Acute Hibernation and Reperfusion of the Ischemic Heart

S. Evans Downing, MD, and Victor Chen, PhD

Background. Recovery from prolonged low-flow ischemia was studied in isolated, isovolumically beating neonatal piglet hearts (n=11) and compared with controls (n=5).

Methods and Results. Hearts were perfused with red blood cell–enhanced Krebs-Henseleit buffer with physiological oxygen-carrying capacity. Left ventricular mechanical function was assessed with a fluid-filled balloon. Measurements of peak systolic pressure, pressure–rate product (PRP), and +dP/dt max were obtained at various filling pressures. Myocardial oxygen delivery and metabolism (MVO2) and lactate uptake were measured at 30-minute intervals. Control data were obtained with coronary flow (CF) set at 2 ml·min⁻¹·g⁻¹. CF was then reduced to 0.2 ml·min⁻¹·g⁻¹ for 2 hours. Thereafter, reperfusion was instituted at control levels. Hearts not subjected to ischemia were studied at identical time intervals. In these, function remained at >80% after more than 3.5 hours of study. Reduction of CF to 10% was accompanied by a abrupt diminution in function (pressure–rate product) and MVO2 to 20% of control and by lactate release. These measures remained constant for the full 2 hours of ischemia. Incremental return of CF caused a lockstep increase in mechanical function and metabolism. At 30 minutes of reperfusion, PRP was 78% of time-matched controls (p=0.05), and dP/dt max did not differ. Increasing calcium to 5 mmol/l returned PRP (and dP/dt max) to preischemia levels. Myocardial ATP and creatine phosphate concentrations were identical in both groups, although glycogen was lower in the ischemic hearts.

Conclusions. Acute hibernation is associated with protection of the in vitro heart from prolonged normothermic ischemia. Systolic function was only modestly lower, and velocity (dP/dt max) did not differ from control hearts. The minimal “stunning” was fully reversible with calcium. (Circulation 1992;82:699-707)

In a recent study,1 we explored sustained low-flow ischemia in isolated neonatal piglet hearts with the objective of defining progressive mechanical and metabolic changes anticipated to occur with time. We found, however, that reducing coronary flow (CF) to 10% of control values was accompanied by an immediate reduction in mechanical function consistent with reduced oxygen availability. After 2 hours of normothermic ischemia, myocardial concentration of glycogen and creatine phosphate (CP) did not differ from the control group; ATP was only 24% lower. We will refer to this phenomenon as acute hibernation consistent with the suggestions of Ross.2 Chronic hibernation is thought to occur in certain instances of long-standing ischemic heart disease in humans.3

It is generally held that myocardial ischemia of brief (5–20-minute) duration in vivo is followed by sustained hypofunction when blood flow to the ischemic muscle is resumed.4–7 Transient interruption of blood flow for this limited period does not cause myocyte necrosis.8–10 Characteristic ultrastructural changes appear, but for the most part these reverse within 1 hour.11 Hence, contractile abnormalities may continue for days in morphologically normal hearts.12 This phenomenon has been ascribed the meaningful, if colorful, label of “stunned myocardium.”13

The present study was undertaken to explore qualitative and quantitative patterns of recovery from 2 hours of low-flow ischemia. The relative efficiency of the acute hibernation mechanism was assessed by comparison of mechanical function and critical metabolic substrate concentrations with nonischemic control hearts studied over the same time base. Differences after 1 hour of recovery were taken to reflect postischemic stunning. To examine the possibility that reduced function could be related to net or compartmental deficiencies in high-energy phosphate concentrations, responses to calcium stimulation were compared before and after induction of low-flow ischemia.6

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Methods

Piglets delivered by sows obtained from a commercial breeder were used. Five hearts from animals ranging from 7 to 15 days old (mean, 10 ± 1) were studied as controls. Eleven hearts ranging from 1 to 18 days old (mean, 8 ± 2) served as the ischemia group. All animals were given sodium pentobarbital (25 mg/kg i.p.) to produce general anesthesia. Anticoagulation was provided with sodium heparin (1,000 units) injected into an ear vein. A tracheostomy was performed, and mechanical ventilation was begun. Hearts were excised rapidly by transection of the pulmonary hila and great vessels and immediately placed in ice-cold Krebs-Henseleit (KH) solution.

