Immediate functional recovery and avoidance of reperfusion injury with surgical revascularization of short-term coronary occlusion

J. VINTEN-JOHANSEN, PH.D., T. ARTHUR EDERGTON, M.D., HAROLD R. HOWE, M.D., PAMELA A. GAYHEART, B.A., STEPHEN A. MILLS, M.D., GEORGE HOWARD, M.P.H.S., AND A. ROBERT CORDELL, M.D.

ABSTRACT  Functional recovery with surgical revascularization of acutely ischemic myocardium has not been compared with its nonsurgical counterpart in experimental preparations of coronary occlusion. This study compares the functional and metabolic recovery of ischemic (1 hr coronary occlusion) segments revascularized either by restoration of coronary patency (simulating nonsurgical recanalization, e.g., angioplasty) or by surgical revascularization with multidose hypothermic potassium blood cardioplegic solution. Twenty-two anesthetized open-chest dogs were instrumented with Millar micromanometer-tip catheters to measure left ventricular and aortic pressures. Piezoelectric ultrasonic dimension gauges were implanted in the subendocardium supplied by the left anterior descending coronary artery to measure segmental contractile function. In five dogs, only biopsy samples were obtained for control measurements of ATP, creatine phosphate, and tissue water content. In the remaining 17 dogs, the left anterior descending artery and collaterals were ligated for 1 hr. The ligatures were removed in eight dogs and coronary perfusion continued for 2 hr, simulating nonsurgical reperfusion. The remaining nine dogs were placed on cardiopulmonary bypass and the hearts were arrested for 1 hr with multidose (every 20 min) blood cardioplegic solution enhanced with glutamate and aspartate, simulating surgical revascularization (coronary artery bypass grafting). The coronary ligatures were not released until the second cardioplegic infusion, simulating graft placement. One hour of coronary occlusion placed 39.4 ± 2.5% of the left ventricle at risk, and converted active systolic shortening to persistent paradoxical bulging (25.2 ± 2.2% to −5.8 ± 1.2% systolic shortening). Surgical revascularization resulted in greater recovery of postischemic systolic shortening (7.95 ± 1.90% vs −6.90 ± 2.01% systolic shortening; p < .05), lower subendocardial water content (79.36 ± 0.50% vs 81.14 ± 0.53%; p < .05), and less gross histochemically apparent (triphenyltetrazolium chloride) tissue damage (4.2 ± 4.2% vs 30.9 ± 6.8% of area at risk; p < .05) compared with hearts reperfused nonsurgically. There were no differences in regional ATP and creatine phosphate levels between surgically and nonsurgically revascularized hearts despite the marked differences in functional recovery. We conclude that (1) myocardium subjected to 1 hr of coronary occlusion is potentially recoverable depending on the modality of the early phase of reperfusion and (2) surgical revascularization avoids reperfusion injury resulting in immediate postischemic functional recovery.


THE GOALS of revascularization of acute myocardial infarcts are to arrest the progression of damage and restore morphologic integrity and functional capacity to the involved segment. The nonsurgical approach of pharmacologic thrombolysis and percutaneous transluminal coronary angioplasty (PTCA) is a strong and popular alternative to restoring coronary patency and perfusion to the previously ischemic segment. However, equivocal success in restoring systolic function globally as well as in the involved segment1–3 has tempered enthusiasm for this approach. Experimental studies have shown that restoration of blood flow after a period of transient coronary occlusion produces myocardial hemorrhage,4–8 advances mitochondrial deterioration,5–8 and produces discrete areas of "no-reflow."9–10 Several studies suggest that many of these manifestations of tissue damage may occur or be exac-
erbated during the reperfusion period itself (reperfusion injury).\(^7\)\(^,\)\(^11\)\(^,\)\(^12\) Whatever the pathophysiologic mechanisms involved, restoration of blood flow after transient occlusion (15 min to 4 hr) fails to fully restore wall motion in the ischemic segment, even after prolonged periods of recovery.\(^13\)\(^\text{-}^15\)

