SUMMARY The effect of global ischemia of different degrees of severity and reperfusion was studied in the isolated working rat heart. Four degrees of ischemia were induced by reducing the control total coronary flow of 8 ml/min to 0, 0.64, 0.4, or 0.8 ml/min for 30 minutes, after which the coronary flow was returned to the control level. After severe ischemia (0 and 0.04 ml/min ischemic coronary flow groups), recovery of contractility was to less than 30% of the control, pre-ischemic value of ventricular developed pressure and dP/dt, and irreversible cardiac contracture and an increased pacing threshold occurred. After moderate ischemia (0.4 and 0.8 ml/min ischemic coronary flow groups), contractile function recovered completely, ischemic contracture was rapidly reversible and the pacing threshold did not increase. The moderately ischemic groups were able to function at a stable, low level of contractility for the 30 minute ischemic period, whereas the severely ischemic groups had no contractile activity. The amount of calculated tissue lactate accumulation correlated with the occurrence of irreversible ischemic injury; the severely ischemic groups which failed to recover with reperfusion accumulated 3–5 times as much lactate as the moderately ischemic groups which recovered completely. The results suggest that relatively small differences in the severity of the ischemic condition can markedly affect the degree of tissue injury.

IN HUMAN OR EXPERIMENTAL coronary occlusion a nonhomogeneous pattern of ischemia occurs, and a heterogeneous peri-infarction zone exists where ischemia is less severe than in the central infarct. This zone is poorly defined geometrically but, after circumflex artery ligation in the dog, approximately two-thirds of the ischemic cardiac tissue was described as borderline or moderately ischemic. In patients who died from cardiogenic shock, diffuse heterogeneous areas of ischemia have been described.

Ischemia is known to cause a rapid decrease in contractility. The magnitude and reversibility of the loss of contractile function is probably dependent upon the severity and duration of the ischemia, but this relationship has not been quantitatively assessed by a dose-response curve. In addition to affecting contractility, ischemia also alters the diastolic properties of the ventricle as well as electrophysiologic function; the relationship between these parameters and the severity of the ischemic condition is also undefined.

The inherent myocardial perfusion heterogeneities of the ventricle with coronary artery disease may be increased by a number of therapeutic interventions which have been shown to protect the ischemic myocardium by favorably affecting the oxygen supply/demand ratio, or by increasing tissue perfusion or diffusion rates, and presumably preserving a portion of the peri-infarction or moderately ischemic zone. These interventions probably shift the spectrum and distribution of the ischemic regions and add a therapeutic heterogeneity to the underlying natural nonhomogeneous pattern of ischemia. Despite the proliferation of a number of therapeutic interventions which have been reported to protect the ischemic myocardium, the sensitivity of ischemic cardiac tissue to small, precisely controlled changes in the ischemic flow rate has not been evaluated. Since the basal oxygen requirements, determined in the potassium-arrested or calcium-chelated heart, are small relative to the oxygen need of the working heart, small increases in oxygen delivery do have the theoretic potential for preventing irreversible injury.

The experiments reported here were performed in order to study the relationship between the severity of induced myocardial ischemia, changes in lactate metabolism, and the depression and recovery of cardiac function. In order to study those factors which cause irreversible injury during myocardial ischemia, a protocol was developed in which the isolated rat heart was subjected to global ischemia of graded severity. The results suggest that irreversible injury was related to the amount of tissue lactate which was calculated to accumulate during the ischemic state.

Methods

Four groups of isolated rat hearts were subjected to different degrees of global ischemia for 30 min, followed by 30 min of reperfusion. Intraventricular pressures and the pacing threshold were monitored throughout the protocol.

An isolated isovolumic working rat heart preparation was utilized to perform these studies. A cannulated fluid-filled balloon was placed in the left ventricle of the isolated heart and attached to a pressure transducer to monitor intraventricular pressure. Contraction was isovolumic, since the balloon was noncompressible. Thus preload, or diastolic fiber length, was held constant and developed pressure and its first derivative (dP/dt) therefore reflected the contractile state of the myocardium.

Surgical and Perfusion Technique

Forty-four albino Sprague-Dawley male rats weighing 220 ± 7 g were utilized. Each animal was decapitated, the thorax rapidly opened, and the heart arrested with chilled saline. The aorta was dissected free, and a cannula was tied...
into the aortic root via an incision made at the level of the right innominate artery. Retrograde coronary perfusion was immediately started from a perfusate reservoir at a level of 75 mm Hg above the heart; thus coronary perfusion was maintained while the heart was being excised from the thorax and only a few seconds elapsed between the time of decapitation and the onset of coronary perfusion.

The perfusate consisted of modified Krebs-Henseleit buffer: 118 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃, 0.4 mM Na₂EDTA, 5.5 mM glucose, and 1.0 mM Na lactate. (Lactic acid was neutralized and added to the perfusate so that aerobic myocardial lactate extraction, as well as anaerobic lactate production, could be measured.) Gassing with 5% CO₂ achieved a pH of 7.4.

The heart was dissected from the thorax while the coronary circulation was maintained from the fixed-pressure perfusate reservoir. An apical puncture decompressed the left ventricle so that minimal contractile work was done during the dissection process. A ventricular apical drain insured that no fluid could collect in the left ventricle from Thebesian drainage during the experiment. The left atrium was opened and a collapsed latex balloon (manufactured in our laboratory) was inserted into the left ventricular chamber.