Cardiac Preparation and Perfusion System

The aorta was cannulated, and coronary perfusion was initiated at 70 cm H2O pressure using a modified Langendorff system. Details of the system have been described previously.14,15 The pulmonary artery was cannulated, and the pulmonary veins and venae cavae were ligated. The time that elapsed between excision of the hearts, weighing, and initiation of retrograde perfusion was less than 10 minutes. Perfusion temperature was maintained at 37°C by a heat exchanger. Arterial blood was oxygenated by passing a mixture of 95% O2−5% CO2 through the perfusate in the Langendorff column.

A fluid-filled balloon was passed into the left ventricle via the left atrium and mitral valve. The balloon was secured with a suture passed through the apex. It was connected to a Gould pressure transducer with a short segment of polyethylene tubing. Left ventricular end-diastolic pressure (LVEDP) was incrementally increased by changing the volume of fluid in the balloon. The corresponding changes in ventricular systolic pressure (SP) and dP/dt max were recorded. Curves representing these relations were plotted. Heart rate was controlled by a pacing electrode secured to the right atrium. Frequencies chosen were generally 10–20 beats per minute greater than intrinsic heart rate. The isolated heart was enclosed in a water jacket to maintain constancy of myocardial temperature. An in-line Swank transfusion filter (model IL-200, Extracorporeal Medical Specialties, Beaverton, Ore.) was incorporated into the system to filter microaggregates.

Perfusate Composition

Packed human red blood cells were washed three times with KH containing 0.5% bovine serum albumin (BSA) (Cohn Fraction V, United States Biochemicals, Cleveland, Ohio) and twice with a 2% BSA–KH solution just before use. The perfusate consisted of a modified KH solution containing 2% BSA, 5 mmol/l glucose, 1.5 mmol/l lactate, 0.15 mmol/l free fatty acids, 50 units insulin, and the following ions in mmol/l: NaCl 118, KCl 4.7, MgSO4 2.4, KH2PO4 1.2, NaHCO3 25, and CaCl2 2.4. The washed erythrocytes were suspended in this solution to provide a hematocrit of 20%, comparable to that of newborn piglets.16

Metabolic Measurements

CF was measured by timed collections of the pulmonary arterial (coronary venous) effluent. Samples were obtained anaerobically in syringes from the pulmonary artery and aortic perfusion cannula simultaneously. Myocardial oxygen consumption (MV02) was calculated from the product of the measured arteriovenous oxygen content difference and CF. Oxygen content was measured directly with a Lex-O2-Con M analyzer (Lexington Instruments Corp., Lexington, Mass.).

Lactate uptake/release was calculated from the product of the measured arteriovenous substrate concentration difference and CF. Lactate concentrations were determined enzymatically according to the method of Lowry and Passonneau.17

At the conclusion of the experiment, the apical half of each heart was freeze-clamped and powdered at liquid nitrogen temperature. The samples were stored in liquid nitrogen until used. A portion was deproteinized with perchloric acid and used for the determinations of CP and ATP concentrations.17 Glycogen content was measured colorimetrically.18

Experimental Protocol

Shortly after the mechanical preparation of the heart was completed, coronary flow was switched from a passive controlled mode to a pump-controlled system using a precalibrated Masterflex pump. Flow was set at approximately 2 ml·min⁻¹·g⁻¹ total heart weight. This value was chosen because it provided more than 200 µl/min/g oxygen delivery to the heart (Table 1) with the red blood cell concentration we used (20%). This is comparable to values found with our neonatal lamb model.19 Measured coronary venous return differed less than 5% from the selected pump (input) setting. A ventricular function curve was then obtained by progressively increasing balloon volume in increments over a range of EDP from 0 to 15 cm H2O. Arterial and venous perfusate samples were drawn for the various analyses. A second control run was obtained 30 minutes later (C30), and these data were used for comparison with subsequent measurements.