On the other hand, results of experiments with surgical revascularization of globally ischemic hearts emphasize that the consequences of ischemia and reperfusion can be reversed or avoided, resulting in recovery of aerobic metabolism, limitation of intramyocardial edema, preservation of the ultrastructural integrity, and substantial recovery of immediate postischemic function.\(^16\)\(^\text{-}\)\(^18\) In contrast to nonsurgical recanalization techniques, the surgical approach affords complete control of the conditions (unloaded ventricle, coronary perfusion pressure) and composition (cardioplegia) of the reperfusionate during the initial phases of reperfusion. This study tests the hypothesis that appropriate modification of the reperfusion phase during surgical revascularization of short-term (1 hr) coronary occlusion reverses or avoids reperfusion damage. We will show that appropriate management of reperfusion allows less edema formation, less histochemically apparent tissue damage, and immediate functional recovery compared with nonsurgical revascularization.

### Methods

Experimental procedures complied with the “Guiding Principles in the Use and Care of Animals” approved by the Council of the American Physiological Society as well as with state and federal regulations.

Twenty-two dogs weighing between 14 and 24 kg were anesthetized with 30 mg/kg iv sodium pentobarbital (Nembutal), supplemented as necessary to maintain deep anesthesia. After endotracheal intubation, positive-pressure ventilation was initiated (Harvard volume cycle respirator) with 100% oxygen. The dogs were then divided into two groups: those receiving unmodified reperfusion by restoring coronary patency and those revascularized by surgical procedures with blood potassium cardioplegic solution.

**Nonsurgical reperfusion group.** In eight dogs the chest was opened in the fifth left intercostal space. A No. 7.5F thermolotion catheter (Criticor, Inc.) was introduced into the right external jugular vein and manipulated into the pulmonary artery for measurement of pulmonary arterial wedge pressure and cardiac output in triplicate (3 ml iced saline), and for volume and pharmacologic management. The dogs were given 2 to 3 mg/kg sodium heparin.

**Surgical revascularization group.** In nine dogs the chest was opened by median sternotomy and the pericardium was tented. After systemic heparinization (2 to 3 mg/kg), cardiopulmonary bypass with right heart bypass capability (to generate Sarnoff function curves) was established by cannulating the inferior and superior venae cavae and directing the venous return to a Shiley S-100A bubble oxygenator through which flowed oxygen and carbon dioxide mixed appropriately for adequate blood gases. Oxygenated blood was returned by a cannula either in the left subclavian artery (total bypass) or in the main pulmonary artery (right heart bypass). Cannulas were placed in the left ventricle and the left atrium via the left atrial appendage to either vent the heart during total bypass or measure mean left atrial pressure, respectively. Total coronary effluent was drained continuously by a cannula in the right ventricle.

In all dogs, Millar MPC-500 pressure transducers were placed into the aortic arch via the right carotid artery or the left internal mammary artery (aortic pressure) and through the apical dimple to measure left ventricular intracavitary pressure. A segment of the left anterior descending coronary artery (LAD) distal to the first diagonal branch was dissected free and a ligature was placed loosely around it for subsequent occlusion. A pair of 2.5 mm diameter ultrasonic piezoelectric dimension transducers were implanted in the subendocardium of the myocardium served by the LAD to measure segmental systolic shortening with a Triton Model 120 Sonomicrometer (Triton Technology, Inc., San Diego).

All measurements were recorded on a Hewlett Packard 7758B thermal recorder and simultaneously digitized and printed by a Buxco Datalogger (Buxco Electronics, Sharon, MA). The end-diastolic and end-systolic segment lengths from the ultrasonic crystals were timed off the upstroke of the instantaneous left ventricular pressure trace and the dicrotic notch of the aortic pressure pulse, respectively. The percent systolic shortening was calculated as 100 × (EDL – ESL)/EDL where EDL and ESL are end-diastolic and end-systolic segment lengths in millimeters, respectively. A positive value indicates active shortening and a negative value represents passive bulging (paradoxical wall motion) during systole.