The heart was then excised from the thorax and suspended in a water-jacketed, constant-temperature chamber which was maintained at 37°C with a circulating pump. (The intraventricular temperature was monitored when control and ischemic coronary flow rates were present and it was constant at 37 ± 1°C in this apparatus.) Coronary perfusion was then switched to a constant flow pump (Harvard Apparatus No. 1203 or Technicon Instruments Proportioning Pump No. 1). A right ventricular pacemaker wire was inserted via a right atrial incision. The right ventricular ejectate consisted of coronary sinus drainage (there was no other flow through the right side of the heart), and was collected by a cannula inserted into the pulmonary artery or the body of the right ventricle. Some of the venous efflux drained via the cut vena cavae and was collected and pooled with the pulmonary artery efflux for any metabolic measurements. Only samples drawn from the cannulated pulmonary artery or from the body of the right ventricle were used for the measurement of coronary venous PO₂ (fig. 1). The perfusate efflux from the heart was not recirculated.

**Measurement of Mechanical Function**

Ventricular pressures were measured by using a 30 cm length of rigid Intramedic Polyethylene Tube PE 160 (internal diameter 0.045 in; outside diameter 0.062 in) attached to a Statham P23Db pressure transducer.

The frequency response to this pressure recording system was assessed with the balloon filled with a volume in the range used during the experiments. A sudden distortion and release of the balloon resulted in a 16% overshoot with aftervibrations of 47 Hz. The damping ratio was 0.54, and the calculated natural resonant frequency was 75 Hz. The system was therefore critically damped; the amplitude of the recorded pressure accurately reflected the amplitude of the true pressure between 0 and the natural resonant frequency of 75 Hz. It is possible that a minor frequency component of the pressure curve may have been greater than 75 Hz, since all hearts were paced at 300/min (5 Hz). This would be reflected in a slightly underestimated ventricular maximum dP/dt measurement since the highest frequency components would be reflected in the most rapid rise of the pressure curve. However, even though absolute maximal ventricular dP/dt may have been slightly underestimated in our recording system, since the same technique was used in all hearts, comparisons before and after ischemia in the same heart, and between groups of hearts, should be valid. Furthermore, since the pacing rate was kept at 5 Hz throughout all experiments, the major frequency components of the ventricular pressure trace should be constant relative to the natural resonance of the recording system.

A photographic recorder with a high frequency response was utilized (Electronics for Medicine Model DR8 or Hewlett Packard Model 4560). Left ventricular dP/dt was obtained via the differentiator output circuit of the Electronics for Medicine SGM Strain Gauge Meter/Amplifier or with an RC differentiator circuit manufactured in our laboratory for use with the Hewlett Packard pressure carrier amplifier Model No. 760-3000.

The collapsed intraventricular balloon was slowly filled with fluid while left ventricular pressures were recorded; balloon volume was adjusted to give a peak left ventricular systolic pressure of 55–80 mm Hg, with a diastolic pressure less than 12 mm Hg. Hearts which could not achieve this level of performance were discarded during the pre-experimental stabilization period (approximately 10% of the preparations). Left ventricular pressure and dP/dt were monitored continuously throughout each experiment.

The relationship between the balloon and left ventricular size is critical in this perfusion technique. The balloon must be slightly larger than the ventricular cavity, or a rise in intraballoon pressure will be recorded as the balloon is filled as
a result of increasing balloon wall tension rather than ventricular wall tension. We have manufactured a number of balloons of slightly different size so that in each experiment the ventricular cavity was always slightly smaller than the balloon capacity, as measured by recording the pressure-volume filling curve of the isolated balloon; thus the experiments were always performed on the flat portion of the balloon's pressure-volume curve.

**Experimental Protocol**

The isolated heart was perfused with well-oxygenated buffer at a total coronary flow rate of 8 ml/min for an initial 15 to 20 min, the time required for mechanical performance to become stable. This coronary flow rate resulted in a mean tissue perfusion rate of 14.96 ± 0.44 ml/min/g of left ventricle. Intraventricular balloon volume was adjusted to maintain a systolic developed pressure of 55–80 mm Hg during the stabilization period and was then held constant for the duration of the experiment.

In order to test the stability of the preparation six consecutive hearts were perfused under well-oxygenated conditions for a 90 min perfusion period. Left ventricular pressure development and dP/dt were constant over the 90 min perfusion period; developed pressure was 68 ± 5 mm Hg during the control period and 69 ± 3 after 90 min; dP/dt was 1818 ± 106 mm Hg/sec (control) and 2007 ± 100 mm Hg/sec after 90 min. No increase in ventricular diastolic pressure was observed.

Myocardial oxygen consumption was measured every 10 min and was constant over the 90 min period (mean 150.6 ± 10.0 μl O₂/g wet wt/min). The heart extracted only 72% of the available oxygen (mean “arterial” perfusate PO₂ was 542 ± 6 mm Hg; mean effluent PO₂ was 153 ± 9 mm Hg), suggesting that tissue oxygenation was adequate. When catecholamines were added to increase oxygen demand in this preparation, the isolated heart extracted more oxygen than occurred in this control series (unpublished observation), indicating that under our coronary flow conditions the myocardium can extract more oxygen if metabolic demand increases.

The pattern of lactate metabolism also indicated adequate tissue oxygenation. Despite the relatively high coronary flow rates, the hearts extracted an average of 5 ± 2% of the 1.0 mM perfusate lactate content in one passage through the myocardium for a mean rate of lactate utilization of 0.60 ± 0.26 μmoles/g/min.

