The Relationships of High Energy Phosphates, Tissue pH, and Regional Blood Flow to Diastolic Distensibility in the Ischemic Dog Myocardium

Shin-ichi Momomura, Joanne S. Ingwall, J. Anthony Parker, Peter Sahagian, James J. Ferguson, William Grossman

From the Charles A Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital, and the Department of Medicine (Cardiovascular Division), Beth Israel Hospital, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts

SUMMARY. Myocardial ischemia due to increased oxygen demand (pacing tachycardia plus critical coronary stenoses) alters diastolic distensibility and relaxation more than ischemia of comparable duration due to coronary occlusion. To investigate the relationship between myocardial diastolic function and metabolism, we compared myocardial high energy phosphate content, tissue pH, and regional blood flow for these two types of ischemia in anesthetized open-chest dogs. Myocardial biopsies were done with a high-speed air-turbine biopsy drill, permitting rapid (<1-second) freezing of tissue samples from both nonischemic and ischemic areas, while myocardial pH was measured with a hydrogen ion-selective polymer membrane implanted in the subendocardium. After 3 minutes of pacing tachycardia in dogs with critical coronary stenoses (demand-type ischemia, n = 14), regional systolic function (% segment shortening by ultrasonic crystals) was mildly depressed (from 19 ± 2% control to 13 ± 2% post-pacing, P < 0.01), while left ventricular diastolic pressure-segment length relations shifted upward, indicating decreased distensibility of the ischemic myocardial segment. Associated with these changes in function, subendocardial adenosine triphosphate decreased (from 31.3 ± 1.5 to 27.9 ± 1.0 nmol/mg protein, P < 0.01), as did creatine phosphate (53.8 ± 2.1 to 39.6 ± 2.5 nmol/mg protein, P < 0.01), while myocardial pH declined slightly (ΔpH = −0.14 ± 0.02, P < 0.01). In contrast, at 3 minutes of coronary artery occlusion (primary ischemia, n = 14), regional segment shortening was replaced by systolic bulging (% shortening decreased from 17 ± 2% to −2 ± 1% during occlusion, P < 0.01), while left ventricular pressure-segment length relations were not shifted upward, and there was no decrease in diastolic distensibility of the ischemic segment. With coronary artery occlusion, subendocardial adenosine triphosphate declined slightly (33.2 ± 0.5 to 29.2 ± 2.0 nmol/mg, P < 0.05), while creatine phosphate decreased substantially (51.1 ± 2.3 to 7.8 ± 1.4 nmol/mg protein, P < 0.01). Myocardial pH fell strikingly (ΔpH = −0.33 ± 0.03, P < 0.01), and the decline was 236% of that seen with demand-type ischemia. Regional myocardial blood flow (microsphere technique) showed a decreased endocardial:epicardial (endo:epi) ratio (1.04 ± 0.04 control vs. 0.40 ± 0.05 during pacing, P < 0.01) and absolute subendocardial flow (1.02 ± 0.47 to 0.47 ± 0.05 ml/min per g, P < 0.01) with demand-type ischemia. However, subendocardial blood flow in demand-type ischemia was still much greater than flow during coronary artery occlusion (0.10 ± 0.03 ml/min per g, P < 0.01). In summary, diastolic dysfunction was prominent during ischemia caused by increased oxygen demand, but was minimal during ischemia due to primary coronary flow reduction of equal duration. The diastolic dysfunction could not be explained simply by adenosine triphosphate depletion, which was modest and similar with both types of ischemia. Protection against diastolic dysfunction in primary ischemia may reflect the combined effects of hydrogen ion accumulation, loss of coronary vascular turgor, and repeated systolic stretch of the ischemic segment. (Circ Res 57: 822–835, 1985)
a perfused rabbit heart preparation. In contrast to these findings with hypoxia or increased O2 demand with coronary stenoses, primary myocardial ischemia due to brief occlusion of a coronary artery (Tyberg et al., 1974, Paulus et al., 1985) or global reduction in coronary blood flow (Palacios et al., 1976; Serizawa et al., 1981) failed to produce an upward shift in left ventricular diastolic pressure-volume or pressure-segment length relations. These pressure-volume relations may even shift downward, indicating increased diastolic distensibility associated with myocardial ischemia in this setting.

The explanation for the differences in diastolic distensibility in these two different types of ischemia is uncertain. Differences in high energy phosphate content, coronary blood flow, and vascular turgor of the affected coronary bed, and accumulation of hydrogen ion or other metabolites might account for the observed differences. With regard to local accumulation of metabolites after coronary occlusion, there is evidence that accumulation of hydrogen ion associated with primary myocardial ischemia leads to a rapid decline in systolic function of the ischemic myocardium (Katz, 1973; Poole-Wilson, 1975). At the same time, an acid pH may slow myocardial adenosine triphosphate (ATP) depletion by suppressing high-energy phosphate consumption required for systolic cross-bridge cycling, and thus protect the ischemic myocardium from contracture (Bing et al., 1973; Greene and Weisfeldt, 1977).

In the present study, we sought to assess the relationship between myocardial diastolic function and metabolism by measuring myocardial high-energy phosphate content, tissue pH, and regional blood flow during ischemia due to increased O2 demand (pacing-induced ischemia in dogs with coronary stenoses) compared to primary ischemia of comparable duration due to coronary occlusion.

Methods

Pacing-Induced Ischemia

An angina-physiology model which was developed in our laboratory (Serizawa et al., 1980; Paulus et al., 1982, 1985) was used. Mongrel dogs weighing 17-35 kg were anesthetized with intravenous a-chloralose (100 mg/kg), after premedication with a subcutaneous injection of ketamine (10 mg/kg). As in our previous studies, propranolol (0.5 mg/kg) was injected to prevent ventricular fibrillation during pacing and to suppress the heightened sympathetic tone associated with the anesthetized state (Reynolds et al., 1978). Respiration was maintained with room air by a Harvard pump via an endotracheal tube. A left thoracotomy was performed, usually at the 5th intercostal space, and the pericardium was opened wide to make a pericardial cradle. Both proximal left circumflex and left anterior descending arteries were freed from adipose tissue, and electromagnetic flow probes (Biotronex BL-5030, 5025) were placed around the arteries. A high-fidelity micromanometer catheter (Millar instruments PC-480) was inserted into the left ventricle via the right carotid artery.

