myocardial unloading was produced in the experimental cats by transecting the chordae tendinae of a single papillary muscle. This procedure unloaded the papillary muscle without apparent interference with its blood supply, contraction frequency, or innervation. Ultrastructure, cell size, hydroxyproline concentration, and muscle mechanics and energetics were characterized in control and unloaded muscles at 1–3 days after surgery. Blood flow and norepinephrine concentration in control and unloaded dog myocardium were determined after the same operative procedure. Ultrastructural changes included disorientation and loss of contractile filaments as well as loss of Z-line substance. Myocyte cross-sectional area decreased to 66% of control at 3 days after the operation. Hydroxyproline concentration increased to 138% of control at 3 days. Isotonic and isometric contractile function was depressed markedly in the unloaded muscles, with a negative shift of the entire force-velocity and active length-tension relationships. There was a major increase in the passive stiffness of the unloaded muscle. Both active oxygen consumption at equivalent levels of isotonic and isometric force and resting oxygen consumption were the same in the unloaded and control myocardium. Blood flow and norepinephrine concentration were only very modestly reduced in the unloaded myocardium. These data demonstrate that—even in the absence of a stimulus to hypertrophy—cardiac structure, composition, and function are not fixed post-neonatal properties but are instead regulated dynamically by the myocyte loading environment. (Circ Res 50: 788–798, 1982)

THIS investigation was designed to assess the hypothesis that myocardial structure, composition, and function are regulated dynamically by the loading conditions of the heart. We propose that the environment of mechanical stress (the hemodynamic force exerted upon heart muscle) in which the myocardium operates is the primary determinant of these basic properties of post-neonatal mammalian myocardium. Normal adult myocardium, when considered in this way, is not static but is, instead, a plastic tissue defined continuously as the operational result of a rather narrow range of hemodynamic forces within a broad potential spectrum including both excessive and reduced loading conditions.

It is clearly established that excessive hemodynamic loads produce myocardial hypertrophy, with eventual functional deterioration into the congestive heart failure state if an excessive load is maintained. The dynamic nature of this situation is demonstrated by the finding that both the morphological and functional abnormalities of hypertrophied myocardium are fully reversible with early restoration of normal myocardial loading conditions (Cooper et al., 1974; Marcus et al., 1977) before congestive heart failure supervenes (Coulson et al., 1977).

Turning, in contrast, to the other end of the spectrum of myocardial stress, there has been virtually no information available heretofore regarding the potential for dynamic regulation of cardiac morphology and function by reduced hemodynamic loading conditions. Earlier studies of myocardial unloading have been restricted largely to descriptions of cardiac atrophy in various clinical settings (Karsner et al., 1925; Hellerstein and Santiago-Stevenson, 1950). The major findings have been that, in situations where the mean arterial pressure has been reduced chronically or the body tissue mass has decreased, cardiac size and fiber diameter are less than normal. While there is very little information regarding functional performance of these atrophied hearts, a recent report (Gottdiener et al., 1978) suggests that myocardial performance may be normal with respect to body size in chronically starved patients. However, in most of the conditions reported, such as Addison's disease or starvation, it is unclear whether hemodynamic unloading or changes in blood volume, hormone levels, and potassium bal-
ance (Goodof and MacBryde, 1944; Harrison et al., 1972) were responsible for myocardial degeneration. In the one study (Roberts and Beck, 1941) in which relatively straightforward hemodynamic unloading by restrictive pericardial disease was observed to result in decreased myocardial mass and fiber diameter, no functional characterization of myocardial performance is available.

In the present investigation, we produced a model of selective unloading of a discrete segment of ventricular myocardium. In this model there was greatly reduced mechanical stress in the experimental tissue without apparent interference either with its blood supply, contraction frequency, or innervation. Further, the hemodynamic conditions for the remaining, normally loaded ventricular myocardium were not altered. We then examined the response of the experimental tissue to mechanical unloading in terms of myocyte ultrastructure and size, tissue hydroxyproline concentration, and myocardial mechanics and energetics. Additional experiments were performed to assess the possible interference of mechanical unloading with myocardial blood flow, contraction frequency and norepinephrine concentration. These latter experiments were done to determine whether myocardial unloading had a primary effect on cardiac structure, composition, and function or whether any effects observed might instead be secondary to alterations in myocardial perfusion, rate of contraction, or sympathetic innervation.

Methods

Preparation of the Experimental Model

The same basic procedure was used to unload a single papillary muscle in the right ventricle of either the cat or the dog. Adult animals of random sex weighing 1.8-4.1 kg (cats) or 16-25 kg (dogs) were anesthetized with sodium pentobarbital, 25 mg/kg, ip (cats) or iv (dogs). They were then intubated and placed on a respirator adjusted to maintain normal arterial blood gases and pH.