**Production of Ischemia**

Ischemia was induced by reducing the pump-controlled coronary flow rate from the control value of 8 ml/min. Four degrees of ischemia were compared in different groups of hearts; during the ischemic period coronary flow was maintained at either 0, 0.04, 0.4 or 0.8 ml/min.

Heart size in each group was comparable. The mean left ventricular wet weights were 545 ± 24 mg, 511 ± 34 mg, 583 ± 24 mg and 560 ± 37 mg for the 0, 0.04, 0.4 and 0.8 ml/min ischemic coronary flow groups, respectively. Because of the small variability in ventricular size, the absolute rates of tissue perfusion in these groups during ischemia defined four groups with relatively narrow ischemic flow ranges: 0, 0.08 ± 0.01, 0.70 ± 0.03, and 1.50 ± 0.11 ml/min/g of left ventricle, respectively.

Ischemia was maintained for 30 min, after which the coronary flow was readjusted to the 8 ml/min control level.

**Dry: Wet Weight Determination**

Wet and dry weights were determined by opening both ventricles, lightly blotting any surface liquid, and weighing. The tissue was then allowed to dry to constant weight at room temperature and the dry weight determined.

**Pacing Technique**

A right ventricular bipolar pacemaker wire attached to a Grass Model S4 stimulator was utilized. The sinus node was removed and a stimulator rate of 300/min with a 5 msec impulse was used in all experiments since it exceeded the rate of the remaining endogenous pacemakers and thus provided a stable heart rate. Threshold was determined by slowly increasing the stimulator voltage until capture occurred. The stimulator voltage was then adjusted to 0.5 V above threshold. Under well-oxygenated conditions capture was achieved with less than a 2.5 V impulse.

The pacing threshold was checked every 5 min during the experimental protocol and was stable during the oxygenated control perfusion period and during moderate ischemia (the 0.4 or 0.8 ml/min ischemic coronary flow groups). With severe ischemia and reperfusion, the threshold increased (see Results). In order to avoid variations in mechanical work during the period of ischemia as a result of the change in pacing threshold, the stimulator voltage was progressively increased and maintained at 20% above threshold up to a value of 25 V. Preliminary experiments demonstrated that the heart could be paced with a 25 V stimulus for two hours without any deterioration of performance.

**Lactate Metabolism**

Arterial and venous perfusate samples were collected and analyzed for the lactate concentration by a specific enzymatic method. Venous effluent samples were collected during the period of ischemia and every 30 sec during the early reperfusion period. Lactate washout was complete within 3 min of reperfusion; the area under the lactate washout curve of concentration vs time was graphically integrated to calculate the amount of lactate which was washed out with reperfusion. This amount was added to the amount of lactate which had effluxed during the low-flow ischemia periods in order to calculate the total amount of lactate production which had occurred during the period of ischemia. In the 0.4 and 0.8 ml/min ischemic coronary flow groups, venous perfusate samples were collected every 5 min during the ischemic period. In the 0.04 ml/min ischemic coronary flow group the entire venous efflux during the 30 min ischemic period (1.2 ml) was collected and analyzed as a single sample. In the 0 flow ischemic group no efflux occurred during the period of ischemia; in this group the lactate washed out with reperfusion represented the total amount of ischemic lactate production. All perfusate samples were immediately mixed with iced 5% trichloracetic acid solution and kept under refrigeration until chemical analysis.

Metabolic data are expressed per unit of left ventricular wet weight (the free wall of the right ventricle was removed and the left ventricle, including the septum, was weighed). In this experimental preparation the right ventricle performs...
very little contractile work, since it pumps only a portion of the coronary sinus drainage to the cut pulmonary artery. Furthermore, the free wall of the right ventricle contributed only 10 ± 2% of the total heart weight. Therefore, the metabolic processes were considered to be due to the working left ventricle and metabolic data were related to the weight of this chamber.

Data Analysis

Statistical analysis of the data was performed by utilizing the unpaired Student’s t-test,18 the Wilcoxon (Mann-Whitney) rank test,19 or by analysis of variance and Scheffe’s multiple comparison procedure.20 All data are reported as the mean value ± SEM.

Results

Mechanical Performance in a Typical Experiment

The left ventricular pressure tracing from a typical experiment is shown in figure 2. Developed pressure was 75 mm Hg during the control period. Coronary flow was reduced to zero to induce ischemia, and a rapid decrease in developed pressure occurred. Diastolic pressure began to increase progressively after ten minutes of ischemia, and by the end of 25 min it was 18 mm Hg. In this preparation, balloon volume is kept constant, there is no diastolic filling of the left ventricle and the increase in diastolic pressure therefore represents contracture of the left ventricle on the non-compressible intraventricular balloon. With reperfusion after 30 min of ischemia, a marked and rapid increase in contracture occurred. After 30 min of reperfusion, developed pressure had returned to 20% of its control value, but diastolic pressure remained elevated.

Developed Pressure and dP/dt (table 1). Developed pressure rapidly fell to close to zero with the onset of ischemia in all groups. After 30 min of ischemia the 0.4 and 0.8 ml/min moderate ischemia coronary flow groups continued to exhibit 4–13% of control pressure development, while the two groups with more severe ischemia showed no contractile activity, except for a systolic oscillation of the pressure tracing less than 1 mm Hg in magnitude which occurred intermittently in four of the 21 hearts in these two groups. With reflow, the 0.4 and 0.8 ml/min moderate ischemia coronary flow groups recovered to 94% and 85% of control, respectively, while the 0 and 0.04 ml/min severe ischemia coronary flow groups recovered 16–25% of control developed pressure.