To assess regional wall motion, we implanted a pair of ultrasonic crystals subendocardially and parallel to the short axis of the left ventricle in an area perfused by the left anterior descending artery, and another pair in an area perfused by the circumflex coronary artery (Fig. 1). Crystals were placed in the inner third of the myocardium, following the technique of Gallagher et al. (1982), who chose this location because of the relatively homogeneous fibre orientation at this distance from the endocardial surface. The position of these crystals was confirmed at the end of each experiment. Pacing electrodes were sutured on the left atrial appendage.

Blood flow in the left anterior descending and the left circumflex coronary arteries was reduced, using small metal clips, while phasic flow was monitored with an electromagnetic flowmeter. The gap diameter of the clips was adjusted using a commercially available gap gauge so that the peak diastolic deflection of phasic coronary flow was reduced by 50%, which corresponds to more than 90% reduction in diameter (Serizawa et al., 1980; Paulus et al., 1982). If a stenosis of excessive severity was created,
an obvious systolic bulge in segmental wall motion occurred, and the stenosis was immediately reduced in severity until segmental function was restored, as assessed visually and by the amplitude of segmental shortening measured by ultrasonic crystals. Thus, critical stenoses were created on both proximal left anterior descending and left circumflex coronary arteries (Fig. 1). After recording left ventricular pressure, dp/dt, and myocardial segment lengths (Honeywell—Electronics-for-Medicine Research Recorder), rapid atrial pacing (1.9 times resting heart rate) was performed for 3 minutes. Hemodynamic parameters were recorded continuously during and after pacing tachycardia.

**Brief Coronary Occlusion**

After the pacing tachycardia protocol, stenoses clips were removed and hemodynamic parameters were monitored for approximately 30 minutes to allow for complete recovery. The left anterior descending coronary artery was then occluded completely for 3 minutes, at the point where the stenosis clip was placed; then the occlusion was released and hemodynamic recovery during reperfusion was observed.

**Pacing Tachycardia without Coronary Stenosis**

In seven dogs, control experiments were carried out with pacing tachycardia in the absence of coronary stenoses. The heart was paced at 1.9 times the resting heart rate for 3 minutes. Hemodynamic parameters and ultrasonic crystal measurements before, during and immediately after pacing tachycardia were obtained.

**Coronary Occlusion without Preceding Pacing Tachycardia**

To determine whether the first episode of ischemia (pacing tachycardia in the presence of critical coronary stenoses) affected the results observed during the second episode of ischemia (brief coronary occlusion), the proximal left anterior descending artery was occluded for 3 minutes without preceding pacing-induced ischemia, in seven dogs, and hemodynamic changes were compared with those in experiments where coronary occlusion followed preceding pacing-induced ischemia.

**Biopsy Technique**

A high-speed air-turbine biopsy drill connected via a vacuum line to a bottle filled with liquid nitrogen was constructed according to the design reported by Allard et al. (1981) and used to take 2-mm (diameter) transmural myocardial biopsies. Using this rapid-freezing transmural biopsy drill, Allard and co-workers (1981) have demonstrated good preservation of high-energy phosphates, since biopsy sample transit through the drill and tissue freezing all occur in less than 1 second. In 14 dogs, biopsies were taken before pacing tachycardia from a normally perfused area and an area perfused by the left anterior descending artery distal to the stenosis. Immediately after pacing tachycardia, after observing 5–10 beats and recording hemodynamic parameters, another biopsy was obtained from the left anterior descending coronary artery region. In the coronary occlusion protocol, a myocardial sample was obtained from the left anterior descending coronary artery region before and after 3 minutes of the left anterior coronary artery occlusion. Before each biopsy, the epicardium was stained with gentian violet or methylene blue so that the epicardial end of the tubular biopsy specimen could be distinguished from the endocardial end. Myocardial transmural biopsy samples were small (<2 mm in diameter) and bleeding was easily stopped by pressing a finger over the biopsy hole gently, without impairment of regional wall motion. Since ischemia is usually more profound in subendocardium than in subepicardium, each frozen sample was divided using tweezers into subendocardial and subepicardial halves in a plastic saucer filled with liquid nitrogen, and then stored at −70°C. Location and sequence of biopsy samples is indicated in Figure 1.

**Myocardial pH**

In 10 dogs, a hydrogen-ion selective polymer membrane pH electrode (LifeSpan 100 TM pH monitor, Biochem International, WI) was implanted into the subendocardium in the left anterior descending artery region (Fig. 1) and myocardial tissue pH was measured continuously. The pH sensing element consists of a silver wire coated with silver chloride at the tip (0.4 mm in diameter), which is coated with a gel electrolyte layer and encapsulated by an ion-specific polymer. A built-in silver to silver chloride reference is contained in the sensor. The electromotive force of the sensors is a function of the hydrogen ion concentration of the media being measured. The sensor yields a linear end response over pH range of 4.00–9.00. Time response of this pH sensor is rapid, so that a 90% step change is achieved in less than 2 seconds. The pH sensor was calibrated using buffers with pH 6.8 and 7.4 before insertion into myocardium, and again at the end of each experiment. Respiration was controlled so that arterial blood pH was 7.40 ± 0.04 (Radiometer blood gas system PMH72; Radiometer).

**Microsphere Protocol**

To study regional myocardial blood flow in the pacing-induced ischemia and coronary occlusion models, we used radioactive microspheres in 13 dogs. Microspheres (diameter = 15 μm, New England Nuclear) labeled with five species of radioactive material (57Co, 113Sn, 103Ru, 95Nb, and 85Sc) were suspended in 0.01% Tween-80 and 5% dextran solution and agitated in an ultrasonic bath for 5 minutes before injection. Thirty to 50 μCi (1.5–2.5 × 10⁶ microspheres) of each different isotope-labeled microsphere preparation were injected into the left atrium via a short (10–15 cm) 6F catheter: (1) before creation of coronary stenoses, (2) with stenoses, (3) during the latter half of 3 minutes of pacing tachycardia plus stenoses, and (4) during coronary occlusion. A blood sample was collected for 90 seconds via a catheter placed in the aorta, using a withdrawal pump with a rate of 10–15 ml/min, for each injection of microspheres. At the end of each experiment, methylene blue dye was injected at the level of the stenoses, first into the left anterior descending and then the left circumflex coronary arteries to confirm areas perfused by coronary arteries distal to the level at which stenoses were created. Then, the heart was excised, and muscle samples were obtained from the following areas: (1) the left anterior descending artery territory distal to the stenosis, (2) the left circumflex artery territory distal to the stenosis, and (3) the normally perfused (nonischemic) area. Usually, myocardium in border zones between these three areas was discarded. Six small muscle pieces weighing...
Measurement of High Energy Phosphate Content