**Protocol.** In dogs receiving normal blood reperfusion (nonsurgical reperfusion), hemodynamic and functional data were obtained in the control state with the LAD patent. In the surgical revascularization group, Sarnoff function curves\(^17\) were obtained in the control state by incrementally increasing cardiac output from 1.5 to 5.0 liters/min while maintaining mean arterial pressure at 80 mm Hg via the subclavian cannula. The cardiac workload was then adjusted to 100 ml/kg cardiac output and a pressure of 100 mm Hg.

In all dogs, 2 to 3 mg/kg lidocaine was administered as a bolus while 0.5 mg/kg/hr was continuously provided as an intravenous drip. The obvious collateral arteries encroaching on the LAD territory and finally the LAD were ligated for a total of 1 hr. Hemodynamic and segmental function data were recorded every 20 min. Blood gases were maintained at a pH between 7.38 and 7.42 and at PCO\(_2\), 30 to 40 mm Hg. Arrhythmias were treated with lidocaine as necessary. Ventricular fibrillation, when encountered, was electrically converted; data were not accepted if (1) the ischemic segment was not dyskinetic for the entire hour, (2) ventricular fibrillation persisted for more than 3 min, or (3) the cardioplegic solution was not within ranges stipulated.

In nonsurgically revascularized dogs receiving normal blood reperfusion, all ligatures were removed after the 1 hr ischemic period and reperfusion continued for a total of 2 hr. In dogs in which revascularization was accomplished surgically, total cardiopulmonary bypass and ventricular decompression was established after the 1 hr occlusion period. Thermistor probes were placed in ischemic (anterior) and nonischemic (posterior) zones. The aorta was cross-clamped and blood cardioplegic solution enriched with glutamate (13 mM) and aspartate (13 mM) (table 1) was delivered with a Buckberg-Shiley Cardioplegia Delivery device (4:1 blood to cardioplegia components ratio) in the aortic root through a preplaced double-lumen catheter (inner lumen for measurement of cardioplegic infusion pressure, outer lumen for delivery of cardioplegic solution) at 50 mm Hg for a total of 10 min. The first 5 min of this period was normothermic (37°C) and the final 5 min hypothermic (4°C).\(^18\)
**TABLE 1**

**Composition of amino acid-enhanced cardioplegic solution**

<table>
<thead>
<tr>
<th>Principle</th>
<th>Component</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenation</td>
<td>Blood</td>
<td>CO₂ 11.84 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hct 17.6 ± 1.2</td>
</tr>
<tr>
<td>Buffer acidosis</td>
<td>Tromethamine</td>
<td>8.028 ± 0.067</td>
</tr>
<tr>
<td>Appropriate pH</td>
<td></td>
<td>@4°–8° C</td>
</tr>
<tr>
<td>Substrates</td>
<td>Glutamate, aspartate</td>
<td>13 mM each</td>
</tr>
<tr>
<td>Reduce calcium</td>
<td>CPD chelation</td>
<td>0.80 ± 0.02 meq</td>
</tr>
<tr>
<td>Avoid edema</td>
<td>Hyperosmolality</td>
<td>381 ± 3 mOsm</td>
</tr>
<tr>
<td></td>
<td>Low pressure</td>
<td>50 mm Hg</td>
</tr>
<tr>
<td>Reduce O₂ demands</td>
<td>Immediate arrest</td>
<td>Hyperkalemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.1 ± 2.3 meq K⁺</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4°–8° C delivery</td>
</tr>
</tbody>
</table>

CaO₂ = cardioplegia oxygen content in ml O₂/100 ml (volume percent); CPD = citrate-phosphate-dextrose; Hct = hematocrit.

Simultaneous with the delivery of hypothermic cardioplegic solution, systemic temperature was decreased to 28°C. Intermittent infusions of 4°C C blood cardioplegic solution were given at 20 and 40 min for a total arrest time of 60 min. The coronary ligatures were removed just before the 20 min infusion, simulating delivery of cardioplegic solution down a graft with distal anastomosis complete or infusion into the aortic root of cardioplegic solution after completion of both proximal and distal anastomoses in a single graft.19 After 1 hr of cardioplegia, systemic temperature was raised to 37°C and reperfusion cardioplegia was administered for 3 min at 50 mm Hg and 9°C above septal temperature.20 Systemic pressure was lowered to 50 mm Hg and the cross-clamp was removed. When electromechanical activity was visible, systemic pressure was gradually raised to 100 mm Hg over 3 min. The heart was maintained in the beating empty state for 30 min and was subsequently placed in the working (right heart bypass) state at 100 ml/kg cardiac output and 100 mm Hg systemic pressure for an additional 30 min. Postischemic hemodynamics and regional and global function were then determined.