After thoracotomy, azygous vein ligation, and venous inflow occlusion, a right ventriculotomy was performed; major epicardial vessels were avoided. Under direct visualization, the chordae tendinae of a single papillary muscle were transected. Direct visualization through the right ventriculotomy allowed selection of an appropriate papillary muscle (long, thin, and discrete) and assured complete chordae transection. The ventriculotomy was excluded from the rest of the ventricle by a vascular clamp immediately after chordae transection. Inflow occlusion then was released after a total duration never exceeding 2 minutes, and the ventriculotomy was closed at leisure. Depending on the particular experimental protocol followed subsequently, the animal either underwent further investigative procedures immediately, or the thoracotomy was repaired and the animal allowed to recover for later study. Control tissue consisted, again depending on the particular subsequent experimental protocol, of either a normally loaded papillary muscle from the same right ventricle or a right ventricular papillary muscle from an animal that had had an otherwise identical sham operation with the exception of chordae transection.

Evaluation of the Experimental Model

Right Ventricular Volume Overload

The critical question to be answered in this regard was whether or not chordae transection resulted in significant tricuspid regurgitation, and thus whether the papillary muscle was unloaded in a normal or in a volume-overloaded right ventricle undergoing hypertrophy. This question was addressed by measuring in the cats at the time of postoperative study (1) right atrial pressure in the anesthesized animal (sodium pentobarbital, 25 mg/kg, ip) obtained via the right external jugular vein from a fluid-filled catheter system, (2) the ratio of the wet weight of the right ventricular free wall exclusive of septum to the preoperative weight of the cat, (3) the ratio of the wet weight of the liver to the preoperative weight of the cat, (4) the ratio of left ventricular wet weight to preoperative body weight to serve as a control for any independent difference in body weight, and (5) myocyte cross-sectional area in normally loaded papillary muscles removed from right ventricles also containing an unloaded muscle and this area in papillary muscles from right ventricles of sham-operated cats.

Myocardial Blood Flow

Perfusion of myocardium and skeletal muscle was measured in dogs rather than cats in order to obtain enough tissue for accurate quantitative analysis. Flow was determined immediately before and after the unloading procedure described above and, in some animals, somewhat later following a volume infusion sufficient to restore arterial pressure to the pre-unloading level. The radioactive microsphere technique was used in accordance with a standard protocol (Marcus et al., 1975) detailed below to measure blood flow.

Each animal was prepared surgically for papillary muscle unloading up to the point of caval occlusion and right ventriculotomy. A cannula was placed in the left atrium for the injection of microspheres, and catheters were placed in the right brachial and both femoral arteries for the withdrawal of reference arterial blood samples and the measurement of pressure via a strain gauge zeroed at the mid-chest level. The dogs then were given heparin, 500 U/kg, iv.

Microspheres 7-10 μm in diameter, labeled with 141Ce, 51Cr, and 85Sr, were employed. For each flow measurement, the 1.3 × 106 to 3.0 × 106 microspheres to be injected were suspended in 0.5 ml of saline. Before injection, the vial containing the microspheres and one drop of polysorbate 80 was agitated vigorously for 4 minutes. Starting 1 minute before microsphere injection and continuing until 3 minutes after injection, blood was withdrawn simultaneously at a constant rate of 2.06 ml/min from the right brachial and right femoral arteries. The microspheres were injected over a 15-second period, and the cannula was flushed with 5 ml of warm saline during the subsequent 20 seconds.

Microspheres were injected at the following times: (1) just before caval occlusion and right ventriculotomy, (2) 5 minutes after chordae transection, caval release, and closure of the ventriculotomy, and (3) in some animals, immediately after restoration of arterial blood pressure to the level observed before the first injection of microspheres by means of intravenous saline infusion. No significant drop in arterial pressure was noted after any of these injections. After each study, the dog involved was killed with an injection of potassium chloride, and the heart and gracilis muscle were excised. The eight samples specified in Table 2 were then obtained. After these blotted specimens were weighed...
quickly to the nearest milligram, they were placed in glass tubes containing 10% formalin and counted for 5 minutes each in a 3-inch well-type γ counter. The reference blood samples were divided into aliquots so that their counting geometry was similar to that of the tissue samples. The energy windows used were 113Ce 126-175 keV, 51Cr 270-370 keV, and 85Sr 400-600 keV. Isotope separation was performed by standard (Rudolph and Heyman, 1967) techniques.

Blood flow was calculated using the following formula:

$$\text{TBF} = \frac{C_0 \times 100 \times \text{RBF} + C_r}{C}$$

where TBF = tissue blood flow in ml/100 g per min, $C_0$ = counts per gram of tissue, RBF = reference blood flow (withdrawal rate from reference arteries) and $C_r$ = total counts in reference blood. If they were in fairly close agreement, the counts in the femoral and brachial blood samples were averaged, but flow values where these reference counts deviated by more than 20% were disregarded.