Measurement of myocardial tissue concentrations of the primary nucleotides and nucleosides in heart was made using high pressure liquid chromatography on Waters model 440 and 450 chromatographs equipped with manual and automatic sample injectors and Waters model M730 and Hewlett-Packard model 338A integrators. Frozen portions of the biopsies were homogenized in 0.6 M HClO₄. The homogenate was neutralized with saturated K₂PO₄ and precipitated salts and proteins were removed by centrifugation. Aliquots of a supernatant were applied to a Partisil SAX ion exchange column, 4.6 mm × 25 cm (Whatman). Nucleosides and nucleotides were eluted at room temperature isocratically on 0.16 M KH₂PO₄ with 0.1 M KCl at pH 6.5 and flow rate of 1.4 ml/min. Resulting chromatograms were analyzed at 254 nm for ATP, ADP, GDP, GTP, UDP, and CTP contents, and amounts calculated using external standards. By means of reverse phase column chromatography, nucleotides and nucleosides were eluted isocratically from a Bondapak C-18 column in 0.05 M (NH₄)₂HPO₄ at pH 4.3 with flow rate of 2.0 ml/min. The column effluent was analyzed at 254 nm for contents of AMP, hypoxanthine, inosine, and NAD⁺ again, using external standards for calibration. Creatine phosphate (CrP) was analyzed by the fluorometric coupled-enzyme method of Lowry and Parsonneau (1972). The protein concentration of the perchloric acid extract was determined by the method of Lowry et al. (1951), and the concentration of each metabolite was expressed as nmol/mg of non-collagen cardiac protein.

Data Analysis

Left ventricular pressure and myocardial segment lengths were digitized at 5-msec intervals, and pressure-segment length loops were constructed using a Tektronix 4052 computer system. Time constant T of left ventricular isovolumic relaxation was calculated from both the logarithm of pressure (Weiss et al., 1976) and the first derivative of pressure (Carroll et al., 1985). These time constants were termed Tₑ and Tₑ, respectively. Hemodynamic data before and after pacing, and before and after coronary occlusion, were compared by Student's t-test. Metabolite contents, myocardial pH, and myocardial blood flow were compared by analysis of variance. Differences were considered to be statistically significant at P < 0.05. All values were expressed as mean ± SEM.

Hemodynamic Changes during Pacing Tachycardia in Dogs without Coronary Stenosis (Table 1)

During pacing tachycardia, end-diastolic segment lengths in both left anterior descending and circumflex areas were decreased, while end-systolic segment lengths were unchanged. Therefore, the percent shortening of both segments was decreased. Following pacing tachycardia, these hemodynamic parameters returned to pre-pacing values. Pre- and post-pacing left ventricular diastolic pressure-segment length loops in dogs without coronary stenosis are shown in Figure 2. Post-pacing pressure-segment length loops were almost identical to pre-pacing loops, and an upward shift was not seen.

Hemodynamic Changes in Pacing-Induced Ischemia and Coronary Occlusion

Hemodynamic data for all experiments in which biopsies were done are summarized in Table 2. A typical hemodynamic recording is shown in Figure 3. During pacing tachycardia with coronary stenoses, left ventricular minimum diastolic pressure decreased initially and then increased gradually. Left

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-PAC</th>
<th>PAC 3 min</th>
<th>Immediately post-PAC</th>
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<tbody>
<tr>
<td>HR (beats/min)</td>
<td>124 ± 3</td>
<td>212 ± 6*</td>
<td>123 ± 4</td>
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<tr>
<td>LVP (mm Hg)</td>
<td>129 ± 7</td>
<td>120 ± 5</td>
<td>122 ± 7</td>
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<tr>
<td>Peak systolic</td>
<td>4 ± 1</td>
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<td>4 ± 1</td>
</tr>
<tr>
<td>EDP</td>
<td>6 ± 2</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
</tr>
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<td>SL in LAD region</td>
<td>10.0 ± 1.1</td>
<td>9.4 ± 1.1*</td>
<td>10.2 ± 1.2</td>
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<tr>
<td>End-diastolic (mm)</td>
<td>8.2 ± 0.7</td>
<td>8.2 ± 0.8</td>
<td>8.4 ± 0.7</td>
</tr>
<tr>
<td>% shortening</td>
<td>17.1 ± 2.3</td>
<td>12.6 ± 2.0*</td>
<td>16.5 ± 3.1</td>
</tr>
<tr>
<td>SL in LCX region</td>
<td>11.1 ± 0.9</td>
<td>10.4 ± 0.9†</td>
<td>11.1 ± 0.9</td>
</tr>
<tr>
<td>End-diastolic (mm)</td>
<td>9.4 ± 0.9</td>
<td>9.0 ± 0.9</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>% shortening</td>
<td>15.4 ± 2.7</td>
<td>13.3 ± 1.4†</td>
<td>16.0 ± 2.0</td>
</tr>
<tr>
<td>LV dp/dt (mm Hg/ sec)</td>
<td>1920 ± 190</td>
<td>2100 ± 250</td>
<td>1910 ± 320</td>
</tr>
<tr>
<td>Peak positive</td>
<td>2550 ± 190</td>
<td>2540 ± 240</td>
<td>2330 ± 210</td>
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<tr>
<td>Peak negative</td>
<td>31 ± 2</td>
<td>34 ± 3</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Time constant (msec)</td>
<td>46 ± 3</td>
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</table>

*P < 0.01, †P < 0.05 compared to pre-pacing.
ventricular end-diastolic and end-systolic segment length also decreased initially and subsequently increased during the 3 minutes of tachycardia. At the end of pacing tachycardia, however, end-diastolic segment length was still smaller than control segment length in both left anterior descending and left circumflex areas, with LV diastolic pressure higher than during control. Immediately after cessation of pacing tachycardia, left ventricular end-diastolic pressure was elevated at twice its control value (from 8 ± 1 mm Hg pre-pacing to 17 ± 2 mm Hg post-pacing, \( P < 0.01 \)) and both ventricular end-diastolic and end-systolic segment lengths were increased, with decreased systolic shortening of the ischemic segments. Peak negative dP/dt decreased, and the time constant of isovolumic relaxation was prolonged (\( T_L \), pre-pacing 35 ± 1 msec vs. post-pacing 51 ± 3 msec, \( P < 0.01 \); \( T_D \), pre-pacing 42 ± 2 msec vs. post-pacing 68 ± 5 msec, \( P < 0.01 \)).