Immediately after the second control run was completed, CF was reduced to 0.2 ml·min⁻¹·g⁻¹ (ischemia group). Studies were repeated at 30-minute intervals for a total of 2 hours of ischemia. Reperfusion was then initiated by doubling of CF in 5-minute intervals to 1.6 and then approximately 2.0 ml·min⁻¹·g⁻¹. Measurements were repeated 30 and 60 minutes after initiation of reperfusion. Control hearts were treated identically, except that CF was maintained at approximately 2 ml·min⁻¹·g⁻¹. After completion of the study, the hearts were freeze-clamped, and myocardium subsequently was analyzed as described above.
Table 1. Oxygen and Lactate Metabolism

<table>
<thead>
<tr>
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<th>I50</th>
<th>I120</th>
<th>R50</th>
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<tr>
<td>Oxygen delivery (µl · min⁻¹ · g⁻¹)</td>
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<tr>
<td>Mean</td>
<td>226</td>
<td>202</td>
<td>219</td>
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<tr>
<td>SEM</td>
<td>15</td>
<td>21</td>
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<td>p</td>
<td>NS</td>
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<td>&lt;0.001</td>
<td>NS</td>
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<td>Oxygen extraction (%)</td>
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<tr>
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<td>NS</td>
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<tr>
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<td>&lt;0.001</td>
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<td>Lactate uptake (µmol/1 · min⁻¹ · g⁻¹)</td>
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<tr>
<td>Mean</td>
<td>243</td>
<td>305</td>
<td>212</td>
<td>[204]</td>
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<tr>
<td>SEM</td>
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</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
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</table>

C, control group (n=5); I, ischemia group (n=11); C30, control values 30 minutes after start of experiment; I50 and I120, values obtained 60 and 120 minutes after onset of ischemia or equivalent time in controls; R50, values 30 minutes after return of normal coronary flow. Brackets indicate negative lactate uptake (release).

Statistical Analyses

By regression analysis of data from the ventricular function curves (Figure 1), peak systolic pressure (PSP₁₀) and dP/dtₘₐₓ were calculated for a uniform EDP value of 10 cm H₂O. For nearly all curves, values of r>0.95 were found. Values of r>0.90 were found in all cases. The product of PSP₁₀ and heart rate, the pressure–rate product (PRP₁₀), was calculated. PRP₁₀ provides a method for quantification of the performance of a given heart, which can be compared at various points in time with other hearts. Moreover, this assessment of contractile function is tightly linked with oxygen consumption.¹

Data were analyzed with the aid of a Digital Rainbow computer and are presented as mean ± SEM. CF, oxygen delivery MVO₂, and lactate uptake have been expressed per gram total wet heart weight. A two-way analysis of variance with one grouping factor (ischemia versus control) and one repeated-measures factor (time) was conducted within the framework of a general multivariate analysis of variance. This analysis was followed by the Newman-Keuls test for multiple comparisons when appropriate. Simple group comparisons not involving time were by a Student’s unpaired t test. Values of p < 0.05 were considered significant.

![Figure 1](http://circ.ahajournals.org/fig?doi=10.1161/01.CIR.79.4.827)

**Figure 1.** Left ventricular function curves from a representative control heart. Data were obtained at time points corresponding to those of experimental hearts subjected to ischemia and reflow. The curves shown were obtained at times equivalent to the 30-minute control (CONTROL₃₀), 2 hours of ischemia ("ISCHEMIA"₁₂₀), and after 1 hour of reperfusion ("RECOVERY"₆₀). Values at end-diastolic pressure of 10 cm H₂O (arrows) were used to quantify the performance of each heart. HR, heart rate (beats per minute). For all curves, r>0.95 by linear regression analysis (p<0.01).
**Effects**

**Stability of Preparation**

The protocol for this study required that the preparation maintain stable mechanical and metabolic function for a period of at least 3.5 hours. Estimates of mechanical function were derived from multipoint curves drawn from measurements obtained over a range of LVEDP. Examples from an individual control heart are illustrated in Figure 1. Estimated PSP at an EDP of 10 cm H$_2$O (PSP$_{10}$) at the 30-minute control point was 127 mm Hg (Figure 1, left panel). Two hours later, calculated PSP$_{10}$ was 115 mm Hg, or 90% of the control value. After an additional hour, PSP$_{10}$ was 103 mm Hg, or 81% of control. A similar pattern is evident for measurements of $dP/dt_{max}$ (Figure 1, right panel).