**Contraction Frequency**

The question addressed here was whether the unloaded papillary muscle continued to contract in synchrony with the rest of the ventricular myocardium. This was readily determined by direct observation at the time of unloading and at the time of study in each case.

**Myocardial Catecholamines**

The possibility of myocardial catecholamine depletion in response to mechanical unloading was assessed qualitatively in the cat and quantitatively, using the larger tissue mass available, in the dog.

Fluorescence histochemistry was used for the qualitative demonstration of catecholamines in cat myocardium. Three days after the unloading procedure, rapid cardiectomy was performed under sodium pentobarbital (25 mg/kg, ip) anesthesia. The following cardiac specimens then were frozen immediately in liquid propane cooled with liquid nitrogen: (1) the unloaded right ventricular papillary muscle, (2) a normal right ventricular papillary muscle, (3) right ventricular free wall, and (4) left ventricular free wall. The tissue was processed (Falck et al., 1962), and the presence of catecholamines was assessed by a microspectrofluorometric technique (Bjorklund et al., 1972). The preparations were examined with a fluorescence microscope using incident light excitation at 490 nm.

Quantitative analysis of total myocardial norepinephrine in the dog was performed by a fluorometric aluminatrihydroxyindole procedure (Anton and Sayre, 1962; Haggendal, 1963; Mayer et al., 1968). Two to 6 days after the unloading procedure, the dogs were anesthetized with sodium pentobarbital (25 mg/kg, ip), and rapid cardiectomy was performed. The nine tissue samples identified in Table 3 were then frozen immediately in liquid propane cooled with liquid nitrogen. The frozen samples were crushed in a glass-to-glass homogenizer, hydrolyzed, and used to estimate collagen concentration by a spectrophotometric method for determining hydroxyproline (Bergman and Loxley, 1963; Lund et al., 1979).

**Myocardial Ultrastructure**

One day after the unloading procedure, cats were anesthetized with ketamine HCl (25 mg/kg, im), intubated, and artificially ventilated. A midsternal thoracotomy was performed, and a catheter connected to a gravity perfusion apparatus was inserted through the brachiocephalic artery into the ascending aorta. Following the injection of heparin, 1000 U/kg, iv, the heart was arrested in diastole with procaine and perfused at 120 mm Hg pressure with 100 ml of Locke's solution followed by 1 liter of 1.5% glutaraldehyde fixative. The onset of fixation occurred less than 1 minute after cardiac arrest (Tomanek and Karlsson, 1973). The unloaded papillary muscle and a control papillary muscle from the same right ventricle were excised, transsected longitudinally, fixed in the glutaraldehyde solution for an additional 2 hours, postfixed in 1% OsO4, dehydrated, and embedded in Epon. Ultrathin sections for electron microscopy were cut from the mid-portion of the papillary muscles. Both longitudinal and cross-sections were obtained from each muscle, stained with uranyl acetate and lead citrate, and then studied and photographed with an electron microscope.

**Myocyte Size**

Transverse 1-μm sections were cut from the mid-portion of the same papillary muscles used for ultrastructure. These sections were stained with Richardson's solution and then studied and photographed with an electron microscope.

**Evaluation of the Unloaded Myocardium**

**Myocardial Contractile and Energetic Function**

One to 3 days after the unloading operation or the sham-operation, the cats were anesthetized with sodium pentobarbital (25 mg/kg, ip), and rapid cardiectomy was performed. An unloaded or a control muscle was excised and mounted in a flow respirometer (Cooper et al., 1981). The chordal end of each muscle was fixed to the lever of a photoelectric displacement transducer mounted above the muscle by a tie at the chordae-muscle junction. The ventricular end of the muscle was enclosed rigidly in a sharp clip sintered to a metal rod; the other end of this rod was screwed directly onto a semiconductor strain gauge. Tension generated by the muscle was measured with very little stray compliance, <0.7 μm/mN over the range of force studied, and the enclosed clip produced only discrete end-segment damage of similar extent in control or unloaded muscles.
and excluded the damaged tissue from the metabolic measurements. Details of the transducer system and associated equipment are reported elsewhere (Cooper, 1976).

After the muscles had been mounted in the respirometer, they were superfused at 29°C by a solution of the following composition (mm): CaCl2, 2.5; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.1; NaHCO3, 24.0; Na acetate, 20.0; NaCl, 98.0; and glucose, 10.0; with 10 units of zinc insulin added per liter. This solution was equilibrated with 95% O2-5% CO2 with a resultant pH of 7.4 and circulated past the muscle from a 1-liter reservoir. Each muscle was preloaded lightly and stimulated at 0.2 Hz until a stable mechanical response was obtained. Field stimuli 5-10% above threshold of alternating polarity with no D.C. offset between stimuli were employed to minimize electrolytic contamination.

Myocardial oxygen consumption was measured polarographically according to well-defined criteria (Carlson et al., 1950). The dimensions and other characteristics of the respirometer were the same as those described before (Cooper, 1976). The configuration of the transducer system and associated equipment is reported elsewhere (Cooper, 1976). Details of the transducer system and associated equipment are reported elsewhere (Cooper, 1976).