Left ventricular maximum systolic dP/dt paralleled developed pressure. During the period of ischemia the 0 and 0.04 ml/min severe ischemia coronary flow groups did not generate enough pressure to permit accurate measurements of dP/dt; the 0.4 and 0.8 ml/min moderate ischemia coronary flow groups maintained 6 ± 1% and 13 ± 1% of the pre-ischemic control values, respectively. With reperfusion the hearts which had been subjected to 0.4 and 0.8 ml/min moderate ischemia coronary flow rates recovered completely, while the more severely ischemic groups recovered to less than 30% of the pre-ischemic control level.

The differences in developed pressure and dP/dt during ischemia and after 30 min of recovery were significant (P < 0.001) when either of the severely ischemic groups (0 or 0.04 ml/min ischemic coronary flow rate) was compared to either of the moderately ischemic groups (0.4 or 0.8 ml/min ischemic coronary flow rates). The small differences between the 0 and 0.04 ml/min ischemic coronary flow groups were not significant. Throughout the ischemic period both developed pressure and dP/dt were significantly greater in the 0.8 ml/min ischemic coronary flow rate group than in the 0.4 ml/min group (P < 0.005); after 30 min of reperfusion at the control coronary flow rate, there was no significant difference between these two moderately ischemic groups.

Diastolic Contracture Pressure (table 1)

During the ischemic period, all groups developed significant contracture. The onset of severe contracture (> 15 mm Hg) was delayed in the moderately ischemic groups, but by the end of the 30 min ischemic period the severity of contracture was comparable in all groups.

With reperfusion, the severely ischemic groups underwent an intensification of contracture which was irreversible. In contrast, the ischemic contracture which had occurred in the moderately ischemic groups rapidly resolved and the diastolic ventricular pressure returned to the control value by the end of the 30 min recovery period.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Left ventricular pressure with ischemia and reflow. Segments of the left ventricular pressure tracing from a typical experiment are shown. The heart was paced at 300/min. During the 30 min ischemic period coronary flow was reduced to zero. With reflow coronary flow was returned to the 8 ml/min control level.
## Table 1. Cardiac Function with Graded Ischemia and Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Ischemic coronary flow</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>10 min</th>
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<tbody>
<tr>
<td><strong>Developed Pressure (mm Hg)</strong></td>
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<tr>
<td><strong>Severe</strong></td>
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<tr>
<td>0 ml/min (N = 13)</td>
<td>75 ± 3</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
<td>12 ± 5</td>
<td>19 ± 7</td>
<td>19 ± 6</td>
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<tr>
<td>0.04 ml/min (N = 8)</td>
<td>69 ± 4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>3 ± 1</td>
<td>7 ± 1</td>
<td>11 ± 2</td>
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</tr>
<tr>
<td><strong>Moderate</strong></td>
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<tr>
<td>0.4 ml/min (N = 12)</td>
<td>68 ± 2</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>51 ± 5*</td>
<td>63 ± 3*</td>
<td>64 ± 3*</td>
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<tr>
<td>0.8 ml/min (N = 11)</td>
<td>72 ± 3</td>
<td>7 ± 1*</td>
<td>9 ± 1*</td>
<td>9 ± 1*</td>
<td>56 ± 3</td>
<td>61 ± 2</td>
<td>61 ± 2</td>
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<tr>
<td><strong>Left Ventricular Max dP/dt (mm Hg/sec)</strong></td>
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<tr>
<td><strong>Severe</strong></td>
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<tr>
<td>0 ml/min</td>
<td>2300 ± 145</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>197 ± 102</td>
<td>389 ± 133</td>
<td>658 ± 214</td>
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<tr>
<td>0.04 ml/min</td>
<td>2162 ± 280</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>73 ± 30</td>
<td>223 ± 45</td>
<td>372 ± 75</td>
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</tr>
<tr>
<td><strong>Moderate</strong></td>
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<tr>
<td>0.4 ml/min</td>
<td>2006 ± 140</td>
<td>116 ± 20</td>
<td>112 ± 25</td>
<td>102 ± 25</td>
<td>1427 ± 200†</td>
<td>1984 ± 183†</td>
<td>2052 ± 198†</td>
<td></td>
</tr>
<tr>
<td>0.8 ml/min</td>
<td>2191 ± 71</td>
<td>284 ± 33*</td>
<td>257 ± 30*</td>
<td>303 ± 31*</td>
<td>1716 ± 111</td>
<td>2104 ± 46</td>
<td>2180 ± 58</td>
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<tr>
<td><strong>Left Ventricular Diastolic (Contracture) Pressure (mm Hg)</strong></td>
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<tr>
<td><strong>Severe</strong></td>
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<tr>
<td>0 ml/min</td>
<td>— ± 2</td>
<td>7 ± 2</td>
<td>27 ± 4</td>
<td>17 ± 3</td>
<td>51 ± 7</td>
<td>34 ± 9</td>
<td>34 ± 7</td>
<td></td>
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<tr>
<td>0.04 ml/min</td>
<td>— ± 2</td>
<td>3 ± 2</td>
<td>28 ± 5</td>
<td>20 ± 3</td>
<td>55 ± 4</td>
<td>45 ± 5</td>
<td>48 ± 5</td>
<td></td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
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<tr>
<td>0.4 ml/min</td>
<td>2 ± 1</td>
<td>6 ± 2</td>
<td>13 ± 4</td>
<td>30 ± 7</td>
<td>7 ± 3†</td>
<td>4 ± 2†</td>
<td>4 ± 2†</td>
<td></td>
</tr>
<tr>
<td>0.8 ml/min</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>11 ± 4</td>
<td>20 ± 6</td>
<td>7 ± 3</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
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</table>