Data reflecting the three key measures of LV performance obtained from the control hearts are summarized in Figure 2. To allow for comparison of disparate types of data, all values were converted to percentage of the 30-minute control values. It is evident that for each of the three measures, there is a nearly identical linear reduction with time. After 3.5 hours of data gathering, mean values averaged 80–84% of those obtained at the 30-minute control time. There was no evidence for an accelerated decline in ventricular function in the later portions of the protocol.

Mechanical stability of these preparations was also reflected in measurements of oxygen and lactate metabolism. As shown in Table 1, oxygen delivery at the 3-hour point ($R_{30}$) was unchanged from the 30-minute point ($C_{30}$) in the control hearts. $MV_O_2$ was $54.3 \pm 4.6$ and $48.9 \pm 2.2 \mu l \cdot min^{-1} \cdot g^{-1}$ at these same intervals. The difference was not statistically significant. This is consistent with measures of lactate uptake, which averaged $243 \pm 49$ and $239 \pm 43 \mu mol \cdot min^{-1} \cdot g^{-1}$, respectively ($p=NS$).

**Effects of Low-Flow Ischemia**

Reduction of coronary flow to 0.2 ml $\cdot$ min$^{-1} \cdot$ g$^{-1}$, or 10% of control, was accompanied by a related decline in mechanical function (Figure 3). When measured 30 minutes after the onset of ischemia ($I_{0}$), PSP was $44 \pm 3$ mm Hg, compared with $110 \pm 5$ mm Hg in the control period ($p<0.01$). Similarly, $dP/dt_{max}$ fell from $1,220 \pm 0$ to $370 \pm 20$ mm Hg/sec, and PRP$_{60}$ fell from $18.4 \pm 0.9$ to $3.4 \pm 0.2$ ($\times 10^3$) units. The latter included a substantial reduction in heart rate. As shown in Table 1, these changes were associated with a reduction in oxygen delivery from $202 \pm 6$ to $21 \pm 1 \mu l \cdot min^{-1} \cdot g^{-1}$. Oxygen extraction doubled from $27 \pm 1\%$ to $56 \pm 3\%$. Hence, oxygen consumption diminished from $53.9 \pm 1.9$ to $11.6 \pm 0.7 \mu l \cdot min^{-1} \cdot g^{-1}$, or about 20% of control. This closely mirrors the reduction in PRP$_{10}$ (to $18.5\%$).

The several mechanical and metabolic measurements were repeated at 30-minute intervals for 2 hours of low-flow ischemia. Data obtained after 120 minutes are shown in Figure 3 and Table 1. No significant further reduction in any of the mechanical measurements was identified. Oxygen metabolism remained stable throughout the ischemia period. Lactate uptake, which averaged $305 \pm 45 \mu mol \cdot min^{-1} \cdot g^{-1}$ in the control period, was consistently negative (release) throughout the ischemia period (Table 1).
Patterns of Recovery After Prolonged Low-Flow Ischemia

After 2 hours of ischemia, CF was increased in steps, being doubled every 5 minutes from 0.2 to 1.6 ml·min⁻¹·g⁻¹ and reaching full (preischemia) flow after 15 minutes. Original traces from a representative heart are shown in Figure 4. At the 30-minute control point, LVSP was 92 mm Hg, LVEDP was zero, +dP/dtmax was 907 mm Hg·sec⁻¹, and heart rate was 158 beats per minute. After 2 hours of ischemia (second panel), LVSP was 28 mm Hg, +dP/dtmax was 324 mm Hg·sec⁻¹, and heart rate was 62 beats per minute. When reperfusion was instituted, with each doubling of CF there was a progressive increase of mechanical function. At 30 minutes (after approximately 15 minutes of full CF), LVSP had increased to 75 mm Hg, +dP/dtmax to 851 mm Hg·sec⁻¹, and heart rate to 158 beats per minute.

The relation between increments in CF after 2 hours of ischemia and recovery of mechanical function defined by the PRP is summarized in Figure 5. PRP averaged 2.06±0.20 (×10³) units when CF was 0.19±0.1 ml·min⁻¹·g⁻¹. There was a linear increase in PRP to 8.24±0.71 (×10³) units when CF reached 1.60±0.07 ml·min⁻¹·g⁻¹. Resumption of full flow caused an insignificant further rise in PRP (R₉₀ in Figure 5). An identical pattern was observed with +dP/dtmax. For each of these measures of mechanical function, regression analysis indicated a strong correlation with CF (r>0.90).