Forces and force-shortening curves were constructed during 0.5-Hz contractions of each muscle as follows: first, a preload of about 5 mN/mm2 muscle cross-sectional area was used to define the first point on these curves; successive afterload increments of 5 mN/mm2 were then added to define further points on these curves until a maximum isometric force was reached. The maximum velocity and extent of shortening at each load were measured. Following this, isometric length-tension curves were constructed by beginning at a relatively short muscle length at which active tension generation was first noted and then proceeding in 0.2-mm increments in length until maximum isometric tension, Lmax, was exceeded slightly. Further details of these techniques have been described before (Cooper et al., 1973).

At each isotonic load and isometric length, 120 contractions during a 4-minute period were studied. The oxygen consumption associated with each group of contractions was calculated from the solubility of oxygen at 29°C, a calibration curve of oxygen cathode current vs. different oxygen concentrations, and the deflection in the oxygen cathode current record produced by each intervention at a constant flow.

After each experiment, muscle length was measured by a micrometer with a known preload attached to the muscle. This length, along with the passive tension portion of the length-tension relationship, allowed calculation of muscle length at Lmax. Muscle cross-sectional area was calculated from this length and from the dry weight obtained as the constant weight reached at 100°C. A wet-to-dry weight ratio of four and a specific gravity of one were assumed. That is, area (mm2) = dry weight X 4 (mm/mm2)/length (mm). Results were normalized in terms of muscle length at Lmax and cross-sectional area.

Statistical Analysis

All values are expressed as mean ± se. For comparisons between groups, Student's unpaired t-test was employed; for comparisons of the same variable within a group, Student's paired t-test was used; for comparisons of the same variable among multiple groups, one way analysis of variance was employed (Dixon and Massey, 1979). A significant difference was said to exist when \( P \) was less than 0.05.

### Results

#### Characteristics of the Experimental Model

**Right Ventricular Volume Overload**

The data in Table 1 demonstrate that there was no apparent tricuspid regurgitation as a result of chordal transection, and, thus, no right ventricular hypertrophy in response to a volume overload. That is, mean right atrial pressure and the ratios of both right ventricular weight and liver weight to body weight were the same in the sham-operated control and in the unloaded cats. While some of the earlier experimental animals (Cooper and Tomanek, 1979), whose data are not included in this report, did demonstrate right ventricular volume overload and hypertrophy, it was found subsequently that the selection of a small, single right ventricular papillary muscle for unloading obviated this problem. The fact that myocyte cross-sectional area in normally loaded papillary muscles removed from right ventricles also containing an unloaded papillary muscle was the same as in papillary muscles from the right ventricles of sham-operated cats further substantiates this point.

#### Myocardial Blood Flow

The data in Table 2 show that the unloading procedure produced only a moderate selective reduction of blood flow to the unloaded dog papillary muscle immediately after chordal transection. While there was a substantial percentage reduction of blood flow to each tissue sample as the immediate result of this intracardiac procedure, flow to the unloaded papillary muscle was still 76% of that to the normally loaded papillary muscle following the operation and remained at more than 10 times the level of preoperative flow to the sample of resting gracilis muscle. In four of these dogs, the blood flows were measured a third time after mean arterial pressure had been restored to the pre-unloading level by intravenous saline infusion. Flows to the unloaded and normally loaded papillary muscles were increased, both to a compa-
TABLE 2
Myocardial and Gracilis Blood Flow (ml/100 g per min) in Dogs