After each experiment, transmural biopsy specimens were taken by high-speed drill from the nonischemic and ischemic zones, divided into subepicardial (EP) and subendocardial (ENDO) halves, and quick-frozen in liquid nitrogen for later analysis of tissue ATP and creatine phosphate (CP).21 Parallel biopsy specimens were also taken from each zone for analysis of tissue water content (percent water = 100 [1 – (dry tissue weight/wet tissue weight)]). Biopsy sites were repaired with pledgeted sutures and all coronary ligatures were replaced. Gentian violet stain (0.2 ml/kg) was infused either into the left atrium (normal blood reperfusion group) or into the aortic root cardioplegia cannula (surgical revascularization group) to demarcate normally perfused from ischemic myocardium. Hearts were arrested with KCl, excised, and cut into 5 mm thick transverse slices, and the ischemic (unstained) and normally perfused (stained) areas were determined by planimetry. The area placed at risk by coronary occlusion was calculated as 100 × (unstained area/total left ventricular area). The slices were incubated in triphenyltetrazolium chloride (TTC) at 37°C for 15 to 20 min.22 TTC stains normal myocardium brick red while leaving damaged or necrotic myocardium pale. The “area of necrosis” was determined by planimetry and calculated as 100 × (TTC unstained area/total left ventricular area). The left ventricular tissue damage is expressed as the ratio of the area of necrosis/area at risk, expressed in percent.

Five dogs used to obtain control biopsy samples were instrumented as in the medical reperfusion group for the purpose of obtaining measurements of myocardial ATP, CP, and water content in the control state.

Data were analyzed with the Statistical Analysis System (Cary, NC) biostatistical package. Differences with respect to time, myocardial region, and group were analyzed by the general linear model multivariate analysis followed by Wilk’s lambda test.

**Results**

Ligation of the LAD and encroaching collateral vessels placed 39.9 ± 4.3% of the left ventricular mass at risk in the nonsurgical group and a similar value of 39.0 ± 2.6% of the left ventricle in the surgically revascularized group.

Hemodynamic data for both groups during control, 1 hr of coronary occlusion (ischemia), and reperfusion are summarized in table 2. Ischemia was accompanied by modest decreases in heart rate and systolic pressure whereas left ventricular end-diastolic pressure increased significantly. In the nonsurgical reperfusion group, cardiac output decreased by 20% during ischemia and by 33% during reperfusion from control levels (p < .05). At the end of 2 hr of reperfusion, cardiac output in the nonsurgical reperfusion group was 16% (p < .05) below that in the surgical revascularization group, in which cardiac output was held constant by the extracorporeal bypass circuit.

End-diastolic length and systolic shortening were similar in both groups during the control period (table 3). Ligation of the LAD immediately replaced active contractile shortening with paradoxical systolic bulging, which persisted for the entire ischemic period. The degree of paradoxical segmental wall motion was equivalent in both groups and averaged −25.3% of control systolic shortening for all hearts.

Hearts revascularized surgically arrested within 1.5 min of initiation of cardioplegia. As expected, the anterior ischemic border zones showed fine fibrillation up to 1.5 min into the cardioplegic period while the posterior segment arrested within 45 sec. This persistence of asynchronous electromechanical activity in the ischemic segment is most likely a result of maldistribution of cardioplegic solution beyond the total occlusion; the anterior ischemic segment remained warmer by 13.9°C than the posterior segment, which received unimpeded flow at 4°C to 8°C (figure 1).