Each heart was studied for an additional 30 minutes of recovery. Values for each measure of mechanical function at LVEDP of 10 cm H₂O were determined for R₃₀ and R₆₀. The latter values are shown in Figure 3. In none of the animals did mechanical function increase further with time after resumption of full CF. Values obtained at R₃₀ were statistically identical with those measured 30 minutes later (R₆₀).

Comparison of Control and Ischemia Groups

The intrinsic rate of deterioration of the preparation with normal CF was modest. Nonetheless, it must be considered in the analysis of the effects of ischemia when dealing with an overall time base of several hours. Mean values for each group at selected time points in the protocol are compared in Figure 3. PSP₁₀ at R₆₀ averaged 90±5 mm Hg in controls and 75±3 mm Hg in those subjected to 2 hours of ischemia. Thus, SP in the latter group averaged about 83% of controls, and the difference is significant (p<0.02). PRP₁₀ was also significantly less in the ischemia group, averaging about 78% of controls. In contrast, +dP/dtmax averaged 980±80 mm Hg·sec⁻¹.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Original traces from a 4-day-old piglet heart. LVSP, full left ventricular pressure trace from which systolic pressure was measured. LVEDP, left ventricular end-diastolic pressure. dP/dt, rate of change of LV pressure. Chart speeds on first and last panels, 50 mm·sec⁻¹; middle panels, 25 mm·sec⁻¹. All traces were obtained when LVEDP was 0–1 cm H₂O.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Average values for the pressure-rate product (upper panel) in relation to coronary flow (lower panel) in hearts after 2 hours of low-flow ischemia (I₁₂₀). Each increment of flow was accompanied by a corresponding increment in the pressure-rate product. Vertical brackets, SEM.
Responses To Calcium (2x)

Before Ischemia  |  After Ischemia
--- | ---
\[C_{30}\]  |  \[C_{Ca^{2+}}\]  |  \[R_{60}\]  |  \[R_{Ca^{2+}}\]

<table>
<thead>
<tr>
<th>Pressure - Rate Product - Units (x10^3)</th>
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<tr>
<td>25</td>
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![Graph](image_url)

**Figure 6.** Mechanical function of hearts perfused with 2.5 mmol/l calcium (open bars) or 5.0 mmol/l calcium (hatched bars). Responses before (left panel) and after 2 hours of ischemia and 1 hour recovery (right panel) are shown. Pressure–rate product increased during calcium stimulation to the same level as before ischemia. Vertical brackets, SEM. ***, p<0.001. \[C_{30}\], preischemia control values 30 minutes after beginning of protocol; \[R_{60}\], values measured 60 minutes after resumption of full coronary flow.

Changes in controls and 900±50 mm Hg · sec⁻¹ in the ischemia group (p=NS). Thus, a ±20% reduction in force generation can be ascribed to the consequences of ischemia, but no changes in velocity aspects of systolic function were demonstrated.