<table>
<thead>
<tr>
<th>Right ventricle</th>
<th>Left ventricle</th>
<th>Gracilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unloaded papillary muscle (0.37 ± 0.12 g)</td>
<td>Endocardium (1.52 ± 0.13 g)</td>
<td>(1.62 ± 0.40 g)</td>
</tr>
<tr>
<td>1. 108.72 ± 5.54</td>
<td>1. 138.90 ± 7.07</td>
<td>1. 4.16 ± 0.27</td>
</tr>
<tr>
<td>2. 58.95 ± 4.73</td>
<td>2. 91.63 ± 7.09</td>
<td>2. 2.88 ± 0.27</td>
</tr>
<tr>
<td>% Δ -45.85 ± 3.27</td>
<td>% Δ -34.17 ± 3.85</td>
<td>% Δ -29.73 ± 4.60</td>
</tr>
<tr>
<td>Normal papillary muscle (0.41 ± 0.08 g)</td>
<td>Mid-wall (1.79 ± 0.21 g)</td>
<td></td>
</tr>
<tr>
<td>1. 105.42 ± 5.35</td>
<td>1. 120.31 ± 8.04</td>
<td></td>
</tr>
<tr>
<td>2. 77.11 ± 4.99</td>
<td>2. 90.61 ± 6.74</td>
<td></td>
</tr>
<tr>
<td>% Δ -26.55 ± 3.05</td>
<td>% Δ -24.37 ± 2.74</td>
<td></td>
</tr>
<tr>
<td>Endocardium (1.15 ± 0.14 g)</td>
<td>Epicardium (2.25 ± 0.34 g)</td>
<td></td>
</tr>
<tr>
<td>1. 103.97 ± 5.67</td>
<td>1. 113.43 ± 6.27</td>
<td></td>
</tr>
<tr>
<td>2. 74.53 ± 5.68</td>
<td>2. 88.82 ± 6.45</td>
<td></td>
</tr>
<tr>
<td>% Δ -27.71 ± 3.82</td>
<td>% Δ -22.53 ± 2.54</td>
<td></td>
</tr>
<tr>
<td>Epicardium (1.32 ± 0.18 g)</td>
<td>Endocardium / epicardium</td>
<td></td>
</tr>
<tr>
<td>1. 87.87 ± 8.23</td>
<td>1.24 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>2. 74.90 ± 5.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Δ -17.53 ± 3.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardium / epicardium</td>
<td>1.17 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. There were 20 dogs in this group, and eight samples were removed from each animal. Sample weights are given in parentheses. Percent change (%Δ) indicates the difference between the initial flow measurement (#1, before unloading) and the second flow measurement (#2, after unloading). The ratios (endocardium/epicardium) are those of initial blood flow in the two specified regions.

Contraction Frequency
Following chordal transection, deformation of the slack right ventricular papillary muscle during its contractions was readily observed in both the dog and the cat. The unloaded papillary muscle was noted to contract in synchrony with the remaining ventricular myocardium in each instance, both immediately after chordal transection and at the time of subsequent cardectomy prior to its removal.

Myocardial Catecholamines
A qualitative assessment of any effect of the operative procedure used to produce unloading or of unloading itself on myocardial catecholamines was made in the cat. In each of two cats studied 3 days after the unloading procedure, fluorescence histochemistry showed a marked reduction in sympathetic innervation of the right ventricle: no sympathetic nerves were seen in either the normally loaded or the unloaded right ventricular papillary muscles, and the right ventricular free wall showed only very few nerves. Each left ventricle appeared to be normally innervated. Thus, the operative procedure, which included a right ventriculotomy, caused a marked reduction in right ventricular sympathetic innervation at a postoperative interval similar to that used in the other sections of this study, but no selective catecholamine depletion in unloaded myocardium was observed.

The data in Table 3 provide a quantitative assessment of catecholamine content in the myocardium of five dogs studied 2-6 days after the unloading procedure. There was a significant reduction in the mean right ventricular norepinephrine concentration when compared to the mean left ventricular value in the same dogs. There was a modest further reduction in the concentration of norepinephrine in the unloaded right ventricular papillary muscle when this sample was compared to each of the other right ventricular samples, but this difference was not significant by one-way analysis of variance.
were associated with a significant increase in the broblast infiltration of the unloaded myocardium unloading procedure in the cat, the atrophy and fibroblast were also apparent at this time. More detailed information about this early stage, as well as characteristics of the unloaded myocardium.

**Myocardial Ultrastructure**

The electron microscopic changes typically observed in the cat at one day after the unloading procedure are illustrated in Figure 1 and described in its legend. At this early time after unloading, some of the unloaded myocytes appeared normal, but the consistent changes found in the affected myocytes were the disorientation of the contractile filaments and the focal loss of Z-line substance shown in Figure 1. By 3 days after unloading, these ultrastructural changes were much more common in individual myocytes, and the myocytes were more uniformly affected. An increase in sarcoplasm, enlarged intercellular spaces, and the appearance of macrophages and fibroblasts were also apparent at this time. More detailed information about this early stage, as well as a full description of the progressive changes in unloaded cat myocardium with time, are presented elsewhere (Tomanek and Cooper, 1981).

**Myocyte Size**

The data in Table 4 show that at 3 days after the unloading procedure in the cat, the atrophy and fibroblast infiltration of the unloaded myocardium were associated with a significant increase in the hydroxyproline content of this tissue. Since Table 4 also shows that the wet-to-dry weight ratio of the unloaded myocardium was normal, the increase in hydroxyproline content probably represents an increase in the proportion of collagen present in the unloaded myocardium at this time. The time course of a further increase in the hydroxyproline content of unloaded myocardium with time is presented elsewhere (Tomanek and Cooper, 1981).

**Hydroxyproline Content**

The data in Table 4 show that at 3 days after the unloading procedure in the cat, the atrophy and fibroblast infiltration of the unloaded myocardium were associated with a significant increase in the hydroxyproline content of this tissue. Since Table 4 also shows that the wet-to-dry weight ratio of the unloaded myocardium was normal, the increase in hydroxyproline content probably represents an increase in the proportion of collagen present in the unloaded myocardium at this time. The time course of a further increase in the hydroxyproline content of unloaded myocardium with time is presented elsewhere (Tomanek and Cooper, 1981).