With 2 hr of reperfusion initiated by simple release of the ligature (nonsurgical), there was no significant restoration of systolic function in the previously ischemic segment. In sharp contrast, those hearts given amino acid–enriched blood cardioplegic solution surgically as the initial reperfusate showed recovery of immediate systolic shortening averaging 32.8% of its
preischemic level (figure 2). This segmental shortening was significantly greater than posts ischemic wall motion in hearts reperfused nonsurgically (p = .00008).

**High-energy phosphates.** ATP and CP levels in control hearts were similar between EPI and ENDO regions (p = .67 and .71, respectively) in both anterior and posterior walls. ATP levels were depleted comparably in the nonsurgical reperfusion group (EPI = 61.8%, ENDO = 30.4% of control) and the surgical revascularization group (EPI = 52.0% and ENDO = 42.8% of control) (figure 3) despite differences in their functional recovery. A slight “overshoot” in CP levels was observed in both groups. No significant changes in high-energy phosphate levels occurred in the posterior, normally perfused myocardium.

**Tissue water content.** Myocardial water content in control hearts averaged 78.18 ± 0.12% and 78.60 ± 0.14% in anterior EPI and ENDO regions and averaged 78.10 ± 0.08% and 78.44 ± 0.12% in posterior EPI and ENDO regions, respectively. There were no differences between anterior and posterior wall or between EPI and ENDO regions. With nonsurgical reperfusion, anterior myocardial water content rose significantly by 2.76% in the EPI and 2.70% in the ENDO region (figure 4). There was no significant tissue water accumulation in the posterior wall, with the EPI value averaging 78.40 ± 0.50% and the ENDO value averaging 78.54 ± 0.53%. With surgical revascularization in which the conditions and composition of the initial reperfusion were controlled, there was a 1.95% gain in EPI water content that was not different from the value in the nonsurgical group. However, edema in the ENDO water content was impaired by .05.

![Graph showing myocardial temperatures in the anterior (ischemic) and posterior (nonischemic) zones after each infusion of cardioplegic solution. Myocardial temperature was significantly higher in the ischemic zone during the induction phase when its distribution was impaired by the ligation. Thereafter, myocardial temperatures were identical in ischemic and nonischemic zones. *p < .05.](image-url)

### TABLE 2
**Hemodynamic variables during control, coronary occlusion, and reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>Arterial pressure (mm Hg)</th>
<th>Cardiac Output (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>LVSP (mm Hg)</td>
</tr>
<tr>
<td><strong>Blood reperfusion</strong> (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>158 ± 7</td>
<td>132 ± 4</td>
</tr>
<tr>
<td>Ischemia</td>
<td>150 ± 7</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>157 ± 6</td>
<td>115 ± 6</td>
</tr>
<tr>
<td><strong>Surgical revascularization</strong> (n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>180 ± 7</td>
<td>133 ± 4</td>
</tr>
<tr>
<td>Ischemia</td>
<td>169 ± 5</td>
<td>126 ± 5</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>164 ± 4</td>
<td>131 ± 5</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.

HR = heart rate; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure.

Significantly different from group control values.

Significantly different from group ischemic values.

### TABLE 3
**Segmental length data during control, ischemia, and reperfusion**

<table>
<thead>
<tr>
<th>Group/time</th>
<th>End-diastolic (mm)</th>
<th>End-systolic (mm)</th>
<th>% Shortening</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonsurgical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.55 ± 1.06</td>
<td>8.40 ± 0.78</td>
<td>26.28 ± 3.36</td>
</tr>
<tr>
<td>Ischemia</td>
<td>12.72 ± 1.44</td>
<td>13.30 ± 1.56&lt;</td>
<td>-4.50 ± 1.68</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>12.09 ± 1.27</td>
<td>12.94 ± 1.44&lt;</td>
<td>-6.90 ± 2.01&lt;</td>
</tr>
<tr>
<td><strong>Surgical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.03 ± 1.00</td>
<td>10.61 ± 0.73</td>
<td>24.24 ± 3.16</td>
</tr>
<tr>
<td>Ischemia</td>
<td>17.53 ± 1.36&lt;</td>
<td>18.76 ± 1.47&lt;</td>
<td>-6.99 ± 1.59&lt;</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>15.31 ± 1.20&lt;</td>
<td>14.23 ± 1.35&lt;</td>
<td>7.95 ± 1.90&lt;</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.