After a 30-minute period of recovery, without coronary stenoses, the left anterior descending coronary artery was abruptly occluded; hemodynamic changes are summarized in Table 2. Systolic function of the ischemic segment deteriorated quickly.
FIGURE 3. Left ventricular pressure (LVP, mm Hg), left ventricular dP/dt (mm Hg/sec) and segment length (SL, mm) recordings in pre-pacing control with coronary stenoses, during pacing tachycardia, immediately after cessation of pacing tachycardia, control before without stenoses before coronary (LAD) occlusion, and at 3 minutes of LAD occlusion. During pacing tachycardia left ventricular diastolic pressure began to increase, and immediately post-pacing, left ventricular end-diastolic pressure was substantially elevated. See text for discussion. SL_{LAD} = segment length in the anterior descending area, SL_{LCX} = segment length in the left circumflex area.

and at 3 minutes of occlusion, systolic shortening of the ischemic segment was replaced by systolic lengthening. Fractional shortening of the segment length in the left anterior descending artery region thus became negative (control 17 ± 2% vs. occlusion −2 ± 1%, P < 0.01). Left ventricular end-diastolic pressure increased mildly (control 7 ± 1 mm Hg vs. occlusion 11 ± 1 mm Hg, P < 0.05), and the time constant of isovolumic pressure decline was slightly prolonged (Table 2).

Left ventricular diastolic pressure-segment length relations in a representative experiment with pacing-induced ischemia are shown in Figure 4. During pacing tachycardia, the diastolic pressure-segment length relation shifted upward. This upward shift was maintained immediately after pacing tachycardia, and persisted for 30-60 seconds. Diastolic pressure-segment length relations during coronary occlusion compared to pacing-induced ischemia are shown in Figure 5. Immediately after pacing tachycardia, the left ventricular diastolic pressure-segment length relation shifted upward. However at 3 minutes of coronary occlusion, an upward shift did not occur, and the diastolic pressure-segment length curve shifted rightward.

Left ventricular end-diastolic pressure-segment length points in pre-pacing (A), post-pacing (B), control before coronary occlusion (C), and coronary occlusion (D) are shown in Figure 6. In both pacing-induced ischemia and coronary occlusion ischemia, left ventricular end-diastolic pressure and segment length increased. However, the increase in end-diastolic pressure was greater (pacing-induced ischemia = A → B; 9.3 ± 1.6 mm Hg vs. coronary occlusion = C → D; 3.8 ± 0.8 mm Hg, P < 0.01), and the increase in end-diastolic segment length was smaller (pacing-induced ischemia 4.6 ± 0.7% vs. coronary occlusion 10.6 ± 2.0%, P < 0.01) with pacing-induced ischemia.

Thus, diastolic distensibility and relaxation abnormalities were prominent following pacing tachycardia in dogs with coronary stenoses. In contrast, with coronary occlusion ("primary" ischemia), systolic dysfunction was predominant and diastolic dysfunction was minor or absent.

Hemodynamic Response to Coronary Occlusion without Preceding Pacing-Induced Ischemia (Table 3)

In these experiments (n = 7), hemodynamic data before coronary occlusion were not different from those in experiments where ischemia due to pacing tachycardia plus coronary stenoses had been imposed as the initial stress. With coronary occlusion, left ventricular segment lengths increased markedly, especially at end systole, and systolic shortening was replaced by systolic lengthening. Left ventricular end-diastolic pressure increased mildly (+3.1 ± 0.1
mm Hg) compared to end-diastolic segment length (+10.3 ± 4.5%) and there was no upward shift of the diastolic pressure-segment length relationship. These hemodynamic changes did not differ from those in response to coronary occlusion with a preceding episode of pacing-induced ischemia (Table 2).

**Myocardial High-Energy Phosphate Content**

Creatine phosphate, ATP, and ATP catabolite contents in the biopsy samples from 14 dogs are shown in Table 4. Placement of the stenoses (without pacing) did not alter ATP and creatine phosphate contents (LAD area, pre-pacing vs. non-ischemic area in Table 4). Immediately after pacing tachycardia, in the presence of coronary stenoses,
subendocardial creatine phosphate was decreased by 26% (P < 0.01) in the ischemic area, compared to pre-pacing measurements in the same area. Subendocardial ATP was decreased by 11% (P < 0.05), and the sum of adenine nucleotides (ATP + ADP + AMP) was decreased by 7% (P < 0.05). In ischemic myocardium, ATP is catabolized to ADP, AMP, adenosine, inosine, and hypoxanthine. Consistent with the decrease in subendocardial ATP content, there was a small increase in tissue contents of hypoxanthine. Changes in the normal distribution of other nucleotides also occurred in the subendocardium. The concentration of the other major purine nucleotide, GTP, was 18% lower (1.10 ± 0.05 to 0.97 ± 0.08 nmol/mg protein, P < 0.05), whereas the content of the pyridine nucleotide NAD⁺ was 9% lower (3.35 ± 0.22 vs. 3.05 ± 0.20 nmol/mg protein, P < 0.05) than in the nonischemic area. The contents of the pyrimidine nucleotides UTP and CTP were not significantly changed (not shown).

After 30 minutes of recovery, tissue content of CrP, ATP, and other measured compounds all returned to control values. There were essentially no changes in the normal distribution of CrP, purine, pyrimidine, and pyridine compounds in the subepicardium either during or after pacing tachycardia.

Following coronary occlusion, creatine phosphate content was markedly depleted in both the subepicardium (−68%, P < 0.01) and subendocardium (−85%, P < 0.01). Compared to the control period before occlusion, ATP content was lower in the subendocardium (−11%, P < 0.05) but not in the subepicardium. Consistent with this decrease in ATP content, there were small increases in the subendocardial content of ADP (P < 0.05), inosine (P < 0.01), hypoxanthine (P < 0.01) and the sum of inosine and hypoxanthine (P < 0.01). For both the subendocardium and subepicardium, the sum of adenine nucleotides (ATP + ADP + AMP), GTP, CTP, UTP, and NAD contents were essentially the same in ischemic and control tissue.