**Pacing Threshold (Volts)**

<table>
<thead>
<tr>
<th></th>
<th>Ischemic coronary flow</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
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</thead>
<tbody>
<tr>
<td><strong>0 ml/min</strong></td>
<td>1.8 ± 0.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>14.3 ± 2.3</td>
<td>10.0 ± 2.0</td>
<td>8.8 ± 2.2‡</td>
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<tr>
<td><strong>0.04 ml/min</strong></td>
<td>0.7 ± 0.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>8.0 ± 2.6</td>
<td>8.1 ± 3.3</td>
<td>12.5 ± 3.5‡</td>
<td></td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
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</tr>
<tr>
<td><strong>0.4 ml/min</strong></td>
<td>1.7 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>2.3 ± 0.4</td>
<td>2.5 ± 0.5</td>
<td>2.7 ± 0.6</td>
<td>2.0 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td><strong>0.8 ml/min</strong></td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>1.8 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.2</td>
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</tr>
</tbody>
</table>

All data expressed as mean ± SEM (% of control pre-ischemic value).

*P < 0.005 for 0.04 ml/min vs 0.8 ml/min during ischemia.

†P < 0.001 for either moderate ischemia group vs either severe ischemia group.

‡P < 0.001 for pacing threshold during reperfusion vs control value.

### Pacing Threshold (table 1)

In the two severely ischemic groups, during the period of ischemia, no systolic activity could be recorded in 17 of the 21 hearts after the first few minutes of ischemia, despite an increase in the pacemaker stimulus to 25 V. The four hearts which exhibited minimal contractile activity required a 25 V stimulus in order to do so. During the recovery period the pacing threshold was significantly increased (P < 0.001) in the severely ischemic groups relative to the control value and relative to the moderately ischemic groups, which did not increase significantly above control, during either ischemia or recovery.

**Myocardial Edema.** Since the perfusate was colloid-free, tissue edema was anticipated. Dry:wet weight ratios were obtained to determine the degree and time-course of edema formation. Immediately after opening the thorax, prior to any perfusion with Krebs-Henseleit buffer, the dry:wet weight ratio of the myocardium was 0.247 ± 0.004 (N = 8). After 30 min of oxygenated perfusion, the dry:wet weight ratio was 0.214 ± 0.007 (N = 9), a significant decrease (P < 0.01). After two hours of oxygenated perfusion the dry:wet weight ratio was 0.211 ± 0.006 (N = 12). Thus, soon after the start of perfusion with the non-colloidal buffer, there was an initial uptake of water by the myocardial tissue, but under oxygenated conditions the degree of tissue edema was constant over the subsequent two hour experimental period.

The dry:wet weight ratio was determined in the four experimental ischemia groups after 30 min of ischemia and 30 min of reperfusion. This ratio was 0.201 ± 0.006 (N = 11), 0.204 ± 0.010 (N = 8), 0.197 ± 0.002 (N = 8) and 0.219 ± 0.012 (N = 8), for the 0, 0.04 and 0.8 ml/min ischemic coronary flow groups, respectively. Thus, the degree of tissue edema was comparable in the four ischemic groups, and was not significantly greater than that which occurred during a comparable period of oxygenated perfusion.

### Lactate Production and Washout during Ischemia (fig. 3)

The metabolic data presented in figure 3 are arranged according to the degree of ischemia which produced irreversible injury (the 0 and 0.04 ml/min ischemic coronary flow groups) or permitted complete recovery (the 0.4 and 0.8 ml/min ischemic coronary flow groups).
Calculated Lactate Accumulation during Ischemia

The second panel from the top (fig. 3) represents the calculated amount of lactate which accumulated in the myocardium during the period of ischemia. These data were obtained by graphically integrating the area under the lactate washout curves during reperfusion. With increasing rates of tissue perfusion, progressively less lactate accumulated during the ischemic period; however, once an ischemic coronary perfusion rate of 0.4 ml/min was reached, there was no further decrease in the amount of calculated tissue lactate accumulation when the ischemic coronary flow was increased to 0.8 ml/min. There was a threefold difference in the amount of calculated tissue lactate accumulation on either side of the transition zone from irreversible injury to complete recovery, which was highly significant \((P < 0.001)\). The degree of calculated tissue lactate accumulation was like a mirror-image of the rate of lactate washout during the period of ischemia (the second panel from the bottom); as the rate of ischemic coronary flow was increased from 0.04 to 0.4 ml/min (the transition between irreversible and reversible injury) there was a marked increase in lactate washout and a concomitant decrease in calculated myocardial lactate accumulation.