Significantly different from group control values.

Significantly different from group ischemic values.
region was significantly less marked (0.76% increase) than that in nonsurgically revascularized segments and was indistinguishable from control ENDO tissue water content (figure 4). Despite the global ischemia experienced during the 1 hr cardioplegic period in the surgical revascularization group, there was no significant alteration in posterior myocardial water content ($EPI = 78.01 \pm 0.35\%$ and $ENDO = 77.62 \pm 0.67\%$).

**Macroscopic tissue damage.** Tissue damage expressed as the area of necrosis demarcated by TTC staining relative to the area involved in the coronary occlusion (area at risk) averaged 30.9 $\pm$ 6.8\% in nonsurgically revascularized hearts. The apparent tissue damage ranged from confluent involvement of the ENDO region to a diffuse transmural “patchy” appearance with islands of stained (normal) tissue interdigitating closely with large discrete nonstained (damaged) areas. In contrast, segments revascularized surgically showed significantly less damage (4.2 $\pm$ 4.2\%), with seven of the eight hearts histochemically analyzed demonstrating no area of TTC-sensitive damage.

**Discussion**

We examined the benefits of surgical revascularization using modification of conditions and composition of the initial reperfusionate in restoring metabolism, morphologic integrity, and function in the involved segment. The results emphasize that the eventual fate of myocardium exposed to ischemia is not only related to the duration and severity of the occlusion but is also dependent on the initial reperfusion phase. The severity and duration of ischemia was identical in both groups before reperfusion. This “reperfusion injury” is

**FIGURE 2.** Systolic shortening in the myocardium perfused by the LAD is shown at control, after 1 hr of coronary occlusion (ischemia), and after reperfusion. Negative values indicate paradoxical bulging during systole. Paradoxical bulging persisted in the nonsurgical revascularization group in contrast to the 32.8\% of control active systolic shortening regained in the surgical revascularization group. *p < .05.

**FIGURE 3.** ATP (top panel) and CP levels (bottom panel), in EPI and ENDO regions of the anterior (ischemic) zone. Control values were obtained from the anterior zone in five dogs not subjected to coronary occlusion. ATP was significantly depleted in EPI and ENDO regions in both nonsurgical and surgical groups relative to controls, avoidable when appropriate management of the reperfusion phase is observed. Avoidance or reversal of reperfusion injury, by modification of the conditions and composition of reperfusionate as is possible during

**FIGURE 4.** Tissue water content in the anterior (ischemic) EPI and ENDO regions. *Significant (p < .05) differences from controls in comparable regions.
coronary artery bypass grafting, leads to immediate recovery in tissue that was previously thought to be metabolically and functionally “stunned.” Although we could not identify the factor or factors responsible for myocardial stunning in this study, our findings emphasize that this delay or failure in recovery may be overcome by modification of the reperfusion.

We chose the model of 1 hr of ischemia on the basis of the total lack of recovery obtained when unmodified blood was the reperfusionate and the subtotal recovery shown with surgical revascularization when blood cardioplegic solution was the initial reperfusionate. The clinical correlate of an occlusion of such a short duration is a short-term occlusion occurring either spontaneously in the hospital or during attempted angioplasty. Studies currently in progress in this laboratory have extended the duration of this ischemic period to 3 hr.