**Myocardial pH**

With coronary stenoses plus 3 minutes of pacing tachycardia, subendocardial tissue pH decreased slowly from 7.23 ± 0.02 to 7.09 ± 0.03 (P < 0.01), and after cessation of pacing tachycardia, pH recovered gradually but completely. With 3 minutes of coronary occlusion, myocardial pH decreased rapidly and profoundly from 7.30 ± 0.02 to 6.97 ± 0.04 (P < 0.01). With reperfusion, myocardial pH recovered to resting levels within 5 minutes. Subendocardial pH for control, during two types of ischemia (stenoses plus pacing tachycardia vs. complete coronary occlusion) and recovery phases were compared. At 1, 2, and 3 minutes of ischemia, the change in pH was substantially greater with coronary occlusion (Fig. 7). Hemodynamic changes in this series of experiments were nearly identical to those in the biopsy experiments.

**Regional Myocardial Blood Flow**

Myocardial blood flow per gram of tissue in epi-, mid-, and endocardial layers, and transmural blood flow ratio (endo:epi) are shown in Table 5. Stenoses on the left anterior descending and left circumflex coronary arteries did not decrease resting myocardial flow in these areas. However, by superimposing pacing tachycardia, absolute subendocardial blood flow and the endo:epi ratio were significantly de-

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Nucleotide and Nucleoside Content in Biopsy Samples (nmol/mg Protein)</th>
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<tbody>
<tr>
<td></td>
<td>CrP</td>
</tr>
<tr>
<td>Nonischemic area (n = 14)</td>
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<tr>
<td>Pre-pacing</td>
<td></td>
</tr>
<tr>
<td>epi</td>
<td>60.2 ± 3.1</td>
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<td>endo</td>
<td>55.3 ± 2.5</td>
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<td>LAD area</td>
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<td>Pre-pacing (n = 14)</td>
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<tr>
<td>epi</td>
<td>56.3 ± 2.4</td>
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<td>endo</td>
<td>53.8 ± 2.1</td>
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<td>Immediately post-pacing (n = 14)</td>
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<tr>
<td>epi</td>
<td>50.0 ± 3.1</td>
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<tr>
<td>endo</td>
<td>39.6 ± 2.5</td>
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<tr>
<td>Control before occlusion (n = 7)</td>
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</tr>
<tr>
<td>epi</td>
<td>57.3 ± 2.3</td>
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<td>endo</td>
<td>51.1 ± 2.3</td>
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<td>Occlusion 3 min (n = 7)</td>
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<tr>
<td>epi</td>
<td>18.3 ± 2.9</td>
</tr>
<tr>
<td>endo</td>
<td>7.8 ± 1.4</td>
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</table>

CrP = creatine phosphate, TANP = total adenine and nucleotide, LAD = left anterior descending artery, epi = subepicardium, endo = subendocardium. Adenosine was not detected in the biopsies analyzed, and thus no data are shown for adenosine. Zeros were entered where contents of hypoxanthine or inosine were below the limits of detectability.

* P < 0.02, † P < 0.01 vs. nonischemia area; † † P < 0.05; † † † P < 0.01 vs. control before occlusion; † † † † P < 0.05, † † † † † P < 0.01 vs. epi; § P < 0.05, §§ P < 0.01 vs. pre-pacing.
A) coronary stenosis + pacing tachycardia (PAC)
B) coronary (LAD) occlusion

*p < 0.05  **p < 0.01

FIGURE 7. Changes in pH (ΔpH) during pacing tachycardia (PAC) with coronary stenoses (A), compared to changes during left anterior descending (LAD) coronary occlusion (B). At 1, 2, and 3 minutes ('), pH changes were greater with coronary occlusion ischemia.

increased, while subepicardial blood flow increased in both the left anterior descending and left circumflex regions. In a nonischemic area (Fig. 1) surrounded by two ischemic areas, the endo:epi ratio decreased during tachycardia. With complete occlusion of the left anterior descending coronary artery, myocardial blood flow in the left anterior descending artery region was markedly decreased in all three layers, and was much lower than during pacing tachycardia plus coronary stenoses. During occlusion of the left anterior descending coronary artery, regional blood flows in the left circumflex coronary artery region and nonischemic area were unchanged.

**Discussion**

In this study, pacing-induced ischemia in dogs with coronary stenoses was compared with ischemia due to coronary artery occlusion. As previously demonstrated, patterns of hemodynamic changes in these two types of ischemia were quite different, especially with regard to alterations of diastolic properties. With ischemia due to pacing-induced tachycardia in the presence of coronary stenoses (primary increase in O₂ demand relative to supply, as in exertional angina pectoris), diastolic dysfunction is a major characteristic, as indicated by an upward shift in the diastolic pressure-segment length relation and prolongation of the time constant (T) of LV isovolumic pressure decay. In contrast, systolic dysfunction was the major finding in experiments where ischemia was due to coronary occlusion (ischemia due to a primary decrease in coronary blood flow), and diastolic dysfunction was minimal: the time constant T was only mildly prolonged, and diastolic pressure did not shift upward relative to segment length. The relation between preservation of systolic function and the development of altered diastolic distensibility during ischemia has been described recently by Paulus et al. (1985).

**TABLE 5**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Stenoses (n = 7)</th>
<th>Pacing tachycardia (n = 7)</th>
<th>LAD occlusion (n = 6)</th>
</tr>
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<tbody>
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<td>LAD area</td>
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<tr>
<td>epi</td>
<td>0.87 ± 0.06</td>
<td>1.05 ± 0.10</td>
<td>1.22 ± 0.15*</td>
<td>0.26 ± 0.12*</td>
</tr>
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<td>mid</td>
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<td>1.08 ± 0.09</td>
<td>0.73 ± 0.07†</td>
<td>0.11 ± 0.04†</td>
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<td>endo</td>
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<td>1.02 ± 0.07</td>
<td>0.47 ± 0.05*§</td>
<td>0.10 ± 0.03*§</td>
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<td>0.40 ± 0.05*†</td>
<td>0.50 ± 0.11†</td>
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<tr>
<td>epi</td>
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<td>1.06 ± 0.08</td>
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<td>0.40 ± 0.09*§</td>
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<tr>
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<td>Nonischemic area</td>
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<td>0.71 ± 0.10*</td>
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</table>