Total Amount of Ischemic Lactate Production

The top panel (fig. 3) represents the total amount of ischemic lactate production in each of the four groups. This value was calculated by adding the amount of lactate which effluxed during the ischemic period (second panel from the bottom) to the amount of lactate washed out with reperfusion (calculated tissue lactate accumulation, third panel from bottom). The marked group differences in the amounts of lactate washout during ischemia and reperfusion almost completely balanced each other so that the total amounts of lactate production were not significantly different between the 0.04, and 0.4 ml/min ischemic coronary flow groups. Although the 0.04 ml/min ischemic coronary flow group

was no such washout. As the ischemic coronary flow rate was progressively increased, there was a progressive increase in the amount of lactate washed out. The difference in this amount on either side of the transition zone from irreversible injury to complete recovery was particularly striking. The total amount of lactate washed out during ischemia in the 0.04 ml/min ischemic coronary flow group was 9.4 ± 3.1 \(\mu\)moles/g compared to 53.7 ± 2.3 \(\mu\)moles/g \((P < 0.001)\) in the 0.4 ml/min group; thus, six times as much lactate was washed out during ischemia despite the same mean effluent lactate concentrations in these two groups. These data are consistent with a feedback inhibition effect,\(^{11, 22}\) as the washout rate increased, the rate of lactate formation increased in parallel, and the mean effluent concentration was maintained at a relatively constant level. In the 0.8 ml/min ischemic coronary flow group the amount of lactate washout during ischemia was significantly greater than in the 0.4 ml/min ischemic coronary flow group; in this range of tissue perfusion, as the ischemic coronary flow rate increased from 0.4 to 0.8 ml/min, the increased rate of washout apparently was greater than any increase in rate of lactate formation and the mean effluent lactate concentration decreased (bottom panel).

**Effluent Lactate Concentration**

The bottom panel in figure 3 represents the mean effluent lactate concentration step-up or increase above the 1.0 mM perfusate level; i.e., the mean effluent concentration of endogenously generated lactate during the ischemic period. (In the 0 ml/min ischemic coronary flow group, where there was no efflux during the ischemic period, this value was approximated by extrapolating from the lactate washout curve to the time of the onset of reperfusion.) There was a general trend toward a lower effluent lactate concentration as the ischemia progressed from severe to mild, but a significant decrease in effluent lactate concentration did not occur until the 0.4 and 0.8 ml/min ischemic coronary flow groups were compared. There was no significant difference in the mean effluent concentration on either side of the transition from irreversible injury to complete recovery (the 0.04 and 0.4 ml/min ischemic coronary flow groups).

**Lactate Washout during Ischemia**

The net amount of lactate which was washed out during the period of ischemia is shown in the second panel from the bottom. In the 0 ml/min ischemic coronary flow group there

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**Figure 3.** Lactate metabolism with graded ischemia. Several parameters of lactate metabolism are shown for four groups of rat hearts subjected to different degrees of global ischemia for 30 min. The two severely ischemic groups (0 and 3% of normal perfusion) underwent irreversible injury, while those perfused at 29 and 63% of normal perfusion recovered completely (table 1). The asterisk indicates that the mean effluent lactate concentration in the zero flow group was approximated by extrapolation from the washout curve, since no efflux occurred in this group during the ischemic period itself.
had less lactate efflux than the 0.4 ml/min ischemic coronary flow group during the ischemic period, the latter group accumulated much less lactate to be washed out with reperfusion. Accordingly, there was no significant difference in the total amount of lactate production between these two groups, one of which underwent irreversible injury and the other of which recovered completely.

The highest rate of ischemic tissue perfusion was associated with a significantly greater total amount of lactate production; the 0.8 ml/min ischemic coronary flow group produced 89.4 ± 7.5 μmol lactate/g, which was significantly greater than the 0.4 ml/min ischemic coronary flow group value of 67.8 ± 3.3 (P < 0.025).

Discussion

The fundamental difference between the ischemic and hypoxic condition is the level of tissue perfusion. The effects of cardiac hypoxia (oxygen deficiency) where anaerobic coronary flow was maintained, or where diffusion provided washout from thin anaerobic papillary muscles, have received a great deal of study. Experimentally, it is difficult to separate the effects of a decreased rate of tissue perfusion from those of a decrease in oxygen delivery, yet it is this crucial difference which distinguishes the pathophysiology of ischemia from that of hypoxia.

Our experimental protocol was designed to model the degree of ischemia which exists in the clinical setting of myocardial infarction or cardiogenic shock. Accordingly, we utilized coronary flow rates which provided “per gram” tissue perfusion rates in the range which has been determined in experimental models of myocardial infarction. In a hemoglobin-free perfusate such as ours, in vivo levels of ischemic flow and oxygenation cannot be reproduced simultaneously. By utilizing ischemic perfusion rates based on in vivo determinations we subjected our hearts to more severe hypoxia (oxygen deficiency) than would occur for a similar perfusion rate in the blood-perfused in vivo state. Our studies were therefore directed toward the pathophysiologic behavior which results from differences in ischemic tissue perfusion rates, rather than from hypoxia per se.

In this study we adjusted the control coronary flow rate, pacemaker rate, and intraventricular balloon volume so that all hearts would have a comparable and narrow range of contractile work and oxygen demand prior to the onset of ischemia. We have observed that greater variation in control cardiac performance occurs if the isolated heart is allowed to autoregulate the amount of coronary flow derived from a fixed aortic perfusion pressure. The uniform coronary flow rate of 8 ml/min resulted in a mean control tissue perfusion rate of 14.96 ± 0.44 ml/min/g, comparable to values obtained by other workers utilizing similar preparations, in which either coronary flow18, 23 or coronary perfusion pressure19, 23 was held constant. Though it would be ideal to provide the same rate of ischemic tissue perfusion on a “per gram” basis for each heart, our hearts were not weighed until the end of the experiment, and the resulting intra-group ischemic tissue perfusion rates therefore had a small, but acceptable, variation (fig. 3).