Ischemia caused by total coronary occlusion initiates a cascade of biochemical and morphologic changes, the severity of which has been related to the duration and degree of flow inadequacy. Contractile function and high-energy phosphate pools are rapidly lost,23, 24 and with more prolonged periods of occlusion glycogen stores are depleted,25 ionic and cell volume homeostasis are lost,26, 27 sarcolemmal integrity and subcellular architecture deteriorate.25 Cell damage and eventual death begin first in the more vulnerable subendocardium and progress with time to the outer, subepicardial tissue in a “wavefront” pattern.28 These alterations in cellular integrity and metabolism predispose the involved myocardium to further deleterious changes that occur with the resumption of coronary blood flow, specifically (1) the influx and accumulation of calcium in the contractile apparatus and mitochondria29 resulting in further deterioration of contractile and metabolic capacity, (2) peroxidation of membrane structures by oxygen radicals,29 30 (3) washout of metabolic precursors and metabolites of high-energy phosphates, and (4) intramyocardial hemorrhage3, 4 and edema. Therefore, myocardium that is potentially reversibly damaged and salvageable before reperfusion may become irreversibly damaged by the deleterious sequelae just outlined. Although the pathophysiologic factors involved in determining the transition from reversibly injured to irreversibly injured myocardium are not yet clear and the limits of cell viability not defined, myocardium made transiently ischemic for relatively short durations (15 min to 4 hr) remain metabolically34 and functionally13-15 “stunned” for up to 4 weeks of reperfusion. The major thrust of research effort has been directed toward the reduction of infarct size and severity by altering the oxygen supply-demand balance pharmacologically. Relatively few studies have addressed the issue of reperfusion injury and how this specific injury after a given duration of ischemia can be minimized. Our results from this study confirm the concept of reperfusion injury as a separate entity and show that this injury can be avoided or reversed, resulting in immediate restoration of contractile function by specifically modifying the reperfusion phase appropriately.

The principles of appropriate modification of the initial reperfusionate have evolved from studies of myocardial protection with potassium cardioplegic solution during global ischemia encountered in cardiac surgery. The basic tenets of reperfusionate modification developed by proponents of blood cardioplegia are summarized in table 1. These principles may apply as well to the setting of regional ischemia produced by coronary occlusion. First, immediate cardiac arrest and subsequent hypothermia reduce myocardial energy requirements to allow channeling of available energy to reparative processes. This may be important in the setting of coronary occlusion, since studies suggest that the oxygen demands of the ischemic segment exhibiting paradoxical motion are high by virtue of passive wall tension during systolic bulging although active tension (contraction) is absent.31 Second, the accumulation of protons (H+) during ischemia is buffered by the relative alkalosis created by tromethamine,16, 19 while the gradient favoring calcium influx from the extracellular space is reduced by chelation with citrate-phosphate-dextrose. The deleterious effects of tissue acidosis and calcium accumulation on myocardial oxidative metabolism, glycolysis, and the cellular oxidation-reduction state are well known.7, 32, 33 Third, the amino acids glutamate and aspartate provide substrates for anaerobic metabolism during global arrest and replace Krebs cycle intermediates metabolized oxidatively once sufficient oxygen is restored.

The addition of the amino acids L-glutamate and L-aspartate to the blood cardioplegic solution was based on previous results in global models of ischemia and reperfusion where postischemic recovery of function and oxygen utilization were improved in hearts given amino acid–enhanced blood cardioplegic solution.17, 34 Oxygen utilization is characteristically impaired in hearts damaged by ischemia and reperfusion.9, 17, 18 Other studies have substantiated the depletion of these amino acids during ischemia35 and their uptake and incorporation when exogenously supplied.36-38 The salutary effects of glutamate and aspartate in hearts sub-
jected to cardioplegia may be exerted by three possible mechanisms. First, during ischemia these amino acids may be anaerobically metabolized ultimately to succinate to yield two additional moles of high-energy phosphate each, independently of glycolysis. Second, glutamate and aspartate incorporated into the cell replenish Krebs cycle intermediates lost during ischemia. Third, glutamate and aspartate participate directly in the malate-aspartate shuttle. This shuttle is the primary mechanism by which reducing equivalents (protons) are transported from the cytosol to the mitochondria, thereby maintaining the normal intracellular oxidation-reduction state. The malate-aspartate shuttle is tightly linked to and coordinates glycolysis and the Krebs cycle to maintain a balanced oxidation-reduction state. We suspect that glutamate and aspartate provided a better metabolic environment during cardioplegia, which allowed better cellular repair and functional recovery. Others have also found amino acid supplementation to improve postischemic myocardial function. However, the salutary effects of amino acid supplementation could not be assessed independent of the other modifications in this study.