epi = subepicardial blood flow; mid = blood flow in mid layer; endo = subendocardial blood flow; endo:epi = transmural blood flow ratio values are expressed as mean ± se.
* P < 0.01 compared to control; † P < 0.05 compared to control.
‡ P < 0.01 compared to stenoses; †† P < 0.01 compared to pacing tachycardia.
§ P < 0.01 compared to epi.
The goal of this study was to investigate the cellular mechanisms which might be responsible for the myocardial diastolic abnormalities seen in the angina-physiology model (coronary stenoses plus pacing tachycardia) in contrast to coronary occlusion ischemia. Incomplete relaxation due to persistent interaction of contractile proteins (actin and myosin) in diastole was suggested previously as a possible mechanism for the decreased distensibility (Grossman and Barry, 1980). The term "incomplete relaxation" has been used by us (Grossman and Barry, 1980; Serizawa et al., 1980, 1981; Momomura et al., 1984), as well as by others (Shine et al., 1978; Nayler et al., 1979), to describe the rise in end-diastolic ventricular pressure relative to volume, or muscle tension relative to length, that develops in the beating heart or cardiac muscle preparation between systolic contractions. Others have used the term "contracture" (Greene and Weisfeldt, 1977; Lewis et al., 1979, 1980) or "rigor" to describe this rise in resting tension with ischemia or hypoxia. We have chosen to avoid these latter terms, in order to emphasize the transient and completely reversible nature of the diastolic abnormalities observed both in the clinical setting of angina pectoris (McLaurin et al., 1973; Barry et al., 1974, Mann et al., 1977) and in animals with coronary stenoses subjected to brief pacing tachycardia.

There are several possible cellular mechanisms which could account for the diastolic abnormalities seen in the present study. Rigor-like bonding of actin and myosin, without normal cross-bridge cycling, has been postulated to occur in the hypoxic contracture of rat myocardium (Holubarsch et al., 1982; Bing and Fischbein, 1979; Lewis et al., 1980). This type of diastolic alteration, although readily demonstrable in the rat, is difficult to produce in other species without prolonged ischemia or hypoxia (Lewis et al., 1979), and is only partially reversible with reoxygenation. This type of diastolic dysfunction does not appear to be related to increased cytosolic Ca++ (Allen and Orchard, 1983; Lewis et al., 1980), but, rather, to severe ATP deficiency, although ATP levels were not measured in most reported studies (Lewis et al., 1979, 1980; Bing and Fischbein, 1979; Holubarsch et al., 1982). Severe ATP depletion was not observed in either model of ischemia described in our study.

A second possible mechanism for the diastolic abnormalities seen in the present study might be persistent cross-bridge cycling throughout diastole in the affected myocardium, either due to impaired sarcoplasmic reticular Ca++ uptake with increased cytosolic free Ca++ in diastole (Nayler et al., 1979), or altered sensitivity of the contractile proteins to a given Ca++ concentration (Allen and Orchard, 1983). It has been shown that mammalian cardiac muscle normally exhibits diastolic Ca++-dependent myofilament interaction (Lappe et al., 1980; Lakatta et al., 1981; Stern et al., 1983), which may be responsible for an active component of myocardial diastolic compliance. Nayler and co-workers (1978, 1979) studied the relationship between high-energy phosphate content and rising resting tension in the hypoxic rabbit heart. They demonstrated that ATP and CrP decreased prior to the rise in resting tension, and suggested that impaired relaxation in hypoxic heart muscle is due to insufficient ATP for normal Ca++ uptake by sarcoplasmic reticulum. If a common mechanism is responsible for increased resting tension during hypoxia in the isolated perfused heart and for decreased left ventricular diastolic distensibility in the angina-physiology dog model of our experiments, then depletion of high-energy phosphate content in ischemic myocardium would be expected during and/or immediately after pacing tachycardia, corresponding to the upward shift in left ventricular diastolic pressure-segment length relation. In fact, ATP and CrP were depleted in association with the diastolic relaxation abnormalities observed in our angina-physiology model; however, depletion of ATP and CrP was mild. This is similar to Nayler's (1978) observation that ATP decreased 28% after 10 minutes of hypoxia in the rabbit heart, associated with a substantial increase in resting tension. Although not tested by the studies reported here, the mild decrease in total ATP observed in our study could reflect depletion of a specific ATP pool critical for sarcoplasmic reticular Ca++ uptake and/or inactivation of cross-bridge cycling. In this regard, it is of interest that Bricknell et al. (1981) previously reported that ATP produced by glycolysis was better able to delay or prevent decreased diastolic distensibility than ATP produced by oxidative phosphorylation in global ischemia in the perfused rat heart preparation. They suggested that there could be strict cytosolic compartmentation of ATP so that glycolysis provides a small but crucial supply of cytosolic ATP that is accessible to the contractile proteins, or glycolytically produced ATP may meet the energy requirements for calcium uptake by sarcoplasmic reticulum maintaining relaxation. It seems possible that in demand ischemia, the contribution of glycolytic ATP to total tissue ATP is low (as in normoxic myocardium), whereas, in low-flow (coronary occlusion) ischemia, glycolytic ATP is relatively more important.

The upward shift, however, may be attributed to mechanisms that are not related to altered cellular metabolism, such as inward "creep" or the opposite phenomenon to stress relaxation, based on viscoelastic properties of the myocardium. During pacing tachycardia, left ventricular diastolic segment length was decreased because of shortened diastole and inadequate filling. If inward "creep" occurs during pacing tachycardia and is exposed by rapidly increased filling immediately after pacing tachycardia, an upward shift might occur without any fundamental diastolic defect. However, this is unlikely because, in the control experiments without coro-
nary stenoses (Fig. 2), an upward shift was not seen following pacing tachycardia, though the segment length decreased during pacing.