**Table 3**

<table>
<thead>
<tr>
<th>Unloaded papillary muscle</th>
<th>Normal papillary muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>102.40 ± 38.47</td>
<td>228.95 ± 60.42</td>
</tr>
<tr>
<td>Normal papillary muscle</td>
<td>Endocardium</td>
</tr>
<tr>
<td>160.95 ± 38.92</td>
<td>230.17 ± 63.48</td>
</tr>
<tr>
<td>Endocardium #1</td>
<td>Mid-wall</td>
</tr>
<tr>
<td>156.24 ± 40.73</td>
<td>202.50 ± 43.18</td>
</tr>
<tr>
<td>Endocardium #2</td>
<td>Epicardium</td>
</tr>
<tr>
<td>166.79 ± 21.82</td>
<td>157.51 ± 31.49</td>
</tr>
<tr>
<td>Epicardium</td>
<td></td>
</tr>
<tr>
<td>163.16 ± 42.22</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. There were five dogs in this group, and nine samples were removed from each animal. Endocardium #1 indicates a sample adjacent to the unloaded papillary muscle. Endocardium #2 indicates a sample adjacent to the normal papillary muscle.

**Myocardial Contractile and Energetic Function**

The dimensions of the right ventricular papillary muscles selected from cats for the study of contractile and energetic behavior are shown in Table 4; they were similar in the control and unloaded groups. The upper limits of papillary muscle cross-sectional area for metabolic support by diffusion at 29°C has been found to be about 1.10 mm² (Cooper, 1979). No muscle larger than this was used in the present study.

The mechanical data for isotonic contractions, shown in Figure 2, demonstrate a decrease in both the velocity and extent of shortening at all loads studied for the unloaded muscles. Less external work, the product of shortening and load, was performed by the unloaded muscles.

The mechanical data for isometric contractions are presented in Figure 3 and in Table 5. The active and resting length-tension relationships depicted in Figure 3 show both a reduction in the active tension developed during contraction at all muscle lengths studied in the unloaded muscles, and an increase, more pronounced at longer muscle lengths, in the tension required to stretch the resting muscle. Table 5 provides a summary of isometric contractile function at Lmmax, that muscle length at which developed tension is greatest. There were reductions for the unloaded muscles in active contractile force, the integral of active force and time during contraction (Ŝ active force), the maximum rate of tension development and relaxation, and the time-to-peak tension and the relaxation time. Both resting force and the ratio of resting to total force were increased in the unloaded muscles. It was found that the mechanical performance had deteriorated to such an extent at times later than 3 days after unloading that quantifiable mechanical data could not be obtained.

The metabolic data for the isotonic contractions are shown in Figure 4. Along with the reduction in contractile state shown for the same contractions in Figure 2, there was a slight reduction, not statistically significant, in the associated oxygen consumption. The oxygen consumption associated with isometric contractions is shown in Figure 5. The upper panel defines the linear regression data of MVO₂ vs. active tension for the contractions of each of the control muscles used to define the active length-tension relationship in Figure 3; the lower panel shows the same data for the unloaded muscles. The two sets of linear regression data do not differ statistically (Dixon and Massey, 1979); MVO₂ for equivalent levels of active force was similar. The oxygen consumption of the
resting, noncontracting muscles was not significantly different in the two groups of muscles. These values were $3.71 \pm 0.3 \, \mu_l/mg$ dry weight per hour for the control muscles and $3.43 \pm 0.38$ for the unloaded muscles.

### TABLE 4

<table>
<thead>
<tr>
<th>Myocardial Characteristics of the Experimental Cats</th>
<th>Control</th>
<th>Unloaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary muscle length (mm)</td>
<td>7.28 ± 0.21</td>
<td>7.06 ± 0.74</td>
</tr>
<tr>
<td>Papillary muscle cross-sectional area (mm$^2$)</td>
<td>0.88 ± 0.05</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>Myocyte cross-sectional area ($\mu^2$)</td>
<td>230 ± 7</td>
<td>152 ± 5*</td>
</tr>
<tr>
<td>Hydroxyproline concentration (µg/mg)</td>
<td>5.79 ± 0.76</td>
<td>8.01 ± 0.98*</td>
</tr>
<tr>
<td>Papillary muscle wet-to-dry weight ratio</td>
<td>4.05 ± 0.11</td>
<td>4.15 ± 0.11</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. Papillary muscle size and mass were measured in a single right ventricular papillary muscle from each of 14 control and 15 unloaded cats. Myocyte size was measured in four cats; hydroxyproline was measured in six cats. For the two measurements, one control and one unloaded muscle were removed from each right ventricle. Each asterisk indicates a significant difference between the control and unloaded muscles.