In clinical myocardial infarction and cardiogenic shock, myocardial ischemia is heterogeneous in severity and distribution throughout the ventricle, and mechanical dysfunction also shows regional variations.1-5 Correlations between flow and dysfunction are difficult to study when coronary ligation or stenosis is used as the method of producing ischemia because of this heterogeneity.5 Furthermore, the severity of ischemia may change with time in a given region of the ventricle after coronary artery ligation.27 Accordingly, we attempted to assess the relationship between the severity of the ischemic state and the resulting functional and metabolic abnormalities in a model of global ischemia, where the rate of coronary flow to the entire ventricle can be precisely regulated and held constant. In this preparation, each group of hearts subjected to a given degree of global ischemia is a model of one region of a ventricle which has developed heterogeneous ischemia as a result of myocardial infarction or severe coronary occlusive disease.

The severity of the global ischemia induced in our experimental preparation should be viewed in the perspective of the reduction in myocardial perfusion rate which occurs with coronary artery occlusion. After coronary artery ligation in the dog, tissue perfusion rates range from 0-0.8 ml/min/g in the central infarct region to 0.4-1.4 ml/min/g where the outer edge of the infarct merges into normal tissue.2, 4 Thus, our severely ischemic groups, with weight-adjusted coronary flow rates of 0 and 0.08 ± 0.01 ml/min/g, represent models of the central zones of a canine infarct, and our moderately ischemic groups, with perfusion rates of 0.70 ± 0.03 and 1.50 ± 0.11 ml/min/g, are models of different regions of the peri-infarction zones.

The degree of ischemia induced in our preparation should also be viewed in relation to normal coronary blood flow in rats, which was measured to be 1.5-2.6 ml/min/g using isotopic potassium.28 Using the microsphere technique, myocardial blood flow was measured to be 4.24 to 5.1% of the cardiac output,29, 30 or approximately 2.4 ml/min/g of ventricle for a 250 g rat with a cardiac output of 50 ml/min.31 Thus our severely ischemic groups represent 0-3% of the rat’s normal myocardial perfusion rate, and our moderately ischemic groups approximately 29% and 63% of the normal rat myocardial perfusion rate.

Previous studies of ischemia in isolated rat hearts21, 26, 32-33 have generally employed ischemic tissue perfusion rates which were greater than the values we selected on the basis of in vivo peri-infarction zone measurements. Therefore, our hearts had greater impairment of contractile performance and less tissue washout during ischemia than occurred in the experiments cited above. We were able to correlate the degree of calculated lactate accumulation with loss of contractile function in the reperfusion period (fig. 3); this correlation, although postulated, previously has not been demonstrated to the best of our knowledge.

The correlation between calculated tissue lactate accumulation and irreversible injury does not necessarily mean that the accumulated lactate is responsible per se; this accumulation may reflect a general lack of washout of metabolites, such as carbon dioxide,24 with ischemia, but is consistent with the hypothesis that intracellular acidosis is a primary cause of ischemic tissue injury. In contrast to the correlation between ischemic injury and calculated tissue lactate accumulation, total lactate production (which reflects the total glycolytic flux) appeared unrelated to irreversible injury (fig. 3), suggesting that the amount of ischemic glycolytic ATP synthesis was not a prime determinant of injury.

The greater degree of calculated tissue lactate accumula-
tion in the 0.04 ml/min ischemic coronary flow group, relative to the 0.4 ml/min ischemic coronary flow group which had similar effluent lactate levels, suggests that the intracellular lactate may not come to equilibrium with the extracellular space with more severe ischemia. This phenomenon has been described previously with severe ischemia21, 22, 26, 32-35 and may reflect a carrier-mediated lactate exit mechanism.36

Partial relief of ischemia increases tissue oxygenation in addition to increasing metabolite washout. The increase in oxygen delivery may have been important in protecting the moderately ischemic groups, but it was probably not the sole factor. The 0.4 ml/min ischemic coronary flow group, which recovered completely, received 6.8 μl/min of oxygen or approximately 12 μl O2/min/g of left ventricle during the 30 min ischemic period. An in vivo coronary blood flow rate of 0.07 ml/min/g would provide the same O2 delivery.* This coronary flow rate and oxygen delivery does not prevent necrosis in central canine myocardial infarcts.2 Furthermore, coronary ligation in dogs for 20 min reduced coronary flow and oxygen delivery to this range, and was associated with incomplete recovery of mechanical function.36 Thus, the amount of oxygen delivery present in our moderately ischemic groups cannot entirely account for the complete recovery of these groups.