In contrast to the angina-physiology model, an upward shift in the left ventricular diastolic pressure-segment length relation was not seen with coronary occlusion or "primary" myocardial ischemia: ATP content in the ischemic myocardium decreased slightly at 3 minutes of occlusion, although CrP was substantially decreased. One factor which may partially explain the absence of a decrease in diastolic distensibility with acute primary ischemia is the difference in tissue pH in the two ischemia models. The decrease in pH was more rapid and more profound with coronary artery occlusion than in the angina-physiology model, and marked systolic dysfunction occurred very quickly. It has been observed previously that an acid pH is associated with substantial depression of contractility, as well as protection against hypoxia-induced rises in myocardial resting tension (Bing et al., 1973, 1975; Greene and Weisfeldt, 1977; Poole-Wilson et al., 1975, 1977), whereas an alkaline pH potentiates hypoxic contracture. In our experiments, the rapid and profound decline in pH with coronary occlusion may have contributed to both the complete cessation of systolic contraction and the absence of any increase in diastolic pressure relative to segment length. Also, low tissue pH in this setting could enhance Ca** uptake by sarcoplasmic reticulum, since it has been observed that acidic pH increases Ca** uptake by isolated sarcoplasmic reticulum in the presence of ATP (Nakamura and Schwartz, 1970, 1972). This would decrease the number of Ca** ions available to bind to troponin during diastole and maintain the left ventricular diastolic pressure at a relatively low level during coronary occlusion. In the angina-physiology model (stenoses plus pacing tachycardia), the decline in myocardial pH with stenoses-plus-pacing was only one third that seen with coronary-occlusion ischemia in our study, presumably because a considerable fraction of the hydrogen ion released was washed out. The hypothesis that relative lack of acidosis plays an important role in diastolic abnormalities in the pacing-induced ischemia might be tested by comparing pre- and post-pacing left ventricular diastolic pressure-segment length relations in the myocardium where pH is altered primarily by perfusing with acidic blood.

A second factor to be considered in explaining the absence of a substantial diastolic abnormality during coronary-occlusion ischemia is the profound decrease in systolic activity associated with coronary occlusion, presumably associated with a near-complete cessation of systolic cross-bridge cycling in the affected myocardium. If "incomplete" diastolic relaxation reflects a partial failure of diastolic inactivation of systolic cross-bridge cycling, the possibility of incomplete relaxation would be substantially reduced in the presence of severely depressed systolic activation (no cross bridges to persist into diastole). Thus, the profound decline in systolic activity with coronary occlusion ischemia may have contributed to the absence of abnormal diastolic distensibility. In contrast, in the angina-physiology model, systolic contractile function of the ischemic myocardium was relatively well preserved (Table 2), thus providing the substrate for incomplete relaxation.

A third factor contributing to the absence of abnormal diastolic distensibility with coronary occlusion ischemia may be the decrease in coronary vascular turgor associated with this type of ischemia. Coronary blood flow in all layers fell to extremely low levels (Table 4) following occlusion of the left anterior descending artery, compared to coronary blood flow in the angina-physiology model. Although not measured, coronary pressure distal to the occlusion presumably also declined. Decreases in both coronary flow and pressure induce a decrease in myocardial stiffness by reducing coronary vascular turgor in the so-called "erectile effect" (Vogel et al., 1982). This decreased stiffness should tend to offset increases in stiffness due to the ischemic process.

A fourth possible explanation for the absence of diastolic abnormality during coronary occlusion ischemia is mechanical stretch of ischemic myocardium. Ogilby and Apstein (1980) demonstrated that in the isolated rabbit heart, intermittent myocardial stretch during ischemic arrest can prevent a decrease in the left ventricular compliance without decreasing recovery, by rupturing the rigor or contracture bonds. Systolic stretching observed in ischemic muscle during coronary occlusion in the present study may prevent the rise in the left ventricular diastolic pressure through this mechanism. However, global ischemia due to a generalized reduction in coronary flow is not associated with an upward shift in the diastolic pressure-segment length relation (P'alacios et al., 1981), and there is no systolic stretching of ischemic myocardium in this setting.

The differences and similarities in myocardial high-energy phosphate metabolism in these two models of ischemia (Table 4) deserve further comment. Since, in this study, experiments were done in sequence of critical stenoses plus pacing followed by total coronary occlusion, some of the metabolic changes could be a result of difference between a first and second ischemia run, rather than being due to primary differences between these two forms of ischemia. It is known that an episode of ischemia due to coronary occlusion for 15–20 minutes depletes high-energy phosphate substantially, and requires days for complete recovery of hemodynamic and metabolic parameters. Therefore, if episodes of ischemia are repeated, incomplete recovery from the first episode of ischemia may modify the effects of a second episode of ischemia. However, in our present study, the first episode of ischemia (pacing-induced ischemia) was very short (3 minutes); fur-
thermore, after 30 minutes of recovery, ATP, CrP, ADP, and AMP returned to pre-pacing level (Table 4), corresponding to complete recovery of hemodynamic parameters. Also, we measured a wide spectrum of purine and pyrimidine compounds, including UTP, CTP, hypoxanthine, and GTP, as well as NAD^+, and these returned to control 30 minutes after the brief period of pacing tachycardia. In addition, hemodynamic changes in experiments where brief coronary occlusion followed preceding pacing-induced ischemia were quite comparable to those in experiments without preceding pacing-induced ischemia (Table 3). Therefore, in the present study, it is unlikely that the metabolic and/or functional changes seen with coronary occlusion (second episode of ischemia) were affected by the brief first episode of ischemia, namely, pacing-induced ischemia. Since it might be expected that demand ischemia caused by pacing tachycardia would lead to increased turnover and depletion of ATP, perhaps the most surprising finding in this study is that the subendocardial ATP content in both types of ischemia models was the same: ATP was depleted 12% in coronary occlusion ischemia and 11% in ischemia caused by pacing tachycardia.

Although ATP content was similar, creatine phosphate content (and, hence, total high energy phosphate content) was different in the two models of ischemia. In ischemia caused by coronary artery occlusion, creatine phosphate depletion was rapid and profound (85% depletion in 3 minutes) whereas, in ischemia caused by pacing tachycardia with coronary stenosis, creatine phosphate depletion was relatively mild (26% at 3 minutes). As a consequence, the ratio of creatine phosphate to ATP content remained near normal (1.4 vs. 1.7) in ischemia due to pacing tachycardia, whereas this ratio was low (0.3) in ischemia due to coronary artery occlusion. Since the biopsy following pacing tachycardia was done within several seconds after cessation of pacing tachycardia, creatine phosphate content at the end of 3 minutes of pacing tachycardia could have been lower than after pacing, and similar to that during coronary occlusion. However, the aim of this study was to investigate the relation between metabolic changes and diastolic dysfunction; therefore, we examined the metabolic state immediately after pacing tachycardia, at a time when the diastolic abnormalities are most marked.