### Discussion

The major conclusion to be drawn from this study is that myocardial structure, composition, and function all undergo marked degenerative changes after myocardial unloading. While this report describes the early changes in each of these three properties, we have also described further rapid changes with time in myocardial structure and composition following unloading (Tomanek and Cooper, 1981), and in the present study the contractile function of papillary muscles was found to deteriorate with such rapidity after unloading the meaningful quantitative data could not be obtained more than 3 days after chordal transection. Therefore, the degenerative changes found in unloaded myocardium are not only very extensive but are also swiftly progressive.

In another recent study examining the opposite end of the spectrum of myocardial loading conditions (Cooper et al., 1981), we have found that a chronic pressure overload results in progressive degenerative changes, especially those of myocardial composition and function, that are strikingly similar in type and extent to those observed in the present study of unloaded myocardium. An important conclusion which follows when these two studies are considered...
together is that any substantial and sustained deviation above or below the normal range of myocardial mechanical stress results in progressive myocardial degeneration.

Experimental Preparation

The model employed in the present study has been characterized in several ways in order that we might be certain that the changes described represent the consequences primarily of the mechanical effects of unloading rather than other effects secondary to the operative procedure itself. The four lines of experimental evidence summarized below clearly implicate mechanical unloading itself as the cause of these changes.

First, no evidence of right ventricular volume overloading was found, so that the unloaded papillary muscle was present in a hemodynamically normal right ventricle rather than one undergoing hypotrophy. Furthermore, even a major degree of right ventricular volume overloading does not produce changes (Cooper et al., 1973) such as those found here for unloaded myocardium.

Second, myocardial blood flow was reduced in the unloaded papillary muscle, but it was not reduced to an extent which would be expected to produce the severe degenerative changes that were found. Some reduction of blood flow was expected, both because the oxygen requirement of an isolated papillary muscle contracting only against its own mass is less than 10% of that required by the same muscle for maximum tension generation (Cooper, 1979), and because increased systolic strain, or deformation, of the myocardium produced by unloading is associated with reduced myocardial blood flow (Williams et al., 1981). However, blood flow to the unloaded papillary muscle was always at least 10 times greater than that to the resting gracilis muscle in the same animal, and gracilis blood flow was similar to normal values reported for this muscle when at rest in the anesthetized dog (Takekita et al., 1976). Further, blood flow to the unloaded papillary muscle was within the range reported for non-ischemic left ventricular endocardium and remained well above the mean value reported for ischemic left ventricular myocardium (Marcus et al., 1975). Finally, unloaded papillary muscle blood flow did not fall below the normal range reported for right ventricular free wall or septum (Fixler et al., 1973), and flow to each tissue specimen examined in the present study promptly returned toward normal in those dogs in which blood pressure was normalized with a volume infusion after unloading.

Third, the unloading procedure had no effect on the contraction frequency of the unloaded papillary muscle. This tissue continued to contract in synchrony with the rest of the ventricular myocardium both immediately after chordal transection and at the time of later study.

Fourth, the catecholamine content of the unloaded papillary muscles was not greatly reduced. The qual-
noretanephrine content occurring in the unloaded papillary muscle. However, catecholamine depletion to the levels obtained after extrinsic cardiac denervation was not observed (Cooper et al., 1961), and even virtually complete cardiac catecholamine depletion has not resulted in changes in papillary muscle contractile (Spann et al., 1966) or energetic (Coleman et al., 1970) functions such as those found here in the unloaded myocardium.

The present model of myocardial unloading has several advantages over the other model of mechanical unloading that has been employed (Ditmer and Goss, 1973). This other model utilizes isologous transplantation of an extra donor heart into the abdomen of a histocompatible host animal; the donor aorta and pulmonary artery are joined to the host aorta and inferior vena cava, respectively, by end-to-side anastomoses. However, the residual hemodynamic loading of the donor right and left ventricles is uncertain, only a third of the donor hearts atrophy, and a quarter of the donor hearts undergo necrosis and osteogenesis. In addition to avoiding both these problems and the complexities of transplantation, the present model does not require histocompatible animals.

Unloaded Myocardium

Given this substantial evidence that the present example of reduced mechanical stress is associated with rapid and consistent degenerative changes in the unloaded segment of myocardium, and that these changes result primarily from altered mechanical conditions, the most important further consideration is the mechanism for these load-related effects. The four changes most characteristic of the unloaded myocardium are: (1) altered cardiac ultrastructure, (2) reduced myocyte size, (3) increased tissue collagen content, and (4) myocardial contractile dysfunction.