**Responses to Increased Calcium Concentration Before and After Ischemia**

To assess the effects of low-flow ischemia on the ability of hearts to respond to inotropic stimulation, calcium concentration was transiently doubled from 2.5 to 5.0 mmol/l. The results, expressed as the PRP₁₀, are illustrated in Figure 6. Before ischemia, PRP₁₀ averaged 17.0±0.6 (×10⁵) units (C₃₀). After the increase in calcium concentration, PRP₁₀ rose to 21.9±0.7 (×10⁵) units, or approximately 29% (p<0.001). Results from the same test applied after 2 hours of ischemia and 1 hour of recovery are shown in the right panel of Figure 6. Before calcium augmentation, PRP₁₀ averaged 11.5±0.9 (×10⁵) units (R₆₀). After the addition of calcium, PRP₁₀ rose to 19.3±1 (×10⁵) units, or nearly 68% (p<0.001). In absolute terms, the level of LV performance during calcium stimulation assessed by PRP₁₀ was nearly as great after the ischemia insult as before (88%). Similarly, +dP/dtmax increased 740 mm Hg · sec⁻¹, or 61%, before the ischemia period. One hour after recovery from ischemia, +dP/dtmax increased 890 mm Hg · sec⁻¹, or 99%, in response to calcium. The mean value for +dP/dtmax during calcium stimulation was 1,960±90 mm Hg · sec⁻¹ before ischemia and 1,790±60 mm Hg · sec⁻¹ after recovery from ischemia. By this measure, the level of contractility during calcium stimulation was 91% of that before ischemia, which is consistent with the 88% level measured by the PRP. In the control hearts after 3.5 hours of perfusion, PRP₁₀ increased from 14.9 to 22.2 (×10⁻⁵) units, or 49%, with calcium stimulation, and +dP/dtmax increased from 980 to 2,080 mm Hg · sec⁻¹, or 112%. These values did not differ significantly from the ischemia group.

**Comparison of Diastolic Pressure–Volume Relations and Effects on Systolic Mechanical Function**

Changes in diastolic ventricular compliance would substantially affect measurements of mechanical function dependent on filling pressure. To address this issue, the slopes of the diastolic pressure–volume relation before and after the ischemic period were compared. Similarly, slopes relating PSP to changes in EDP (PSP₁₀) or volume (PSP₁⁺_m) were calculated and expressed as percentage of control values. These data are summarized in Table 2. The pressure–volume slopes were identical at each time point. Whether measured by EDP or volume changes, there was no significant difference in SP generated during either recovery period when expressed as percentage of control (C₃₀). Transient changes in wall stiffness were noted with initiation of reperfusion. These disappeared within 5–10 minutes and were not characterized further. Sustained compliance changes could not be identified (Table 2). However, subtle changes might have been identified by more sophisticated analyses.

**Comparison of High-Energy Phosphates and Glycogen Concentrations and Myocardial Water Content**

Immediately after the measurements with elevated calcium were completed, the perfusate was replaced with that containing normal (2.5 mmol/l) concentration. Approximately 5 minutes was allowed for recovery. The hearts were then freeze-clamped and subsequently assayed for ATP, CP, and glycogen concentrations and water content. The data are shown in Figure 7. In controls, ATP averaged 28.4±1.8 µmol/g and in the ischemia group 23.3±1.9

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**Table 2. Comparison of Diastolic Pressure and Volume Changes in Relation to Systolic Mechanical Function Before and During Respective Recovery Periods After 2 Hours of Ischemia (n=8)**

<table>
<thead>
<tr>
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<th>C₃₀</th>
<th>R₆₀</th>
<th>R₆₀</th>
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</thead>
<tbody>
<tr>
<td>PV_slope</td>
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<tr>
<td>PSP₁₀ (%)</td>
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<td>PSP₁⁺_m (%)</td>
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<td>70.1±4.0</td>
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*Change in pressure (P) per unit change in end-diastolic volume (V), cm H₂O/ml. C₃₀, control values 30 minutes after start of experiment; R₆₀, values 30 and 60 minutes after return of normal coronary flow; PSP₁₀ peak systolic pressure at filling pressure of 10 cm H₂O expressed as percent of control (C₃₀); PSP₁⁺_m peak systolic pressure after 1 ml added to end-diastolic volume at 0 cm H₂O. End-diastolic pressure expressed as percent of control. All values estimated by linear regression analysis. No significant changes in left ventricular compliance are identified.
Discussion

Reduction of CF and oxygen delivery in the piglet heart to 10% of normal for 2 hours was followed by substantial recovery of function and metabolism when full CF was resumed. High-energy phosphate concentrations did not differ from those of control hearts, although glycogen concentrations were lower. Evidence for significant myocardial ischemia was the appearance of sustained lactate production and a significant widening of arteriovenous differences in $\text{PCO}_2$ and pH. These changes reversed when normal CF was resumed after 2 hours of ischemia (Table 3).