Despite the fact that ATP content is similar in both models of ischemia, different mechanisms may be operating to maintain these relatively high ATP levels. In the coronary occlusion model, the rapid decline of systolic activity following coronary artery occlusion would help to decrease the rate of ATP utilization. Since ATP synthesis via oxidative phosphorylation must be limited in coronary artery occlusion, maintenance of ATP during the first few minutes of occlusion is probably supported by the creatine kinase reaction and glycogenolysis. In contrast, since oxygen is still available to the myocardium in the pacing tachycardia model, it seems likely that respiratory ATP remains the dominant source for ATP, and glycolysis with resupply of ATP via creatine kinase may be relatively less important. The transfer of high-energy phosphate from creatine phosphate to ADP via the creatine kinase reaction is a proton (hydrogen ion)-consuming reaction. The greater depletion of creatine phosphate observed for the coronary artery occlusion model (85% vs. 26%) is consistent with the acidic pH observed in the coronary artery occlusion model.

Consideration must be given to the possibility that differences in metabolism between the two types of ischemia studied in our experiments may be simply a result of more severe regional myocardial flow reduction in the coronary occlusion model. Thus, ischemia may well be less severe in the angina-physiology (pacing-induced ischemia) model. However, diastolic abnormalities were more severe with pacing-induced ischemia. This discrepancy between extent of ischemia and diastolic abnormalities suggests that ischemia alone is not a sufficient explanation for the altered diastolic properties in our angina-physiology model.

The changes in myocardial pH seen in our study should also be placed in the perspective of previous studies (Williamson et al., 1975; Ellis et al., 1976; Steenbergen et al., 1977; Ichihara et al., 1979; Jacobs et al., 1982). Cobbe et al. (1980, 1982) demonstrated that myocardial pH decreased by approximately 0.15 unit at 150 seconds of coronary occlusion, a finding similar to that in our study (Table 3). The pH monitored in the present study is not intracellular pH but "tissue" (primarily extracellular) pH; however tissue pH reflects intracellular production of hydrogen ion (Neely et al., 1975).

The myocardial blood flow measurements made in our study demonstrated differences between antegrade coronary flow (flowmeter) and myocardial flow (microspheres). Waters et al. (1977) studied the relation between graded coronary stenosis and regional function of the left ventricular myocardium, and found that antegrade coronary arterial flow (electromagnetic flowmeter) at onset of regional lactate production and segmental mechanical dysfunction was 48%. Stenoses created in the present angina-physiology model [approximately 50% reduction of antegrade coronary flow corresponds to more than 90% stenosis in diameter according to Gould et al. (1974)] are severe, and represent the lower limit of blood flow required for preservation of resting regional function. However, despite reduction in antegrade coronary flow in our studies, regional myocardial blood flow at rest with severe stenoses was preserved as shown by the microsphere studies (Table 5), presumably due to abundant physiological collateralization in dogs. By superimposing pacing tachycardia, absolute subendocardial flow and transmural perfusion ratio (endo:epi ratio)
were significantly decreased in areas distal to stenotic coronary arteries in our experiments. Since coronary arteries and arterioles distal to the stenoses are probably dilated maximally, mechanical factors probably are responsible for the reduction in myocardial flow observed during increased \( \text{O}_2 \) demand-type ischemia. Two mechanisms could be involved: (1) an epicardial coronary steal may have occurred; (2) increased diastolic myocardial compression of the intramyocardial coronary vasculature may have resulted from incomplete diastolic relaxation of the myocardium (Apstein et al., 1977). The effect of tachycardia on myocardial regional blood flow in the presence of coronary stenoses has been studied by Neill et al. (1975), who demonstrated in chronically instrumented dogs that pacing tachycardia decreases the endo:epi ratio in ischemic areas in the presence of mild coronary stenoses, although absolute myocardial flow per weight was not measured in that study. Selwyn et al. (1981) used krypton-81m to assess regional myocardial perfusion during pacing tachycardia in patients with angina pectoris, and found a decrease in radioactivity in a segment perfused by a stenosed artery, accompanied by S-T segment depression and angina. This suggests that a similar change in myocardial flow distribution to that seen in our angina-physiology model occurs in the affected segment in human angina pectoris.

It is interesting that the endo:epi ratio was decreased in the nonischemic area as well as in the ischemic area during pacing (Table 5), suggesting a "steal" from nonischemic to adjacent ischemic myocardium. This steal could occur from subendocardium to subepicardium, because elevated diastolic pressure in this model tends to increase subendocardial extravascular compression, even in the nonischemic area.

Two recent studies support a causative role for demand ischemia in producing impaired relaxation and decreased regional myocardial distensibility. Sassyama et al. (1985) showed that there is an upward shift in left ventricular pressure-segment length relations for ischemic myocardium, but not for adjacent normally perfused myocardium during pacing-induced angina pectoris in patients with coronary stenoses. Carroll et al. (1985) showed that exercise-induced angina is associated with both an upward shift in the left ventricular diastolic pressure-volume relationship and impaired ventricular pressure decay, and that both findings disappear following revascularization surgery. That tachycardia (either induced by electrical pacing or exercise) is not a prerequisite for these diastolic abnormalities is supported by the report of Sharma et al. (1983), who found an upward shift of left ventricular pressure relative to volume during spontaneous angina pectoris.

In summary, in myocardial ischemia due to 3 minutes of complete coronary occlusion, systolic dysfunction was predominant, and diastolic abnormalities were minimal. A marked fall in myocardial pH in the ischemic myocardium combined with preservation of nearly normal ATP (but not CrP) may have contributed to both the systolic dysfunction and the diastolic protection. In contrast, with myocardial ischemia due to coronary stenoses plus tachycardia for 3 minutes (angina-physiology model), diastolic dysfunction was predominant. As in the coronary occlusion model, ATP was mildly depleted, but the fall in pH was much smaller. The relative lack of protection by acidic pH, and the possibility that glycolytic ATP available for myocardial relaxation is limited, may explain the diastolic abnormalities seen in this model of ischemia.

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INDEX TERMS: Diastole • Diastolic distensibility • Compliance • Ischemia
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S Momomura, J S Ingwall, J A Parker, P Sahagian, J J Ferguson and W Grossman

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