Regardless of underlying mechanism, postischemic recovery appears to be critically dependent upon relatively small differences in the degree of ischemia which existed. Therefore, experimental protocols which utilize recovery of function as a measure of protection from ischemia should include precise regulation or measurement of the degree of ischemia which occurs. This is particularly critical in the canine heart since the severity of ischemia induced by coronary artery ligation is highly variable.3, 9

The increase in pacing threshold after severe ischemia suggests the possible in vivo existence of ventricular tissue with latent contractile ability which may remain dormant due to an inadequate stimulus to depolarization. Experimentally, the potential contractile function of postischemic myocardium may be considerably underestimated if an adequate stimulus is not applied. Postischemic cardiac function may be reduced secondary to electrical or mechanical dysfunction; these mechanisms have different implications in terms of irreversible tissue damage or the possibility of improving cardiac function. Our observations suggest that an increased threshold to excitation may be partially responsible for loss of contractile function of the ischemic and postischemic myocardium.

Our protocol of graded ischemia caused progressive degrees of tissue injury. A modest reduction in the severity of the ischemic condition completely protected the normothermic ischemic mammalian left ventricle for at least 30 minutes. The protective effect on contractile function was only minimally manifested during the period of ischemia, but was marked with reperfusion.

*The "arterial" perfusate PO2 was approximately 550 mm Hg. The solubility of O2 in water at 37°C is 0.0239 ml O2/ml H2O for 760 mm Hg, so that the perfusate O2 content is 17 μl O2/ml perfusate and a coronary flow rate of 0.4 ml/min delivers 6.8 μl O2/min. The mean left ventricle wet weight in the 0.4 ml/min ischemic flow group was 0.583 g, hence during ischemia oxygen was delivered at 12 μl O2/min/g of left ventricle. In an intact animal with an arterial O2 content of 20 vol%, 90% extraction would remove 0.18 ml O2/ml arterial blood; hence 12 μl of O2/min/g would be delivered by a coronary blood flow of 0.07 ml/min/g.

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The Effect of Propranolol on Microvascular Injury in Acute Myocardial Ischemia

ROBERT A. KLONER, M.D., PH.D., MICHAEL C. FISHEIN, M.D., RAMZI S. COTRAN, M.D., EUGENE BRAUNWALD, M.D., AND PETER R. MAROKO, M.D.

SUMMARY The purpose of this study was to determine whether propranolol, which has been shown to reduce the extent of myocardial infarction, reduces microvascular injury which may play a role in exacerbating ischemia. Saline (10 dogs) or propranolol (2 mg/kg i.v., 7 dogs) was injected prior to a one hour occlusion of the left anterior descending coronary artery. Carbon black (1 ml/kg), which labels damaged and leaky vessels, was injected 5 min after release of the occlusion and allowed to circulate for two hours. By morphometric analysis of 1 μ thick sections, 75 ± 12% of vessels and 84 ± 7% of myocardial cells showed damage in untreated dogs; only 2 ± 1% of vessels and 9 ± 8% of myocardial cells showed damage in the propranolol-treated dogs (P < 0.001). The number of carbon black-labeled vessels/10 fields/biopsy from comparable areas of ischemic tissue was 55 ± 7 in untreated dogs and 27 ± 3 in propranolol-treated dogs (P < 0.001).

The results suggest that propranolol not only protects the ischemic myocardial cell, but also significantly decreases the ischemic microvascular changes.

ISCHEMIC DAMAGE, following coronary occlusion, occurs not only in the myocardial cells, but in the capillaries, venules, and arterioles supplying the myocardium as well. Although the effect of ischemia on myocardial cells has been examined in detail,2,3 there have been few studies examining the fate of the microvasculature following coronary occlusion and the extent to which it can be salvaged. The investigation of injury to the microvasculature can add still another dimension to the definition of means by which myocardial ischemic injury can be altered.

A number of agents have been used to decrease the size of experimental myocardial infarct.4,5 These agents reduce the number of myocardial cells that die, but their effects on the vascular elements of the myocardium need clarification. Some agents may exert a protective effect against microvascular destruction and hence preservation of collateral flow is one of their mechanisms of action to preserve ischemic myocardium. For example, recently hyaluronidase, an enzyme known to decrease experimental myocardial necrosis,6 has been shown to prevent the decline in collateral blood flow to the ischemic myocardium which occurs in the interval between 15 min and 6 hours following coronary occlusion in the dog.7

The purpose of this study was to determine whether the beta-adrenergic blocking agent, propranolol, which has been shown to reduce the extent of myocardial infarction,4 decreases microvascular ischemic injury. The extent of vessel injury was studied by electron microscopy, morphometric analysis of 1 μ sections, and by quantitating the degree of vascular damage using the method of labeling with colloidal carbon as a marker of endothelial injury.8

Methods

Studies were carried out in mongrel dogs of both sexes that weighed between 19 and 25 kg. They were anesthetized with sodium thiamylal (25 mg/kg i.v.), and ventilated with a Harvard respirator following endotracheal intubation. Arterial pressure was recorded through a saline-filled catheter from the carotid artery (Statham P23Db pressure transducer) and lead aV of the ECG was monitored continuously throughout the experiment on a polygraph (Brush Instruments, Cleveland, Ohio). A thoracotomy was performed in the fifth left intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was isolated from the adjacent tissues approximately 2-2.5 cm distal to the aorta. For carbon black injections, a catheter was placed into the left atrial appendage and tied into place with a purse string suture.

Two occlusions were carried out in each dog. The first occlusion lasted 15 minutes, a time period during which myocardial cell injury can be reversed, as determined by morphologic techniques.2 The first occlusion was performed to demonstrate that the site of occlusion selected resulted in grossly similar areas of ischemia between groups of dogs, as...
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C S Apstein, L Deckelbaum, M Mueller, L Hagopian and W B Hood, Jr

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