The ultrastructural changes, which are shown in their earliest stages in Figure 1 and in their later stages in considerable detail elsewhere (Tomanek and Cooper, 1981), are remarkable not only for the specific changes found but also for the great rapidity with which they occur. That is, previous work with mammalian striated muscle has established that tenotomy of skeletal muscle eventually disrupts the structural integrity of that tissue (Tomanek and Cooper, 1972), but the changes are much more gradual than those found in this study of cardiac muscle. Two of the most prominent early changes in cardiac ultrastructure are contractile filament disorientation and loss of Z-line and then contractile filament substance. The obvious heterogeneity of myofilament orientation, best seen in Figure 1 when the myofilament axes in the sarcomeres of adjacent fibrils are compared in the normal and unloaded tissues, persists during the period of myocardial atrophy until recognizable sarcomeres are no longer present. The early appearance and persistence of this defect throughout the course of a great variety of degenerative changes in the myocytes suggests that the mechanism for the myofilament disorientation may be a simple untethering
of the normal mechanical constraints of the myofilaments.

The loss of structural and contractile proteins in the unloaded myocytes presumably is the net result of some combination of enhanced proteolysis and reduced protein synthesis; the very rapid appearance of changes which are pronounced enough to be apparent micrographically argues for the former, and the eventual profound myocyte atrophy argues for some contribution from the latter. Although a recent review of the relationship of protein metabolism to myocardial stress (Schreiber et al., 1981) does not consider the biochemistry that might obtain during reduced cardiac stress, some information is available, particularly with respect to other striated muscles.

For cardiac muscle, it has been demonstrated (Petersen and Lesch, 1972) that both passive stretch and active tension generation enhance the rate of myocardial protein synthesis; we have extended this observation (Cooper, unpublished observations) in finding enhanced messenger RNA synthesis in the excised, superfused cat right ventricular papillary muscle under the same two mechanical conditions, and this synthesis could be detected as early as 15 minutes after either mechanical perturbation of the quiescent muscle at rest length. Although direct information is lacking, a reasonable inference is that the rate of cardiac protein synthesis varies directly with the magnitude of active and/or passive stress and that proteolysis, whether enhanced or not, would become the dominant factor below a certain level of myocardial loading.

For skeletal muscle, it has been established that inactivity produced by immobilization reduces protein synthesis and enhances proteolysis (Goldspink, 1977). However, the finding that the lack of either isometric contraction or passive stretch also has this effect on protein balance (Goldberg, 1979) is probably more germane to the present study of cardiac muscle unloading, in which atrophy was found despite a normal contraction frequency and extensive shortening during each contraction. The observation (Huet de la Tour et al., 1979) that a reduction of sarcomere number in shortened skeletal muscle is related more to the shorter muscle length than to lesser active tension is also of interest, although these two factors cannot be separated in the present experimental model.

Perhaps the most striking analogy to the present results occurs in metamorphosing insects, where entire striated muscles degenerate entirely within several hours of ecdysis (Lockshin et al., 1980). Here, also, the earliest change is a loss of continuity of the Z-lines, followed by dissolution of the myofilaments; both events occur within the sarcoplasm. In mammalian heart muscle, soluble cytoplasmic proteases which can at least begin the degradation of Z-line and contractile proteins do exist (Clark et al., 1980). Thus, while the initiating stimuli probably are quite different, the general proteolytic processes in such widely disparate involving tissues as insect skeletal muscle and mammalian cardiac muscle appear to be similar.

The initial reduction in myocyte size, which proceeds in a rapid and linear manner with time up through at least 4 weeks after unloading (Tomanek and Cooper, 1981, Fig. 1), is eventually associated with a reduction in myocardium number and macrophage infiltration. Thus, the associated increase in tissue collagen content may reflect actual connective tissue proliferation, substitution of connective tissue for phagocytized contractile tissue, loss of myocyte mass, or some combination of these three processes.

The severe, progressive myocardial contractile dysfunction is characterized both by a loss of active mechanical properties and by increased stiffness of the passive muscle. Each of these changes has structural and possibly functional origins. The structural disorganization of the sarcomeres, so that force and shortening are no longer generated exclusively in parallel with the long axis of the muscle, would necessarily degrade active mechanical performance. The replacement of contractile filaments by sarcoplasm (Tomanek and Cooper, 1981, Figs. 8 and 12) must also contribute to this defect. Both the myofilament disorientation and the increase in collagen content would be expected to increase passive stiffness. Possible functional contributions to these changes are speculative, but might include the differences in systolic contractile protein enzymatic activity and diastolic cross-bridge attachments characteristic of several other abnormal cardiac muscle states.

Conclusion

The major finding of this study is that unloaded myocardium atrophies rapidly. This response, and the associated abnormalities of structure, composition, and function, appear to be based on the reduction in tissue stress rather than other properties of the experimental model. In conjunction with the rapid appearance of similar abnormalities described for pressure overloaded, hypertrophied myocardium, this study shows that the structure, composition, and function of the heart are the ongoing product of a continuum of myocardial loading conditions extending from overloading through normal loading to underloading. Myocardial normality, then, is probably not an innate property of the heart, but instead appears to be the dynamic operational result of a particular, rather narrow range of tissue stress within the broad spectrum of potential hemodynamic loading conditions.

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