We have previously shown that mechanical performance assessed by the PRP$_{10}$ and MVO$_2$ bears an essentially linear relation to CF in the range studied. Moreover, the delay in reestablishing equilibrium after a step change in flow is brief (2–3 beats). This association has long been recognized in acute regional myocardial ischemia and was recently confirmed by more detailed analysis. The signal that transduces this remarkable phenomenon has not been identified. The pattern of changes, when prolonged, is referred to as hibernation and presumably protects myocardial cells from irreversible damage during ischemia.

Although the hibernation mechanism appears tightly coupled with oxygen supply, this interpretation is probably overly simplistic. For example, even though proportional changes in cardiac function and MVO$_2$ were found, significant anaerobic metabolism appeared, as reflected by a shift from positive to negative lactate uptake and a corresponding widening in the arteriovenous pH difference across the heart. Regardless, these changes in function and metabolism remained in stable equilibrium throughout the 2-hour ischemia period (Figure 3 and Table 1).

Patterns of recovery following ischemia were essentially the reverse of those observed previously with incremental reductions in CF. Each increase in CF was accompanied by a virtually lockstep increase in mechanical function (Figure 4), with an estimated delay of only 2–3 beats. However, PSP$_{10}$ and PRP$_{10}$ reached only 83% and 78%, respectively, of values obtained at comparable time intervals in the control group. The differences in the two measures, while small, were statistically significant. They presumably reflect the phenomenon known as stunned myocardium.

The mechanistic basis for persistent myocardial dysfunction after ischemia and reperfusion is not fully defined; it need not be unifactorial. Good correlations between decreased ATP concentrations and contractile...
function have been demonstrated. In this study, the percentage changes in the PRP and ATP were identical after ischemia; both were 83% of values in the control group. This clearly does not indicate a causal relation, however. Altered calcium transport in cardiac sarcoplasmic reticulum has also been described in postischemic myocardium, and damage to the contractile filaments may be a contributory factor.

Regardless of the mechanism, it is evident that the ischemic hearts were able to generate vigorous inotropic responses to calcium (Figure 6). PRP or +dP/dt\textsubscript{max} with calcium stimulation was identical in the two groups. This was true before and after ischemia and recovery or the equivalent time base in the control group. These results appear comparable to those obtained in the adult dog. Myocardial high-energy phosphate concentrations were not measured before reperfusion. It seems unlikely, however, that deficient energy stores are sufficient to explain the reduced levels of postischemic contractile function in view of the findings that these hearts responded to calcium stimulation as well as or better than time-matched controls or their own preischemia performance. Moreover, measured ATP and CP concentrations did not differ from control hearts (Figure 7). Other mechanisms, such as reduced rates of ATP-dependent calcium transport in sarcenemal membrane or sarcoplasmic reticulum, appear to be more likely candidates.

There is considerable discussion regarding the relative vulnerability of neonatal hearts to ischemic injury. Some research has indicated that they are more sensitive to global ischemia than are adult hearts and that this may be ascribed to a more rapid accumulation of lactate as a result of a greater anaerobic metabolic capacity. Other data would lead to the opposite conclusion, that neonatal hearts are more resistant to ischemia. Our studies indicate that prolonged low-flow ischemia causes only a modest impairment of force generation (PSP). Velocity measurements (dP/dt\textsubscript{max}) did not differ from controls (Figure 3). No differences in recovery from ischemia were identified among hearts 1–18 days old used in the present study. This is consistent with our earlier observations that piglet hearts assume metabolic patterns of the adult within a few days of delivery.

Interpretation of these data requires that several specific factors be recognized. First, low-flow ischemia may differ importantly from total ischemia. Tani and Neely showed that even very low flow rates significantly reduced calcium overload in ischemic rat hearts, resulting in improved recovery with reperfusion. Second, we used red blood cell–enhanced perfusate with oxygen carrying capacity typically found in the piglet in vivo. Crystallloid solutions have been used more commonly. There is now abundant evidence, however, that hearts larger than rat hearts are not fully oxygenated by these solutions. Third, it should be kept in mind that isolated hearts are not subjected to the many autonomic, hormonal, and mechanical stresses of the in vivo heart. This point is emphasized by the demonstration that oxygen requirements of dyskinetic myocardial segments may be remarkably high, perhaps related to passive stretching in vivo. Regardless, the phenomena of myocardial hibernation and stunning can be identified and studied in the more restricted environment of the isolated perfused heart.

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References


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