The normothermic induction of cardioplegia was designed to maximize the rate of cellular repair by maintaining the basal metabolic rate (cell maintenance and repair) while avoiding the additional energy requirements of mechanical work. Normothermic induction of cardioplegia in globally ischemic hearts was associated with a greater oxygen uptake during the induction period and improved postischemic oxygen utilization and function. However, the efficacy of this phase depends on the distribution of cardioplegic solution to the involved myocardium. Coronary occlusion offers a significant impediment to the adequate delivery of cardioplegic solution to the myocardium distal to the occlusion. This maldistribution is inferred from figure 1 by the significant temperature gradient demonstrated between the ischemic and normally perfused segment. Therefore the ischemic segment did not receive the benefit of the initial warm phase of cardioplegia and in reality did not receive adequate cardioplegia until the ligation was relieved before the second (hypothermic) infusion. The importance of this initial normothermic phase on postischemic recovery is emphasized by the results of a previous experiment in which an ischemic segment demonstrated greater (55%) recovery of segmental function when given the benefit of the normothermic infusion despite an additional hour (2 hr total) of coronary occlusion.

We suspect that greater postischemic recovery could have been gained in the present study if the segment was initially normothermically perfused. Perhaps a clinical strategy could be developed to ensure the distribution of a normothermic infusion of cardioplegic solution subsequent to completion of distal anastomosis to optimize postischemic recovery.

Tissue levels of high-energy phosphates have in the past been considered an index of the adequacy of myocardial protection. Therefore, cardioplegic techniques and formulations that result in higher ATP levels are considered to be superior in myocardial protection than techniques and formulations resulting in lower ATP levels. Several studies have shown a critical level of ATP below which cell survival is doubtful, and failure of ATP levels to be restored has been speculated in the etiology of myocardial stunning. However, our results contradict this correlation of tissue ATP levels with cell viability and adequacy of myocardial protection. We found no differences in ATP and CP levels between myocardial segments showing functional recovery, less marked edema, and little or no morphologically apparent damage than those exhibiting no functional restoration and gross edema. A similar lack of correlation has been reported by others. Static levels of ATP and CP are only a measure of the size of the pool and do not reflect the turnover rate, which may be independent of the ATP pool size. Oxygen consumption may be a more sensitive index of metabolic rate than static ATP levels. Previous studies have shown a good correlation between oxygen consumption and the functional recovery of myocardium after global ischemia of various durations.

Surgical revascularization of acutely ischemic myocardium has been shown to be feasible. However, quantitative substantiation of the functional and metabolic restoration of ischemic myocardium as well as the mechanisms involved with surgical revascularization are lacking. This study represents our initial efforts to understand the potential mechanisms underlying salvage of myocardium with surgical techniques so that methods may be designed to optimize recovery from more prolonged durations of ischemia.

We are grateful for the expert assistance provided by A. C. Shircliffe and the Surgery Research Unit, and by Kevin S. Harbourne, B.S. Appreciation is extended to Robin Tesh for meticulous preparation of the manuscript and to William E. Johnston, M.D., for his thoughtful comments. Oxygenators and cardioplegia delivery devices were kindly donated by Shiley, Inc., through the efforts of Danny Daniel.

References
VINTEN-JOHANSEN et al.


5. Klone RA, Ganote CE, Whalen D, Jennings RB: Effect of a transient period of ischemia on myocardial cell. II. fine structure during the first few minutes of reflow. Am J Pathol 74: 399, 1974


41. Safer B, Smith CM, Williamson JR: Control of the transport of reducing equivalents across the mitochondrial membrane in perfused rat heart. J Mol Cell Cardiol 2: 111, 1971


46. Jennings RB, Hawkins HK, Lowe JE, Hill ML, Klotman S,


Immediate functional recovery and avoidance of reperfusion injury with surgical revascularization of short-term coronary occlusion.
J Vinten-Johansen, T A Edgerton, H R Howe, P A Gayheart, S A Mills, G Howard and A R Cordell

Circulation. 1985;72:431-439
doi: 10.1161/01.CIR.72.2.431

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/72/2/431

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/